Table 1. Inhibition of CdR kinase by dCTP or 5-AZA-dCTP*

Substrate	Addition	Concn (μM)	Nucleotide formed (nmole)	Inhibition (%)
CdR	None		0.95	0
	dCTP	5	0.81	15
	dCTP	10	0.68	28
	dCTP	20	0.53	44
	5-AZA-dCTP	20	0.83	13
	5-AZA-dCTP	40	0.67	29
	5-AZA-dCTP	80	0.50	47
5-AZA-CdR	None		0.98	0
	dCTP	5	0.96	2
	dCTP	10	0.67	32
	dCTP	20	0.40	59
	5-AZA-dCTP	20	0.74	25
	5-AZA-dCTP	40	0.57	42
	5-AZA-dCTP	80	0.37	62

* The standard reaction mixture contained 100 mM imidazole–HCl, pH 6.8, 0.05 μCi of 20 μM [^{14}C]CdR or [6–^{14}C]5AZA-CdR, as indicated, and the indicated concentrations of dCTP or 5-AZA-dCTP. The reaction mixture was incubated for 5 min at 37° in the presence of 0.3 unit CdR kinase.

32 per cent inhibition of the phosphorylation of 5-AZA-CdR (20 μM) by CdR kinase. 5-AZA-CdR was slightly more sensitive to the inhibition produced by dCTP than the natural substrate, CdR.

5-AZA-dCTP was also an inhibitor of CdR kinase when either CdR or AZA-CdR was used as the substrate (Table 1). The inhibition of this reaction produced by 5-AZA-dCTP was less than an equimolar concentration of dCTP. Thus, this fradulent nucleotide had lower binding affinity for the enzyme than the natural nucleotide, dCTP, analogous to the condition observed for the substrates as mentioned earlier. The concentrations of 5-AZA-dCTP that inhibit CdR kinase in these experiments are probably in the range that will produce some modulation of the phosphorylation of 5-AZA-CdR at the cellular level.

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Biochemical effects of pure isomers of hexachlorobiphenyl—Hepatic microsomal epoxide hydrase and cytosolic glutathione *S*-transferase activities in the rat*

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Some polychlorinated biphenyl (PCB) [1, 2] and polybrominated biphenyl isomers [3] are thought to be metabolized via arene oxides, which are usually substrates for cytosolic glutathione *S*-transferases and microsomal epoxide hydrase. PCBs have been reported to induce microsomal epoxide hydrase [4] and cytosolic glutathione *S*-transferase activities [5]. Recently, inductive properties of PCBs have been shown to be due to the combined effects of two separate classes of compounds [6], one having inducing properties similar to those of phenobarbital and the other having inducing properties similar to those of 3-methylcholanthrene. Since it is known that phenobarbital and 3-methylcholanthrene have differential effects on microsomal epoxide hydrase activity [7], the effects of 3,4,5,3′,4′,5′-HCB,† 2,4,5,2′,4′,5′-HCB and 2,3,5,2′,3′,5′-

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†HCB, hepachlorobiphenyl.

Table 1. Effect of single oral dose (50 mg/kg body weight) of hexachlorobiphenyl isomers on body weight, liver weight, hepatic microsomal protein and cytochrome P-450 or P-488 content in male rat liver*

	Liver wt (g)	Liver wt (g/100g body wt)	Microsomal protein (mg/g fresh liver)	Cytochrome P-450 or P-448 (nmoles/mg protein)
Control†	3.9 ± 0.26	3.67 ± 0.17	17.2 ± 4.0	0.80 ± 0.13†
3,4,5,3′,4′,5′-HBC§	4.68 ± 0.61‡	5.66 ± 0.49‡	19.6 ± 2.8	2.07 ± 0.30‡§
2,4,5,2′,4′,5′-HCB†	4.71 ± 0.26‡	4.76 ± 0.30‡	26.5 ± 2.7‖	2.11 ± 0.31†‡
2,3,5,2′,3′,5′-HCB†	4.84 ± 0.31‡	4.75 ± 0.34‡	24.1 ± 2.9‖	2.29 ± 0.53†‡

* Values in the table are means ± S.D. of the results obtained from six separate animals.
† Cytochrome P-450.
‡ Statistically significant ($P < 0.01$) from controls.
§ Cytochrome P-448.
‖ Statistically significant ($P < 0.05$) from controls.

