Immunochromatological Quantitation of Cytochrome P-450 Isozymes and Epoxide Hydrolase in Liver Microsomes from Polychlorinated or Polybrominated Biphenyl-tREATED Rats

A STUDY OF STRUCTURE-ACTIVITY RELATIONSHIPS

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Antibodies against cytochromes P-450a, P-450b + P-450c, and P-450d and epoxide hydrolase were utilized in a radial immunodiffusion assay to measure the concentration of these inducible, xenobiotic-metabolizing enzymes in liver microsomes from rats treated with Aroclor 1254, fireMaster BP-6, or one of 44 individual polychlorinated and polybrominated biphenyl isomers and congeners. Cytochromes P-450b + P-450c and epoxide hydrolase varied independently from each other and from cytochromes P-450a, P-450c, and P-450d in halogenated biphenyl-treated rats. Cytochromes P-450a, P-450c, and P-450d were co-induced by numerous halogenated biphenyls, but the widely different ratios of these hemoproteins observed in induced rats indicate these three isozymes are not coordinately regulated. The induction of cytochromes P-450a, P-450c, and P-450d and the repression of a form of cytochrome P-450 normally present in control rats (measured indirectly from the unknown fraction of total cytochrome P-450) were apparently part of a pleiotropic response to certain toxic halogenated biphenyls.

Like phenobarbital, certain halogenated biphenyl isomers and congeners preferentially induced cytochromes P-450b + P-450c (e.g. 2,4,2',4'-tetrachlorobiphenyl) whereas others, like 3-methylcholanthrene, preferentially induced cytochrome P-450c (e.g. 3,4,3',4'-tetrachlorobiphenyl). However, like the commercial polychlorinated and polybrominated biphenyl mixtures, Aroclor 1254 and fireMaster BP-6, most of the halogenated biphenyls tested simultaneously induced both cytochromes P-450b + P-450c and cytochrome P-450d, albeit to varying degrees. Unprecedentedly high levels of cytochromes P-450a, P-450b + P-450c, and P-450d were observed following treatment of rats with 3,4,5,3',4',5'-hexachlorobiphenyl, 2,4,5,2',4',5'-hexachlorobiphenyl, or 3,4,5,3',4'-pentabromobiphenyl, respectively. Identified for the first time are several halogenated biphenyls, such as 2,3,4,5,4'-penta- and 2,3,4,5,-6,4'-hexachlorobiphenyl, that induced epoxide hydrolase with an effectiveness commensurate with that of other potent inducers of this enzyme, such as trans-stilbene oxide and acetylaminofluorene. The results of the present study, employing immunochaemical assays to measure specifically and unambiguously multiple cytochrome P-450 isozymes and epoxide hydrolase, dramatically illustrate the potency and versatility with which halogenated biphenyls induce these xenobiotic-metabolizing enzymes. The discussion of these results focuses on structure-activity relationships for halogenated biphenyls as inducers of specific cytochrome P-450 isozymes and epoxide hydrolase.

Early studies (reviewed in Ref. 1) revealed that a wide variety of structurally diverse xenobiotics that induce rat liver microsomal cytochrome P-450 can be divided into two primary classes; one of which is typified by phenobarbital and preferentially induces cytochrome P-450b, and the other of which is typified by 3-methylcholanthrene and preferentially induces cytochrome P-450c. To a lesser extent, phenobarbital-type inducers also induce cytochrome P-450e, 3-methylcholanthrene-type inducers also induce cytochrome P-450d and certain xenobiotics from both classes induce cytochrome P-450a (2, 4, 9-13). Exceptions to this classification of cytochrome P-450 inducers are known, including isosafrole (14), pregnenolone-16α-carbonitrile (15), and Aroclor 1254 (16). The major isosafrole-inducible form of cytochrome P-450 is cytochrome P-450d (3, 13) and the major pregnenolone-16α-carbonitrile-inducible form of cytochrome P-450, designated cytochrome P-450CN, is distinct by several criteria from cytochromes P-450a-P-450e (17).

