ENVIRONMENTAL HEALTH PERSPECTIVES
NO 1 APRIL 1972

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Perspective on PCBs

The discovery of polychlorinated biphenyls PCBs) in fish from the Baltic Sea attracted videspread attention among scientists. Prior to hat event in 1966 the many formulations of 'CBs—some 210 isomers are theoretically ossible—were thought to be used largely in ontrolled or closed systems. That they were resent in fish, and were later discovered in birds, dded to the concern since the inevitable last link n the food chain was man himself.

Concerns were heightened even further by the nowledge that one of the primary characteristics hich made PCBs so useful during the past 40 ears was their extreme stability under a variety f circumstances. Furthermore, their structural esemblance to the persistent organo-chlorine esticides raised other questions about whether ll of our worries over these pesticides were ccurate or whether, as some suspected, PCBs ere the real cause of that concern.

Alert epidemiologists and other scientists in apan confirmed our expectations of the worst in 969 with their startling discovery and description f Yusho, a human disease caused by consumption f large quantities of PCB-contaminated rice oil.

A glance at the table of contents for the first issue of ***Environmental Health Perspectives*** gives the reader some idea about the quantity of research being done to specify the extent of the PCBs story. Furthermore, while the spectrum of work presented is comprehensive, it is neither all-inclusive nor definitive. Rather, our hope is that this volume will contribute a new perspective to the study of PCBs and will help as well to engender a renewed vigilance about other "inert" chemicals which are so pervasive a part of the environment of man.

The reports which follow were originally prepared for a Conference on PCBs, sponsored by NIEHS at the request of an Interdepartmental Task Force on the subject. The Conference was held at the Quail Roost Conference Center in Rougemont, North Carolina, on December 20–21, 1971. The papers presented at that meeting appear here substantially as they were presented. Your comments on them are indeed welcome.

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Some Chemical Aspects of Polychlorinated Biphenyls (PCBs)

by J. William Cook*

In the past few years considerable scientific information has appeared on the chemistry and biological effects of the PCBs and related materials. The amount of chemical investigation, and especially the biological effects investigation that can be done on these products, is almost limitless. Even though the PCBs show a great deal of inertness, a property very useful in their industrial applications, they certainly do change in most instances before they are inadvertently consumed by man. It is important that we learn more about the nature of these products and the changes they undergo. I believe that the choice of scientific investigation that will provide effective answers is going to be made by close cooperation between the scientists in the field of chemistry and those in the various biological disciplines. I hope that conferences like this one will help give us guidance as to the most fruitful avenues to follow in research and will assist in the interpretation of the data obtained. It is simpler to acquire data than to interpret them.

No attempt has been made to make or present a complete survey of literature on chemistry of PCBs. Instead, I have chosen to discuss in a general way some facets of the chemistry which I think are interesting and relevant to the subject of this conference. For the most part I have drawn upon illustrations from our FDA laboratories because of the easily available material.

There are a number of statements in the literature and the public press which liken PCBs to DDTs. If this were the case, we would need to do little research in chemistry of PCBs, because the chemistry of the DDTs and related compounds is well known. Even so, all the biological effects of DDTs are by no means known or fully understood even though there has been much work during the past 25 years.

In contrast to the three well known isomers of DDT—that is, the p,p′, o,p′, and o,o′-dichloro-compounds, the term polychlorobiphenyls or PCBs encompasses a very complex heterogeneous series of chemicals. When manufactured in a certain way, they form technical products which have enjoyed tremendous industrial use over the past 40 years because of the unique chemical and physical properties of the complex mixtures.

The exact chemical composition of these mixtures has not been determined. There probably was no incentive to determine the real nature of all the components as far as the industrial value of the products was concerned, but now that it has been found that there are untoward biological effects from these products and that their residues are widely distributed, there is need for more knowledge than is now available.

