# COMPARATIVE EFFECTS OF POLYCHLORINATED BIPHENYLS AND ORGANOCHLORINE PESTICIDES IN INDUCTION OF HEPATIC MICROSOMAL ENZYMES[1]

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Chlorinated biphenyls (PCB), triphenyls (PCT) and other related compounds have important industrial uses and have been produced in large volumes for many years. Evidently some amounts reach the general environment. Pesticide residue chemists became interested in such materials following a report from Sweden of their presence in wildlife tissues.[1] This has been shown to interfere with the routine residue analyses of many organochlorine pesticides since complex patterns are produced in EC-GLC.[2,3] Moreover, routine cleanup procedures fail to eliminate such interferences unless special precautions are taken. These materials undoubtedly contributed to some of the "apparent" DDT found in biological specimens predating usage of DDT in agriculture or public health and constitute part of the present background of lipoidal organic chlorine in the biota.

Polychlorinated biphenyls produce acute toxic effects in mammals similar to those of chlorinated naphthalenes and other chlorinated aromatics. The special vulnerability of the liver to such agents is well documented.[4] Many lipid-soluble chemicals induce various enzymes of the hepatic endoplasmic reticulum (microsomal enzymes) including the drug-metabolizing hydroxylative system. Several organochlorine pesticides are potent inducers of such enzymes, with the result that treated animals metabolize drugs, insecticides and other foreign compounds more rapidly.[5]

[1] Presented at the 158th meeting, American Chemical Society, Pesticide Chemistry Division, September 8, 1969.

In some situations such stimulation is beneficial as, for example, in causing a more rapid detoxication of dieldrin and a lower residue storage in the body. The induction could likewise be detrimental because certain toxifying reactions, such as activation of organothiophosphate insecticides, are also stimulated. Enhanced steroid degradation also occurs which raises possibilities of disturbing certain physiological processes due to altered endocrine relationships. The latter has been suggested as the basis for the thin egg shell phenomenon and poor reproduction in certain birds chronically exposed to DDT, dieldrin and some other organochlorine insecticides.[6] Some polychlorinated biphenyl materials have already been reported to induce microsomal enzymes.[7] The present investigation was undertaken to compare the induction potencies of several types of polychlorinated biphenyls and triphenyls to those of various organochlorine pesticides, and to obtain preliminary information about tissue storage trends for such materials. The results contribute to our understanding of molecular characteristics required for microsomal enzyme induction and also allow some speculation about the consequences of various types of chlorinated compounds found in biota.

METHODS

Groups of male and female rats were individually fed Purina Laboratory Chow diets containing 25, 50, and 100 ppm concentrations of one of the several PCB and PCT materials tested. The test materials, Aroclors® provided by the Monsanto Co., consist of crude products obtained by chlorination of biphenyl, or triphenyl, to specified limits; each is therefore a complex mixture. The ten products ranged from 21 to 68 percent chlorine. Except for the negative controls, the diets were also treated to contain 1 ppm dieldrin. One group of rats in each experiment was fed diet containing 50 ppm DDT and 1 ppm dieldrin. The diets were

fed for 15 days. During that period tests of drug and insecticide metabolism in vivo were conducted. Later, tests of microsomal enzyme activity were carried out with liver homogenates.

Hexobarbital sleeping time measurements were made on the tenth day of treatment. Hexobarbital sodium was administered at the rate of 100 mg/kg i.p. in aqueous solution. The duration of deep sleep ending with the return of the animal's righting reflex was recorded as the sleeping time. The oxidative detoxification of EPN (O-ethyl-O-(p-nitrophenyl)-phenylphosphonothioate) and the O-demethylation of p-nitroanisole (PNA) were measured by incubation of the respective substrates with the 10,000 X g supernatant of liver homogenates following the method of Neal and DuBois as modified by Kinoshita et al.[8] The ring hydroxylation of aniline was measured by the incubation of aniline hydrochloride with the 10,000 X g supernatant of liver homogenates following the method of Kato and Gillette.[9]

Residual dieldrin in adipose tissue was determined by electron capture gas chromatography on hexane extracts of the tissue. The adipose tissue extracts required special cleanup to eliminate PCB interference in the detection of dieldrin. This was accomplished by passing the extract over a 2-stage chromatography column consisting of 1:1 MgO-Celite (5½") on the bottom and Florisil containing 4% moisture (4") on the top. After eluting with hexane (200 ml) to bring out the PCB components, dieldrin was recovered by eluting with 400 ml of a 1 + 4 dichloromethane + hexane solution.