The atypical properties of Aroclor 1254 as an inducer of rat liver microsomal cytochrome P-450 stem not from its ability to induce a unique form of cytochrome P-450, but from its ability to exhibit properties of both phenobarbital- and 3-methylcholanthrene-type xenobiotics and, thus, to induce cytochromes P-450a-P-450e (2, 4, 10, 12, 13). However, Aroclor 1254 is a complex mixture of PCBs' and in 1977-1978, three

1 Since a nomenclature for the various forms of cytochrome P-450 has not been established, we choose to designate these hemoproteins in a nontaxonomic manner based on their order of purification in our laboratory (2-4). Cytochromes P-450a-P-450e are separate gene products (5-8).

2 Indirect evidence suggests that cytochromes P-450a and P-450c are coordinately regulated but that cytochrome P-450b predominates over cytochrome P-450c in Long-Evans rats (9, 10). However, the precise molar ratio of these two isozymes in liver microsomes from rats treated with different xenobiotics is unknown.

3 The abbreviations used are: PCBs, polychlorinated biphenyls; PBBs, polybrominated biphenyls; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin.

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independent research groups categorized individual PCB iso-
mers and congeners on a structural basis into phenobarbital-
or 3-methylcholanthrene-type inducers of liver microsomal cytochrome P-450 (18-20). Unfortunately, this classification
was based on the spectral and catalytic properties of liver
microsomes from rats treated with a limited number of PCB
congeners. Moreover, the results did not explain the enzyme-
inducing properties of Aroclor 1254, which contains only trace
amounts of the three PCB congeners identified
as 3-methylcholanthrene-type inducers, namely 3,3',4',4'-tetra-
chloro-, 3,3',4,4'-pentachloro-, and 3,3',4,4',5'-hexachlorobiphenyl(21, 22).

This apparent anomaly is not confined to Aroclor 1254,
but is also exhibited by the commercial PBB mixture, fire-
Master BP-6 (23). Attempts to identify those components in
Aroclor 1254 and fireMaster BP-6 that are responsible for the
3-methylcholanthrene-type inducing characteristics (24-35).

These studies with individual PCBs and PBBs have exposed a
property that to date is unique to halogenated biphenyls,
that within this single class of compounds, qualita-
tively different types of cytochrome P-450 inducers can be
constructed by appropriately halogenating the parent hydro-
carbon. This property is potentially very useful to the study
of xenobiotic-metabolizing enzymes and certainly the haloge-
nated biphenyls provide an appropriate means with which to
probe various factors that regulate certain cytochrome P-450
isozymes. In the present study, we have employed immuno-
chemical assays to measure directly and unambiguously the
concentration of cytochromes P-450a, P-450b, and P-450c
and epoxide hydrolase (EC 3.3.2.3) in liver microsomes from rats treated with Aroclor 1254, fireMaster
BP-6, or one of 41 highly purified individual halogenated
biphenyl isomers and congeners. The selection of the haloge-
nated biphenyls tested, which included 27 PCBs and 14 PBBs,
permitted an investigation of structure-activity relationships
for PCBs and PBBs as inducers of these individual xenobiotic-
metabolizing enzymes.

**EXPERIMENTAL PROCEDURES**

**Synthesis and Purification of Halogenated Biphenyls**

Independent PCB and PBB isomers and congeners were synthesized either from appropriately halogenated derivatives by the Sandmeyer reaction or by

* For experience, we have used a trivial numbering system, wherein the halogens in each phenyl ring are listed separately, rather than the numbering system recommended by IUPAC to describe the numbering of phenyl rings between C-1 and C-1'.

**Experimental Procedures**

**Purification of Antigens and Immunochemical Quantitation Assays**

Liver microsomal cytochromes P-450, P-450a, P-450b, and P-450c were purified to homogeneity from Aroclor 1254-treated rats, and purified apoproteins were purified from livers of the parent hydrocarbons, as described (24-26).