HCB pretreatment on hepatic microsomal epoxide hydrase and cytosolic glutathione *S*-transferase activities have been studied to reveal the differential effects of these isomers. It has been reported that chlorination at the *para* position is a prerequisite for a chlorobiphenyl to be an inducer of microsomal enzymes [6]; we included in our study 2,3,5,2′,3′,5′-HCB, not chlorinated at the *para* (4,4′) position, to check this possibility.

Styrene oxide[8-^{14}C] (sp. act. 0.497 mCi/m-mole, radiochemical purity > 99 per cent) was purchased from New England Nuclear, Boston, MS, and unlabeled styrene oxide was a product of Eastman Organic Chemicals, Rochester, NY. Benzo(a)pyrene-4,5-oxide [^{3}H] (sp. act. 10 mCi/m-mole, radiochemical purity > 98 per cent) and unlabeled benzo(a)pyrene 4,5-oxide were purchased from Midwest Research Institute, Kansas City, MO, under a contract with NCI. Glutathione was obtained from the Sigma Chemical Co., St. Louis, MO. Plates for thin-layer chromatography were purchased from the Kontes Glass Co., Vineland, NJ. Hexachlorobiphenyls of 98–99 per cent isomeric purity were synthesized at the Environmental Chemistry Branch of our Institute. The purity of the isomers was checked further by chromatography of PCBs on aluminum oxide with 3% methylene chloride (PCBs) and 20% methylene chloride (Furans) in hexane as eluant. Dibenzofuran fractions were examined by gas chromatography on OV-210 liquid phase, using a ^{63}Ni-electron capture detector. The 3,4,5,3′,4′,5′-HCB had less than 0.7 ppm, 2,4,5,2′,4′,5′-HCB less than 2 ppm, and 2,3,5,2′,3′,5′-HCB less than 5 ppm of tetrachloro- plus pentachlorodibenzofurans.

Charles River CD strain male rats (80–100 g 1-month-old) were used in the present study. Each rat was housed in a separate cage. Twenty-four animals were divided into four groups of six animals each (one control and three experimental). Animals in each experimental group were fed 3,4,5,3′,4′,5′-HBC, 2,4,5,2′,4′,5′-HCB or 2,3,5,2′,3′,5′HCB as a single oral dose (50 mg/kg body weight) dissolved in 0.3 to 0.4 ml cotton seed oil. The animals from the control group received 0.3 to 0.4 ml cotton seed oil. The animals from all the groups were given food and water *ad lib.* for 48 hr after dosing and were then fasted for an additional 24 hr with free access to water during the fasting period. After the total period of 72 hr, the animals were guillotined. Livers were removed immediately and processed.

Livers were homogenized in 4 vol. of 0.15 M KCl, 0.02 M HEPES. Microsomes and 176,000 *g* supernatant fractions were prepared, and glutathione *S*-transferase activity of hepatic 176,000 *g* supernatant fraction was assayed with styrene oxide (SO) and benzo(a)pyrene 4,5-oxide (BPO) as substrates by quantitating product formation as described previously [8]. Microsomal epoxide hydrase was assayed by the method of Jerina *et al.* [9]. Cytochrome P-450 was assayed by the method of Omura and Sato [10]. Protein was estimated by the method of Lowry *et al.* [11] using bovine serum albumin as a standard.

The liver weights and liver:body weight ratios of the rats fed 3,4,5,3′,4′,5′-HBC, 2,4,5,2′,4′,5′-HBC or 2,3,5,2′,3′,5′-HBC (50 mg/kg body weight) for a period of 72 hr were increased significantly (Table 1). A single oral dose of 3,4,5,3′,4′,5′-HCB did not affect the amounts of microsomal

Table 2. Effect of a single oral dose (50 mg/kg body weight) of hexachlorobiphenyl isomers on hepatic cytosolic glutathione *S*-transferase activity and microsomal epoxide hydrase activity in male rats*