Qualitative and semi-quantitative evaluation of PCB storage in adipose tissue was possible after careful evaluation of GLC data comparing parent material and that recovered from tissue.

RESULTS

Short term feeding of polychlorinated biphenyls and triphenyls to rats at rates up to 100 ppm in the diet had essentially no effects on food consumption, weight gains or efficiency of food utilization (Table 1). Liver enlargement was observed with all products tested, increasing in degree with the percentage chlorine in the product. Products with 60 percent chlorine or more caused greater liver enlargement than equivalent doses of the insecticide DDT.

Evidence of PCB induction of liver hydroxylating enzyme activity was obtained with all enzyme tests employed. Hexobarbital sleep times were markedly reduced by the PCB materials at both 50 and 100 ppm in the diet for 10 days (Fig. 1). The rate of decrease was reasonably linear with increasing degree of PCB chlorination.

Enzymatic aniline hydroxylation, EPN degradation and demethylation of p-nitroanisole, as determined with liver homogenates, were increased with preparations from PCB-treated rats (Fig. 2 and Table 2). In general, higher chlorination of the biphenyl resulted in greater enzymatic activities. These enzyme activities were similarly affected in both sexes by the treatment materials, although the male appeared to be more responsive (Table 3).

Dieldrin metabolism in vivo, judging from its residue storage in adipose tissue, was stimulated by the treatment materials, especially those with high degrees of chlorination (Fig. 3). The products containing 60% chlorine or greater reduced dieldrin storage to levels found in untreated control animals in our laboratory. This activity toward dieldrin metabolism had been predicted by analogy to the similar effects of enzyme-inducing drugs and organochlorine pesticides.

Higher dosage rates (100 ppm vs 50 ppm in the diet) produced greater degrees of enzyme induction. The dose-response curve (Fig. 4) obtained with 62% Cl-PCB, in the range from 5 ppm to 100 ppm in the diet, was generally similar to that of DDT or phenobarbital yet that substance was greater in potency at every dosage level. Heptachlor epoxide, by contrast, was considerably more potent than any of these compounds.

The two products containing chlorinated triphenyl components (one 42% Cl-PCT and the other a mixture of PCB:PCT, 60:40, containing 65% chlorine) tested were, in comparison to the PCB materials, less active in terms of effect on the enzyme activities measured in vitro. However, hexobarbital sleep time was more strongly influenced by the 42% Cl-PCT material than by the equivalent PCB, and dieldrin metabolism was highly stimulated by both the PCT-containing materials (Table 4).

The Arochlor products fed and the residual components recovered from adipose tissue were all examined by electron capture gas-liquid chromatography. Some degree of storage of each product was observed although the residues had greatly modified composition in comparison to the parent materials administered. Those products and individual components having the least chlorine appeared to be stored the least in adipose tissue. That can be seen in the comparison illustrated (Fig. 5) for 62% Cl-PCB and its tissue residue. The early GLC peaks (components low in chlorine) in the residue were greatly diminished relative to most of the later peaks in the chromatogram. Peaks C and D in that residue match, in retention time, two of the three major peaks reported by Risebrough et al.[3] in PCB residues from tissues and eggs of birds in the San Francisco bay area. The tissue residues for PCBs lower in chlorination were much lower in proportion to that of the highly chlorinated PCBs and

consisted chiefly of minor components of those materials. Work is in progress to quantitate these residues in terms of organic chlorine.