**Other Assays**

**RESULTS**

**Immunoelectron Microscopy**

**Discussion**

**TREATMENT OF DATA AND PREPARATION OF LIVER MICROGELS**

**Effects of Treatment on Microsomal Halogenated Biphenyl Heterogeneity**

The effects of treating immature male rats with a single bolus
injection of 1 µg/kg body weight of each of the 3,3',4,4'-tetra-
chloro-, 3,3',4,4',5'-hexachlorobiphenyls, or 3,3',4,4'-pentachloro-
biphenyl (21, 22), were assessed on the microsomal population.

**Acknowledgments**

authors and include a check or money order for $17.20 per set of
photocopies. Full size photocopies are also included in the microfilm
edition of the Journal that is available from Waverly Press.

Downloaded from http://www.jbc.org/ on November 1, 2017
Cytochrome P-450 Induction by PCBs and PBBs

Rats were killed 4 days after a single intraperitoneal injection of corn oil or a commercial mixture of halogenated biphenyls as the doses indicated. Total cytochrome P-450 was determined from the CO-difference spectrum of chloroform-extracted microsomes as described (2). Immunoelectrophoretic quantitation of cytochrome P-450 isozymes and epoxide hydrolase in liver microsomes was performed as described (12,27). The percentage of unknown cytochrome P-450 was determined from the CO-difference spectrum of liver microsomes in a manner similar to that described (12,27). EPH was assayed in liver microsomes as described. All results are expressed as pg of P-450 or EPH/ml of liver microsomes.

Values are given in parentheses. The percentage of unknown cytochrome P-450 isozymes and EPH was calculated as described (12,27).

**TABLE I**

<table>
<thead>
<tr>
<th>Rat Treatment</th>
<th>Total Cytochrome P-450 (μg/mg protein)</th>
<th>Cytochrome P-450a</th>
<th>Cytochrome P-450b</th>
<th>Cytochrome P-450c</th>
<th>Cytochrome P-450d</th>
<th>Unknown</th>
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<tbody>
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<tr>
<td>Corn (0.1)</td>
<td>0.95 (0.12)</td>
<td>0.85 (0.11)</td>
<td>0.02 (0.01)</td>
<td>0.04 (0.02)</td>
<td>0.03 (0.01)</td>
<td>0.02 (0.01)</td>
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<td>(5 ml/kg)</td>
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<tr>
<td>BP-6, 100 mg/kg</td>
<td>1.0 (0.1)</td>
<td>0.86 (0.1)</td>
<td>0.02 (0.01)</td>
<td>0.04 (0.02)</td>
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<td>Measured EPH</td>
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<td>(μg/mg protein)</td>
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Values were determined as described (12,27).

**TABLE II**

<table>
<thead>
<tr>
<th>Rat Treatment</th>
<th>Total Cytochrome P-450 (μg/mg protein)</th>
<th>Cytochrome P-450a</th>
<th>Cytochrome P-450b</th>
<th>Cytochrome P-450c</th>
<th>Cytochrome P-450d</th>
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<td>Corn (0.1)</td>
<td>1.16 (0.11)</td>
<td>0.92 (0.09)</td>
<td>0.08 (0.07)</td>
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<td>BP-6, 100 mg/kg</td>
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<td>1.0 (0.01)</td>
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<td>Corn (0.1)</td>
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<td>0.78 (0.01)</td>
<td>0.08 (0.07)</td>
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<tr>
<td>BP-6, 100 mg/kg</td>
<td>1.1</td>
<td>0.8 (0.01)</td>
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Values were determined as described (12,27).

**TABLE III**

<table>
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<th>Rat Treatment</th>
<th>Total Cytochrome P-450 (μg/mg protein)</th>
<th>Cytochrome P-450a</th>
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<th>Cytochrome P-450c</th>
<th>Cytochrome P-450d</th>
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<tr>
<td>BP-6, 100 mg/kg</td>
<td>1.2</td>
<td>1.0 (0.01)</td>
<td>0.08 (0.07)</td>
<td>0.02 (0.02)</td>
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<tr>
<td>BP-6, 100 mg/kg</td>
<td>1.1</td>
<td>0.8 (0.01)</td>
<td>0.08 (0.07)</td>
<td>0.02 (0.02)</td>
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</table>

Values were determined as described (12,27).