	Glutathione *S*-transferase (nmoles product formed/min/mg protein)		Epoxide hydrase (nmoles product formed/min/mg protein)	
	Styrene oxide	Benzo(a)pyrene oxide	Styrene oxide	Benzo(a)pyrene oxide
Control	161 ± 18	23 ± 2	7.10 ± 0.38	7.62 ± 0.40
3,4,5,3′,4′,5′-HCB	228 ± 43†	46 ± 5†	6.25 ± 1.27	7.60 ± 2.18
2,4,5,2′,4′,5′-HCB	234 ± 30†	33 ± 2†	16.58 ± 0.93†	18.26 ± 2.08†
2,3,5,2′,3′,5′-HCB	247 ± 46†	35 ± 4†	16.41 ± 2.73†	17.70 ± 3.68†

* Values in the table are means ± S. D. of the results obtained from six separate animals.
† Significantly different ($P < 0.01$) from controls.

protein, whereas an increase was obtained when the other two isomers were used (Table 1). In addition, we found that 2,3,5,2′,3′,5′-HCB, like 2,4,5,2′,4′,5′-HCB, induced cytochrome P-450 but did not shift the absorption maximum of the CO-difference spectrum from 450 to 448 nm in the rat liver microsomes. The effects of this isomer have not been documented previously. 3,4,5,3′,4′,5′-HCB shifted the absorption maximum of the CO-difference spectrum from 450 to 448 nm in the rat liver microsomes.

Glutathione *S*-transferase activities were induced similarly by all the isomers studied (Table 2). These enzymes are known to be induced by a mixture of PCBs [5] or phenobarbital and 3-methylcholanthrene [12].

Induction of epoxide hydrase by nonplanar isomers of hexachlorobiphenyl corroborates well the earlier report that type I compounds induce epoxide hydrase while type II do so mildly or not at all [7]. Consequently, no induction of epoxide hydrase activity was observed when rats were fed 3,4,-5,3′,4′,5′-HBC (Table 2), which confirms the earlier findings that 3,4,5,3′,4′,5′-HCB behaves like type II compounds [6] and the other two isomers studied behave like type I compounds. The inhibition by PCBs of development of nodular hyperplasias, oval cell infiltration and bile duct proliferation induced in the liver by chemical carcinogens [13] could be explained in part on the basis of the ability of these compounds to induce epoxide hydrase, which would then enhance the degradation of arene oxides generated through oxidative metabolism of the carcinogens. However, since epoxide hydrase plays a dual role, this possibility needs further investigation. A mixture of PCBs has been reported to induce epoxide hydrase activities [5] due possibly to the presence of isomers which cannot assume coplanarity of phenyl rings. It has been suggested recently that metabolism of PCBs may be taking place via the formation of an arene oxide as a metabolic intermediate [2, 14] which could then be expected to be a substrate for glutathione *S*-transferases or epoxide hydrase. While no conjugates of PCBs with glutathione or their derivatives have been demonstrated as yet, 2,5,2′,5′-tetrachlorobiphenyl has been shown to be metabolized to *trans*-3,4-dihydro-3,4-dihydroxy-2,5,2′,5′-tetrachlorobiphenyl, as demonstrated by its isolation from urine of rabbits fed the parent compound [15]. The induction of epoxide hydrase by 2,4,5,2′,4′,5′-HCB or 2,3,5,2′,3′,5′-HCB indicates that these isomers may be inducing their own metabolism as well as that of other isomers. In this connection, it would be interesting to demonstrate whether the activity of epoxide hydrase is induced when arene oxides of chlorobiphenyls are used as substrates.

Recently, the biological half-life of 2,3,5,2′,3′,5′-HCB has been demonstrated to be similar to that of 2,4,5,2′,4′,5′-HCB†. This isomer also has been shown to induce *N*- and *O*-demethylases, microsomal nitroreductase and bromosulfophthalein-glutathione conjugating enzyme [16], and also to cause proliferation of smooth endoplasmic reticulum and to increase the content of phospholipids in rat liver [17]. These observations support our findings that 2,3,5,2′,3′,5′-HCB induces glutathione *S*-transferase, epoxide hydrase and cytochrome P-450, leading to the conclusion that chlorination at the *para* (4,4′) position is not a necessary requirement for a chlorobiphenyl to exert its inducing effects. In this respect, total stereoelectronic properties may be of more importance in determining structure–activity relationships than the position of chlorines in the biphenyl rings.

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