SUMMARY

To summarize all the responses evaluated, hepatic microsomal enzyme induction was minimal with the PCBs of low chlorination (21-32% chlorine) but increased to very high values for those materials averaging over 60% chlorine. The latter exceeded DDT in potency of induction, but not heptachlor epoxide.

Microsomal enzyme induction caused by PCB materials is probably additive with that produced by organochlorine pesticides.[6,10] Hence, if any of the toxicological aspects of organochlorine pesticides in the environment are related to their enzyme inducing action, the presence of highly chlorinated biphenyls and triphanyls would intensify such biological effects of the pesticides.

The very marked influence of the highly chlorinated PCB and PCT materials on dieldrin metabolism (which, by analogy, would affect all other cyclodiene insecticides and DDT as well) could greatly confound the interpretation of exposures of animals and birds bearing mixed residues. The toxicological implications of such exposures would be equally confounded.

This work extends our previous investigations of the structure-activity aspects of microsomal enzyme inducing agents (presented in part at the 156th ACS meeting, Agriculture and Food Chemistry Division) and the results fit well with our working hypotheses regarding the activities of various bridged and unbridged diphenyl compounds.

LITERATURE CITED


1. Jensen, S. New Sci. 32:66(1966)

2. Reynolds, L. M. Bull. Envir. Cont. Toxicol. 4:128-143(1969)

3. Risebrough, R. W., P. Reiche and H. S. Olcott. Bull. Envir. Cont. Toxicol. 4:192-201(1969)

4. Von Oettingen, W. F. U. S. Dept. of Health, Education and Welfare, Public Health Service Publication No. 414, 1955. The Halogenated Aliphatic, Olefinic, Cyclic, Aromatic, and Aliphatic-Aromatic Hydrocarbons Including the Halogenated Insecticides, Their Toxicity and Potential Dangers. p. 306-307.

5. Street, J. C. Ann. New York Acad. Sci. 160:274-290(1969)

6. Peakall, D. B. Nature 216:505(1967)

7. Risebrough, R. W., P. Reiche, D. B. Peakall, S. G. Herman, and M. N. Kirven. Nature 220:1098-1102(1968)

8. Kinoshita, K. F., J. P. Frawley and K. P. DuBois. Toxicol. Appl. Pharmacol. 9:505(1966)

9. Kato, R. and J. R. Gillette. J. Pharmacol. Exptl. Therap. 150:280(1965)

10. Street, J. C., D. J. Wagstaff, and F. Mayer. Proc. 6th Interamerican Conf. on Toxicol. and Indust. Med., Univ. Miami School of Medicine, 1968. (in press)

Table 1. Food consumption, weight gain and efficiency, and liver weights of female rats treated with polychlorinated biphenyls and DDT.[1]