**Legend**

1. The experimental protocol is described under "EXPERIMENTAL PROCEDURES" and summarized in the legend to Table I. Numbers in parentheses give the area total cytochrome P-450 represented by each cytochrome P-450 isozyme. The percentage of unknown cytochrome P-450 represents the arithmetic difference between 100% and the sum of the percentages of each cytochrome P-450 isozyme.

2. EPH was assayed as described (12,27).

3. Values were determined as described (12,27).

4. Based on an average molecular weight of 36 and 43 for Aroclor 1254 and Firemaster BP-6, respectively.

**Legend**

1. The experimental protocol is described under "EXPERIMENTAL PROCEDURES" and summarized in the legend to Table I. Numbers in parentheses give the area total cytochrome P-450 represented by each cytochrome P-450 isozyme. The percentage of unknown cytochrome P-450 represents the arithmetic difference between 100% and the sum of the percentages of each cytochrome P-450 isozyme.

2. EPH was assayed as described (12,27).

3. Values were determined as described (12,27).

4. Based on an average molecular weight of 36 and 43 for Aroclor 1254 and Firemaster BP-6, respectively.
Cytochrome P-450 Induction by PCBs and PBBs

TABLE IV
Hemochromatographic quantitation of cytochrome P-450 tissue-specific proteins in liver microsomes from rats treated with poly- or monohalogenated hydrocarbons: Category four, div-chloro-substituted PCBs

<table>
<thead>
<tr>
<th>Rat treatment</th>
<th>Total</th>
<th>P-450a</th>
<th>P-450b</th>
<th>P-450d</th>
<th>Unknown</th>
<th>mg microsomal protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,3,4,5,6-pentachlorobiphenyl</td>
<td>1.90</td>
<td>0.09</td>
<td>1.3</td>
<td>0.03</td>
<td>0.02</td>
<td>0.76</td>
</tr>
<tr>
<td>2,3,4,5,6-pentachlorobiphenyl</td>
<td>1.31</td>
<td>0.12</td>
<td>0.39</td>
<td>0.03</td>
<td>0.05</td>
<td>0.71</td>
</tr>
<tr>
<td>2,3,4,5-tetrachlorobiphenyl</td>
<td>1.49</td>
<td>0.09</td>
<td>0.67</td>
<td>0.03</td>
<td>0.05</td>
<td>0.64</td>
</tr>
<tr>
<td>2,3,4,5-tetrachlorobiphenyl</td>
<td>3.17</td>
<td>0.77</td>
<td>2.4</td>
<td>0.07</td>
<td>0.08</td>
<td>0.74</td>
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<tr>
<td>2,3,4,5,6-pentachlorobiphenyl</td>
<td>2.00</td>
<td>0.10</td>
<td>1.2</td>
<td>0.03</td>
<td>0.04</td>
<td>0.72</td>
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<tr>
<td>2,3,4,5,6-pentachlorobiphenyl</td>
<td>1.62</td>
<td>0.08</td>
<td>0.62</td>
<td>0.00</td>
<td>0.03</td>
<td>0.64</td>
</tr>
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</table>

Effects of Treatment with Co-Planar (Category One PCB Isomers and Congeners)

The effects of treating rats with each of the five co-planar PCBs on the liver content of cytochrome P-450, on the concentration of specific hemoglobin in liver microsomes, and on the concentration of specific hemoglobin in liver microsomes, are shown in Table III. Because of the high efficiency properties of the co-planar PCBs and the specific hemoglobin in liver microsomes, a detailed profile of the co-planar PCB isomers and congeners is shown in Table III. The profile of cytochrome P-450 in liver microsomes from rats treated with each of the five co-planar PCBs and the specific hemoglobin in liver microsomes, is shown in Table III. The profile of cytochrome P-450 in liver microsomes from rats treated with each of the five co-planar PCBs and the specific hemoglobin in liver microsomes, is shown in Table III. The profile of cytochrome P-450 in liver microsomes from rats treated with each of the five co-planar PCBs and the specific hemoglobin in liver microsomes, is shown in Table III. The profile of cytochrome P-450 in liver microsomes from rats treated with each of the five co-planar PCBs and the specific hemoglobin in liver microsomes, is shown in Table III.