There are also statements in the literature which in effect assert that PCB analytical methods and limits for residues should apply to specific chemicals within the series rather than to PCB undefined. Stated differently, if the toxicity of the PCBs is mainly due to certain compounds in the mixtures, then we could be justified in developing methodology to separate, quantitate, and evaluate these toxic compounds. We will need to know more about the chemistry and biological effects before we can be so specific.

* Director, Division of Chemical Technology, U.S. Food and Drug Administration, BF-420, 200 C Street, S.W., Washington, D.C. 20204

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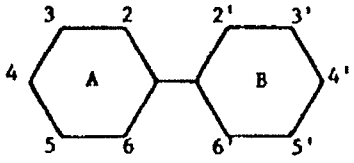
Until we have developed data which, on the one hand, show that essentially all components have about the same biological effects or, on the other hand, show that some are greatly different from others, we have no real scientific basis on which to make a choice. Of course there is evidence that some industrial PCB products contain polychlorodibenzofurans. We need to know how and where these form. Do they form somehow in the environment, and if so, do they survive through the food chain to man? If so, are there great differences in biological effects among the large number of possible polychlorofurans as is the case among the polychlorodioxins? If these compounds are present in foods, we certainly should try to identify them soon, but if they are like the dioxins, they will be difficult to measure in food.

PCBs are manufactured in France, Germany, Great Britain, Italy, Japan, Russia and U.S.A.

Assuming that the starting raw product is only biphenyl, and assuming that no reaction takes place except chlorination of biphenyl during manufacture, 210 different chemicals are theoretically possible from the 10 positions where chlorine can add on as shown in Fig. 1.



Chlorines on B ring \ Chlorines on A ring	0	1	2	3	4	5
0	1	3	6	6	3	1
1		6	18	18	9	3
2			21	36	18	6
3				21	18	6
4					6	3
5						1

FIGURE 1. Structural formula for biphenyl and theoretically possible sites for chlorination.

It is not certain how many of these do appear in significant amounts in the technical products or in food. It is possible that a few molecules of each is present in each of the technical products.

It is important to point out that, in the industrial products, the PCBs are associated with chloroterphenyls. There are three possible terphenyls (the o, m, and p) as starting materials in contrast to one biphenyl as a starting material, and also there are 14 possible points instead of 10 at which each of the 3 isomers may be chlorinated. Therefore, these compounds could result in a considerably more complex mixture than the PCBs. The chloroterphenyls can be associated with the PCBs in the ecosystem, and they are not readily determined by the usual methods of analysis for PCBs and the pesticides. Later some gas chromatograms will be shown that illustrate some of the relationships.

The industrially important products are formed by chlorination of biphenyl to a given chlorine content. Products which contain 21, 42, 48, 54 and 60% chlorine by weight appear to be the most important ones. The end point of the desired average chlorine content is found by making specific gravity or softening point measurements on samples withdrawn during chlorination. The crude products so formed are generally purified to remove color and traces of hydrogen chloride and ferric chloride, largely by distillation. Mixing of distillate is also used to obtain uniform material of the desired composition (1).

Various factors during the manufacturing process can affect the proportion of the various isomers and the proportion of compounds of higher or lower chlorine content than the average (1). Therefore products manufactured by different processes could yield products which give somewhat different amounts of various components. If some components have different biological effects than others, then different samples would produce unequal effects.

Figure 2 shows gas chromatograms of two PCBs; A is Aroclor 1221, and B is 1232. These and the following gas chromatograms (Figs. 3-8) were made by the conditions used in FDA laboratories. The patterns generally look like those we see from other laboratories, so the products seem to be relatively uniform, except for Aroclor 1232. This appears not to have been a fully consistent product in the past.

Figure 3 shows more in the series. A is 1242, and B is 1248.