| Treatment | Food Cons. g | Weight Gain g | Wt. Gain ÷ Food g/100g | Liver Wt. g/100g |
|---|---|---|---|---|
| **Trial A** | | | | |
| Basal | 226 ± 5.6 | 45 ± 1.6 | 20 ± 0.8 | 4.1 ± 0.44 |
| Dieldrin, 1 ppm | 233 ± 3.3 | 49 ± 3.3* | 21 ± 1.6 | 4.3 ± 0.27 |
| + DDT, 50 ppm | 224 ± 6.0 | 44 ± 2.4 | 20 ± 0.9 | 4.5 ± 0.44* |
| + 48% Cl-PCB, 50 ppm | 226 ± 4.7 | 47 ± 3.2 | 21 ± 1.3 | 4.7 ± 0.45** |
| 100 ppm | 225 ± 5.5 | 44 ± 6.5 | 20 ± 3.0 | 4.7 ± 0.46** |
| + 54% Cl-PCB, 50 ppm | 224 ± 5.4 | 47 ± 2.0 | 21 ± 0.7 | 4.7 ± 0.39** |
| 100 ppm | 231 ± 9.0 | 48 ± 2.0* | 21 ± 1.7 | 5.5 ± 0.46** |
| + 60% Cl-PCB, 50 ppm | 226 ± 7.1 | 50 ± 3.1* | 22 ± 1.4* | 4.6 ± 0.28** |
| 100 ppm | 225 ± 5.5 | 59 ± 2.2** | 26 ± 3.0** | 5.3 ± 0.22** |
| + 68% Cl-PCB, 50 ppm | 224 ± 9.6 | 50 ± 5.0* | 22 ± 1.8* | 5.1 ± 0.16** |
| 100 ppm | 227 ± 6.3 | 51 ± 5.5* | 22 ± 1.8* | 5.1 ± 0.46** |
| + 65% Cl (PCT + PCB), 50 ppm | 228 ± 8.3 | 43 ± 3.0 | 19 ± 0.9 | 4.8 ± 0.31** |
| 100 ppm | 232 ± 7.9 | 49 ± 1.7* | 21 ± 1.1 | 5.2 ± 0.37** |
| + 42% Cl-PCT, 50 ppm | 218 ± 4.6 | 40 ± 4.3 | 18 ± 1.8 | 4.4 ± 0.21 |
| 100 ppm | 211 ± 5.7 | 48 ± 5.8* | 18 ± 1.1 | 4.7 ± 0.01** |
| **Trial B** | | | | |
| Basal | 237 ± 3.8 | 39 ± 2.3 | 16 ± 2.5 | 4.0 ± 0.31 |
| Dieldrin, 1 ppm | 234 ± 6.9 | 40 ± 8.0* | 17 ± 3.0 | 4.3 ± 0.32* |
| + DDT, 50 ppm | 221 ± 2.6* | 32 ± 2.3 | 15 ± 1.6 | 4.5 ± 0.21** |
| + 21% Cl-PCB, 50 ppm | 228 ± 6.6 | 33 ± 1.3 | 14 ± 1.7 | 4.1 ± 0.33 |
| 100 ppm | 228 ± 6.9 | 33 ± 5.5 | 14 ± 2.5 | 4.1 ± 0.56 |
| + 32% Cl-PCB, 50 ppm | 225 ± 4.5* | 38 ± 1.7 | 17 ± 1.9 | 4.4 ± 0.52** |
| 100 ppm | 232 ± 4.3 | 39 ± 4.7 | 17 ± 1.3 | 4.7 ± 0.44** |
| + 42% Cl-PCB, 50 ppm | 226 ± 7.7 | 39 ± 2.1 | 17 ± 1.9 | 4.5 ± 0.26** |
| 100 ppm | 211 ± 5.0** | 31 ± 3.1 | 15 ± 3.8 | 4.6 ± 0.28** |
| + 62% Cl-PCB, 50 ppm | 216 ± 5.4** | 35 ± 2.5 | 16 ± 1.1 | 4.8 ± 0.22** |
| 100 ppm | 230 ± 5.3 | 48 ± 3.6** | 20 ± 1.2** | 5.3 ± 0.33** |

[1] All groups except the basal received 1 ppm dieldrin. Data are expressed as means ± S.E. for groups of five rats.

Asterisks, * and **, indicate values judged significantly different from the basal group by LSD analysis with $P \leq .05$ or .01, respectively.

Table 2. Enzyme responses and reduction in hexobarbital sleep time and dieldrin storage in female rats treated with polychlorinated-biphenyls, -triphenyls or DDT. [1]