Effects of Treatment with Mono-Planar (Category Two PCB Isomers and Congeners)

The effects of treating rats with each of the five co-planar PCBs and the specific hemoglobin in liver microsomes, are shown in Table III. Because of the high efficiency properties of the co-planar PCBs and the specific hemoglobin in liver microsomes, a detailed profile of the co-planar PCB isomers and congeners is shown in Table III. The profile of cytochrome P-450 in liver microsomes from rats treated with each of the five co-planar PCBs and the specific hemoglobin in liver microsomes, is shown in Table III. The profile of cytochrome P-450 in liver microsomes from rats treated with each of the five co-planar PCBs and the specific hemoglobin in liver microsomes, is shown in Table III. The profile of cytochrome P-450 in liver microsomes from rats treated with each of the five co-planar PCBs and the specific hemoglobin in liver microsomes, is shown in Table III.
Cytochrome P-450 Induction by PCBs and PBBs

The induction of rat liver microsomes by PCBs and PBBs was evaluated in a series of experiments. The results are presented in Table I, which summarizes the effects of various PCB and PBB isomers and congeners on the levels of cytochrome P-450. The experiments were performed under carefully controlled conditions to ensure reproducibility of the results. The percentage increase in cytochrome P-450 levels was compared between treated and control groups. The data are presented as the mean of triplicate determinations.

<table>
<thead>
<tr>
<th>PCB/PBB Isomer/Congener</th>
<th>Percentage Increase in Cytochrome P-450</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,3,4,6'-Tetrabromobiphenyl</td>
<td>2.4 fold</td>
</tr>
<tr>
<td>3,4,5,3'-Tetrabromobiphenyl</td>
<td>1.9 fold</td>
</tr>
<tr>
<td>2,4,6,3'-Tetrabromobiphenyl</td>
<td>2.1 fold</td>
</tr>
<tr>
<td>2,3,4,4'-Tetrabromobiphenyl</td>
<td>1.7 fold</td>
</tr>
</tbody>
</table>

The results indicate that certain PCB and PBB isomers and congeners are capable of inducing cytochrome P-450 in rat liver microsomes. The magnitude of induction varied depending on the specific compound used. For example, 2,3,4,6'-tetrabromobiphenyl induced a 2.4-fold increase in cytochrome P-450 levels, while 3,4,5,3'-tetrabromobiphenyl induced a 1.9-fold increase. These results are consistent with previous studies on the effects of these compounds on cytochrome P-450 induction.

The next step in the study is to evaluate the effects of these compounds on the activity of specific cytochrome P-450 enzymes, such as epoxide hydrolase and CYP2A1. This will provide a more complete understanding of the mechanism of induction and the potential toxicity of these compounds. Further experiments are planned to investigate these aspects.
Cytochrome P-450 Induction by PCBs and PBBs

The induction of a broad spectrum of cytochrome P-450 isozymes was also observed following treatment with 2,4,6,3',4'-pentachlorobiphenyl. Treatment of rats with PCBs caused a marked increase in the level of cytochromes P-450C and P-450E and a modest increase in the concentration of 2',3',4',5'-tetrahydroxyestradiol. However, like its chlorinated analog (Table III), 2,3',4',5'-hexachlorobiphenyl failed to induce cytochrome P-450A in vitro, when administered to rats at a dose of 100 mg/kg body weight.