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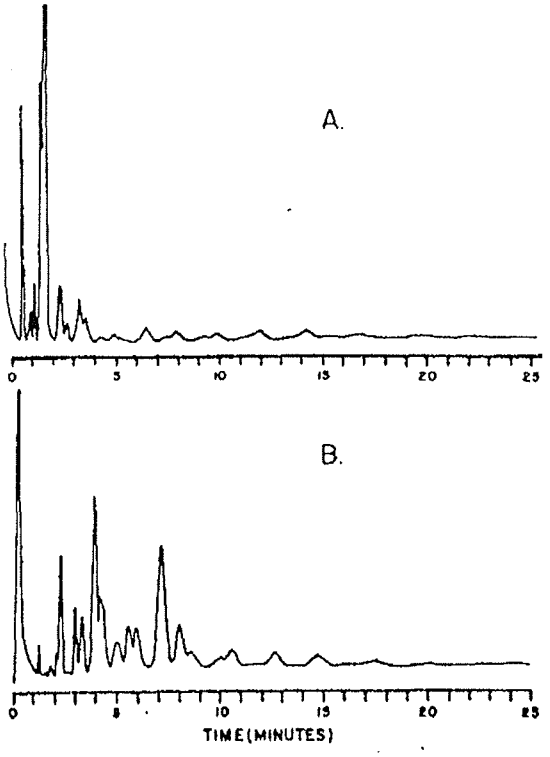

FIGURE 2. Gas chromatograms of PCBs. A is Aroclor 1221. B is 1232.

Figure 4 shows three more in the series; A is 1254, B is 1260, and C is 1262. Note that there is an overlap of peaks. This does not prove that they always represent the same compounds, but in all probability the major part of the peaks is the same. Those which appear in the lower series probably would have been chlorinated to higher chlorine content if the product had stayed in the reaction chamber longer. Generally, as the peaks drop off on the left of the chromatograms, the ones on the right increase, or new ones appear. In all probability there are a few molecules of every possible structure present in each product. Only the predominant ones are in high enough concentrations to give the detector responses. Also note that the time the PCBs are in the gas

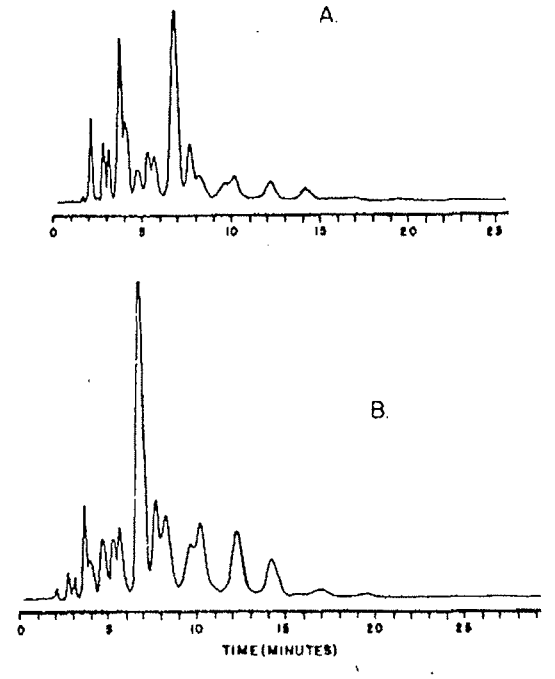

FIGURE 3. Gas chromatograms of PCBs. A is 1242 and B is 1248.

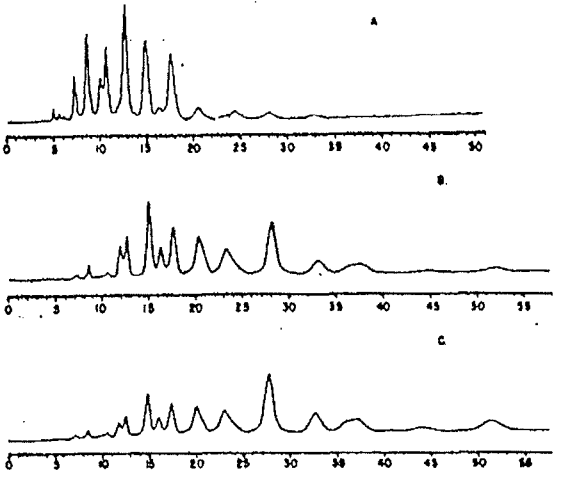

FIGURE 4. Gas chromatograms of PCBs. A is 1254, B is 1260 and C is 1262.