| Treatment | EPN Detox. % | PNA Demeth. % | Aniline Oxidn. % | Hexobarb. Sleep Time -% | Dieldrin Storage -% |
|---|---|---|---|---|---|
| **Trial A** | | | | | |
| Basal | 94 | 98 | 104 | | |
| + Dieldrin, 1 ppm | 100 | 100 | 100 | 0 | 0 |
| + DDT, 50 ppm | 431 | 228 | 205 | 75.0 | 93.4 |
| + 48% Cl-PCB, 50 ppm | 225 | 408 | 193 | 34.6 | 60.7 |
| 100 ppm | 362 | 568 | 295 | 57.7 | 77.5 |
| + 54% Cl-PCB, 50 ppm | 444 | 555 | 309 | 65.4 | 85.8 |
| 100 ppm | 475 | 672 | 346 | 80.0 | 91.6 |
| + 60% Cl-PCB, 50 ppm | 475 | 291 | 231 | 78.8 | 93.4 |
| 100 ppm | 544 | 368 | 273 | 80.0 | 96.5 |
| + 68% Cl-PCB, 50 ppm | 600 | 272 | 266 | 83.7 | 96.5 |
| 100 ppm | 525 | 242 | 240 | 80.0 | 96.0 |
| **Trial B** | | | | | |
| Basal | 92 | 114 | 114 | | |
| + Dieldrin, 1 ppm | 100 | 100 | 100 | 0 | 0 |
| + DDT, 50 ppm | 450 | 300 | 248 | 78.6 | 92.2 |
| + 21% Cl-PCB, 50 ppm | 117 | 148 | 138 | 11.3 | 7.8 |
| 100 ppm | 117 | 128 | 154 | 31.0 | 10.9 |
| + 32% Cl-PCB, 50 ppm | 183 | 257 | 194 | 48.8 | 29.7 |
| 100 ppm | 233 | 411 | 225 | 55.4 | 45.3 |
| + 42% Cl-PCB, 50 ppm | 200 | 386 | 250 | 37.5 | 37.5 |
| 100 ppm | 275 | 474 | 305 | 65.0 | 64.7 |
| + 62% Cl-PCB, 50 ppm | 625 | 403 | 293 | 85.7 | 94.5 |
| 100 ppm | 717 | 474 | 312 | 87.5 | 96.5 |

[1] Each value is the mean percentage increase (or decrease) in the measured response for groups of five rats compared to the groups that received the basal diet plus only 1 ppm dieldrin.

Table 3. Sex comparisons in selected response of rats to various polychlorinated biphenyl materials.[1]

| PCB Treatment | Sex | EPN Detox. % | PNA Demeth. % | Dieldrin Storage -% |
|---|---|---|---|---|
| 48% Cl, 50 ppm | F | 193 | 416 | 46.0 |
| 25 ppm | M | 155 | 265 | 46.7 |
| 54% Cl, 50 ppm | F | 293 | 497 | 74.7 |
| 25 ppm | M | 225 | 341 | 76.6 |
| 60% Cl, 50 ppm | F | 413 | 316 | 91.8 |
| 25 ppm | M | 340 | 331 | 85.0 |
| 62% Cl, 50 ppm | F | 433 | 286 | 91.5 |
| 25 ppm | M | 408 | 363 | 90.0 |
| 68% Cl, 50 ppm | F | 467 | 254 | 92.7 |
| 25 ppm | M | 430 | 359 | 88.7 |

[1] Data from rats fed diets containing 1 ppm dieldrin plus each treatment at the indicated level. Each value is the mean percentage increase (or decrease) in the measured response of groups of five rats compared to groups that received the basal diet containing only 1 ppm dieldrin.

Table 4. Comparisons of responses from polychlorinated biphenyls to polychlorinated triphenyl materials equivalent in chlorination.[1]

| Treatment | EPN Detox. % | PNA Demeth. % | Aniline Oxidn. % | Hexobarb. Sleep Time -% | Dieldrin Storage -% |
|---|---|---|---|---|---|
| 42% Cl-PCB | 200 | 386 | 250 | 37.5 | 37.5 |
| 42% Cl-PCT | 238 | 208 | 145 | 46.2 | 73.6 |
| 68% Cl-PCB | 600 | 272 | 266 | 85.6 | 96.4 |
| 62% Cl-PCB | 625 | 403 | 293 | 85.7 | 95.0 |
| 65% Cl-PCB + PCT | 519 | 249 | 231 | 66.3 | 95.5 |

[1] Data from female rats fed diets containing 1 ppm dieldrin plus each treatment at a level of 50 ppm. Each value is the mean percentage increase (or decrease) in the measured response of groups of five rats compared to groups that received the basal diet containing only 1 ppm dialdrin.