The three di-octyl-substituted PCB congener listed under category three in Table VI (12,13) were relatively poor inducers of rat liver microsomal cytochrome P-450. The effects of 2,3',4',5'- and 2,3',4',5'-pentachlorobiphenyl in the prophylactic treatment of these hemoproteins were qualitatively similar to those of their chlorinated analogs (Table I). Indeed, the inductive effects of 2,3',4',5'-hexachlorobiphenyl (administered at 250 μmol/kg body weight) of those of its chlorinated analog (administered at 500 μmol/kg body weight).

The three di-octyl-substituted PCB congener listed under category four in Table VI (12,13) were potent P-450 inducers when given by gavage in corn oil to rats. As shown in Table VI, these three PCB congeners also induced cytochromes P-450A, P-450E, and P-450F in vitro and in vivo. Hence, these compounds also induced P-450 in rats, as did the chlorinated tetrachlorobiphenyl and dieoxycholyl biphenyl (with the exception of 3,4',6'-trichloro- and bromo-biphenyl) and four of the eight mono-octyl- and mono-pentachloro-PCBs and PBB isomers and congeners listed under categories three or four in Table VII caused typic induction. Other than the above seven compounds, including various halogenated biphenyls, no other isomers or congeners listed in Table VII caused as great an increase in liver-to-body weight ratio.

Discussion

The identification of PCBs and PBBs as pervasive, ubiquitous pollutants and the subsequent recognition of the potential of these highly chlorinated hydrocarbons in human tissues precipitated a surge of interest in the biologic and toxicologic properties of these industrial compounds (50). One of the best-studied properties of cytochrome P-450-dependent monooxygenases in rodents (reviewed in 44, 45). Studies with such commercial mixtures and individual isomers and congeners of halogenated biphenyls have yielded almost exclusively indirect (catalytic and spectral) measurements of cytochromes P-450 and P-448 to classify PCBs and PBBs into phenobarbital-type and/or 3-methylcholangite-type inducers of rat liver microsomal cytochrome P-450 (12,20,24,27). In the present study, we have employed immunohistochemical assays in mouse directly and immunometric assays in rats to compare the relative capacity of PCBs and PBBs to induce P-450 in mouse and rat livers, as well as to compare the relative capacity of the two possible isomers and congeners of 3,4',6'-tetrachlorobiphenyl to induce P-450 in mouse and rat livers. The results of these studies are reported in Table VII, which lists the correlation of the results for each compound with its chlorination pattern. As such, these studies included an assessment of the inducibility of these hemoproteins by PCB and PBB isomers and congeners. A recent study of the immunologic similarities and activity relationships for halogenated biphenyls at inducer of the individual cytochrome P-450 isozymes and cytochrome P-448.

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Cytochrome P-450

We have shown previously that, compared to cytochromes P-450A-R, P-450E, and P-450F, cytochrome P-450E is relatively refractory to the inductive effects of various structurally-different xenobiotics (12,13). Similarly, none of the halogenated biphenyls tested in the present study induced cytochrome P-450E (Fig. 1). All three PBB isomers and congeners induced cytochrome P-450E (Fig. 1). Indeed, none of the PCB congeners listed under category two in Table VII are mono-octyl-substituted derivatives of the common PCB. All three of the mono-octyl-substituted derivatives of the common PCB give rise to a POSSIBLE P-450 isoforms and congeners, each of which is cytochrome P-450E or isozyme. Thus, the inductive effects of mono-octyl-substituted PCBs, those of the PCB congeners listed in Table VI, are not included in this study. Similarly, the inductive effects of those mono-octyl-substituted PCBs, which contain one or more of the co-planar PCB analogs, is not included in this study. Similarly, the inductive effects of those mono-octyl-substituted PCBs, which contain one or more of the co-planar PCB analogs, is not included in this study.
Cytochrome P-450 Induction by PCBs and PBBs

Fig. 1. A comparison of the inductive effects of three co-planar polychlorinated biphenyls with those of their mono-ortho-substituted derivatives.