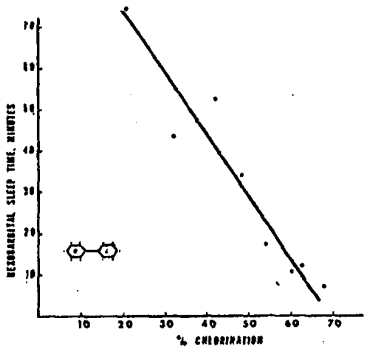


Figure 1. Reduction in hexobarbital sleep time in female rats after receiving PCB materials in the diet (50 ppm) for 10 days.

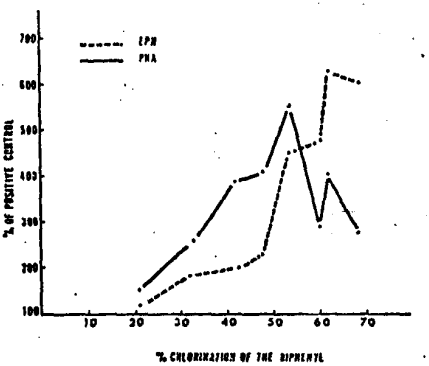


Figure 2. Increases in activity of liver homogenates from female rats in degrading EPN and p-nitroanisole after receiving PCB materials in the diet (50 ppm) for 15 days. Data are presented as percentage increase in activity relative to rats receiving the basal diet containing only 1 ppm dieldrin.

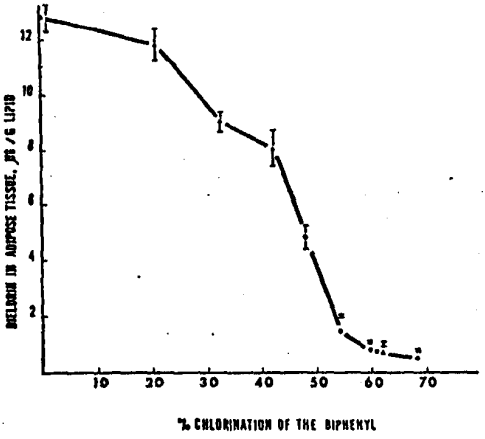


Figure 3. Reduction in dieldrin storage in adipose tissue of female rats fed diets containing 1 ppm dieldrin and various PCB materials (50 ppm) for 15 days.

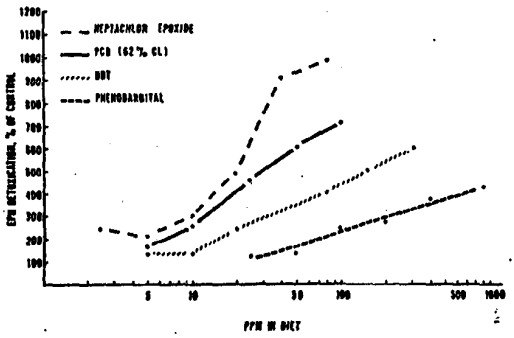


Figure 4. Dose response curves for various microsomal enzyme inducing agents in terms of stimulation of the in vitro degradation of EPN by liver mitochondrial supernatant preparations. The inducing agents were administered in the diets of female rats for 15 days.

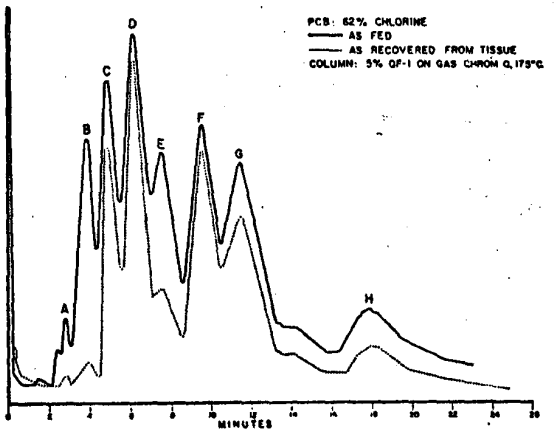


Figure 5. Electron capture gas chromatograms of 62% Cl-PCB material and its residue recovered from rat adipose tissue.