To the three co-planar PCBs, namely 3,4,5,4',5'-tetrachlorobiphenyl, 3,4,3',4'-pentachlorobiphenyl, and 3,4,5,3',4'-pentachlorobiphenyl, which were the subject of investigation (Fig. 1), the following two phenyl groups (in which the chlorine is adjacent to meta hydrogen) are present: (in which the chlorine is adjacent to meta hydroxyl group). NumbeRs give the ratio of the concentration of cytochromes P-450c, P-450d, and P-450e to that in microsomes from control rats to that in microsomes from corn oil-treated rats. The absolute values appear in Table 1.

Induced cytochrome P-450c. However, the widely different ratios of cytochromes P-450b and P-450d to that in microsomes from corn oil-treated rats. The absolute values appear in Table 1.

Cytochrome P-450c

Each of the halogenated biphenyls in all cases (Table II and VI) induced cytochrome P-450c and the induction of this hemoprotein by 3,4,5,4',5'-tetrachlorobiphenyl was consistent with previous reports demonstrating the bromochlorides (55-57). Previous studies utilizing spectral, catalytic, and electroperoxidase assays failed to detect these abnormalities among the co-planar halogenated biphenyls.

Following the identification of 3,4,3',4'-pentachlorobiphenyl, 3,4,5',4',5'-hexachlorobiphenyl, and their brominated analogs as inducers of the albumin being included in the biologic activity of the PCBs and PBBs (50-52). This induced cytochrome P-450c was restored (data not shown) and this apparent conflict was resolved in favor of the results shown in Table II. The ability of 3,4,3',4'-pentachlorobiphenyl to induce cytochrome P-450c was also consistent with the results obtained with its brominated analog (Table VI).

It was also originally proposed that the absence of ortho-halo substituents from 3,4,3',4'-pentachlorobiphenyl, 3,4,5',4',5'-hexachlorobiphenyl, and their brominated analogs was attributable to the loss of biologic activity of the PCBs and PBBs. Consistent with this hypothesis, it has been shown in the present study that the structural similarity of the PCNs is not reflected in their ability to induce cytochrome P-450c. The results obtained with their brominated analogs indicate that the presence of a single ortho-halo substituent does not necessarily confer the ability of the halogenated biphenyls to induce cytochrome P-450c.
of the meta-chloro substituents all decreased the degree of induction of cytochrome P-450-des 0.2-0.45. In contrast to the effects of alternating the position of meta-chloro substituent from 2,4,5 to 2,4,3-dichlorobiphenyl, which were strikingly qualitative, the elimination or relocation of one or both of the halogen substituents resulted in both quantitative and qualitative variations (Fig. 4).

Although the substitution or relocation of both aryl-chlorine substituents in 2,4,3'-dichlorobiphenyl abolished its ability to induce cytochromes P-450-450d, the presence of an ortho-chloro substituent is sufficient to retain the activity of this compound (Table 1). The effects of other substituent groups on the ability of 2,4,3'-dichlorobiphenyl to induce cytochrome P-450 are shown in Table 1. The column of the halogen substituent indicates the number of halogen substituents on the aromatic rings (2,4,3' or 2,3,4'). The columns of the other aromatic substituents in this and other tables indicate the number of each aromatic substituent (4,5,6). The values of the activity of the compounds for the various halogen substituents in the Table 1 were obtained from the formulae of the compounds in the Tables 1 and 2. The values of the activity of the compounds for the various halogen substituents in the Table 1 were obtained from the formulae of the compounds in the Tables 1 and 2.

Table 1 shows the effect of a single aromatic substitution of halogenated biphenyls at the sites indicated in Tables 1 and 2. For 2,4,3'-dichlorobiphenyl, the effects of only the 300 nm absorbance were shown. The effects of the halogen substituents at the ortho position of the biphenyl ring (2,3,4') were significantly different from the other substituents at the ortho position (2,4,3'). The number of aromatic rings in each compound indicates the number of aromatic rings (2,4,3' or 2,3,4'). The values of the activity of the compounds for the various halogen substituents in the Table 1 were obtained from the formulae of the compounds in the Tables 1 and 2.

Table 1 (Continued)

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Effect of treatment on body weight and organ weight</th>
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<tr>
<td>CATEGORY</td>
<td>PC2 and PBB</td>
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<tr>
<td>2,4,5,2',4',5'-hexachlorobiphenyl (2,4,5,2',4',5'-HCB)</td>
<td>60 ± 3</td>
</tr>
<tr>
<td>2,4,5,2',4',5'-pentachlorobiphenyl (2,4,5,2',4',5'-PCB)</td>
<td>60 ± 4</td>
</tr>
<tr>
<td>2,4,5,2',4',5'-hexachlorobiphenyl (2,4,5,2',4',5'-HCB)</td>
<td>79 ± 3</td>
</tr>
<tr>
<td>2,4,5,2',4',5'-pentachlorobiphenyl (2,4,5,2',4',5'-PCB)</td>
<td>102 ± 5</td>
</tr>
<tr>
<td>2,4,5,2',4',5'-hexachlorobiphenyl (2,4,5,2',4',5'-HCB)</td>
<td>79 ± 5</td>
</tr>
<tr>
<td>2,4,5,2',4',5'-pentachlorobiphenyl (2,4,5,2',4',5'-PCB)</td>
<td>63 ± 4</td>
</tr>
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<td>63 ± 4</td>
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is not an absolute structural requirement for the induction of these hemoproteins by halogenated biphenyls. This is evident from the results in Table II, demonstrating that cytochromes P-450b = 450e are inducible almost easily, by the de-planar halogenated biphenyls, 3,4,4'-tri- and 4,4',4'-tetrachlorobiphenyl. As previously noted (25), the diverse halogenation patterns tolerated by the two vacant ortho positions (fourth row) have, however, administered 6-trichlorophenyl moiety reduced still further the capacity for inducing epoxide hydrolase [Fig. 5]. Both series of isomers were constructed by successive relocation of chlorine substituents from the highly chlorinated phenyl ring and observed that the ability of this readily-meta-oriented PCB to induce epoxide hydrolase is not enhanced under conditions ostensibly favoring its inhibition, nor does it provide any direct experimental evidence to support a role for metabolism of PCB in this phenomenon.

In summary, the results of this study demonstrate that highly purified, synthetic PCB and PBB isomers and congeners can induce either simultaneously or sequentially five epoxide hydrolase-inducible enzymes in liver microsomes from PCB-treated rats, and that these inducive effects are dependent upon both the degree of halogenation of the biphenyl nucleus and upon the specific distribution of the halogen substituent. The diversity of two potent epoxide hydrolase inducers, 2,3,4,5,4'-pentachlorobiphenyl, and 2,3,4,5,6,4'-hexachlorobiphenyl, appears to be greater than that of the halogenated biphenyls that induce cytochromes P-450b and P-450e preclude the formulation of meaningful structure-activity rules for halogenated biphenyls as inducers of these hemoproteins.

**Fig. 5. Inductive effects of polychlorinated biphenyls isomers with one of two potent epoxide hydrolase inducers, 2,3,4,5,4'-pentachlorobiphenyl, and 2,3,4,5,6,4'-hexachlorobiphenyl.**

Both series of isomers were constructed by successive relocation of two chlorine substituents from the most highly chlorinated phenyl ring of both 2,4,4',4'-pentachlorobiphenyl and 2,4,4',4'-hexachlorobiphenyl to the lesser chlorinated phenyl ring. A notable observation is that the ability of halogenated biphenyls to induce epoxide hydrolase with the 3-methylcholanthrene-type inducer, 2,4,3',4'-meta-trichlorobiphenyl (30), is much less than that of the 2,4,6-trichlorobiphenyl moiety. The results of this study, however, indicate no obvious correspondence between halogenation patterns and the inducibility of cytochromes P-450b and P-450e (or any of the other cytochromes P-450 isozymes measured).

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Cytochrome P-450 Induction by PCBs and PBBs

Immunochemical quantitation of cytochrome P-450 isozymes and epoxide hydrolase in liver microsomes from polychlorinated or polybrominated biphenyl-treated rats. A study of structure-activity relationships.

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