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chromatograph under these conditions is between 0 and 50 minutes.

Figure 5 shows a mixed biphenylterphenyl product (Aroclor 4465); note that some come out early, and some come out late. Some of the early ones look like 1260 and 1262 in Fig. 4.

Figure 6 shows a polychloroterphenyl-(Aroclor 5442). Note that there are peaks which come out as early as portions of Aroclor 1242 and 1248. The proportion of the early eluters is relatively low; however, if accumulation through the food chain occurred, they may appear as PCBs.

Figure 7 shows a comparison between the gas chromatograms of Kanechlor 400 from Japan and Aroclor 1248 from U.S.A. This is presented to show that the two products look remarkably alike, even though one was made across the sea. The one from Japan may be manufactured by the same processes as the U.S. one.

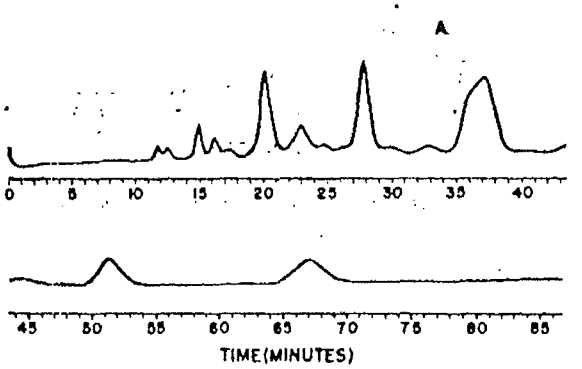

FIGURE 5. Gas chromatogram of a mixed biphenyl-terphenyl product (Aroclor 4465).

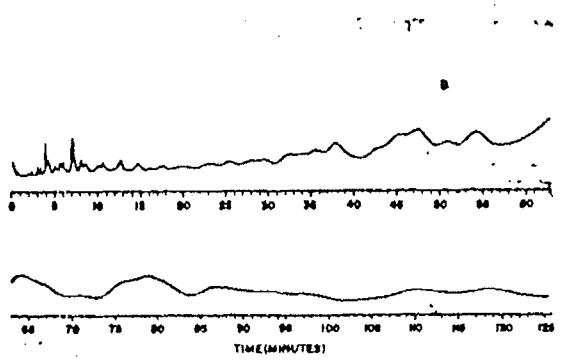

FIGURE 6. Gas chromatogram of a polychloroterphenyl (Aroclor 5442).

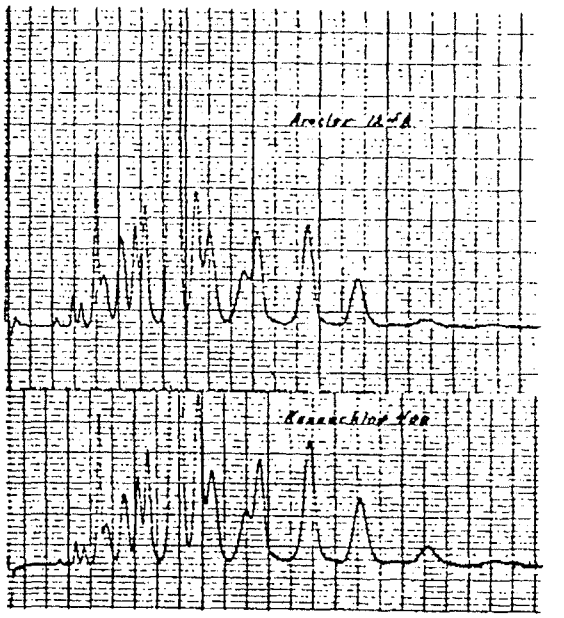

FIGURE 7. Comparison of gas chromatograms of Kanechl 400 (Japan) and Aroclor 1248 (USA).

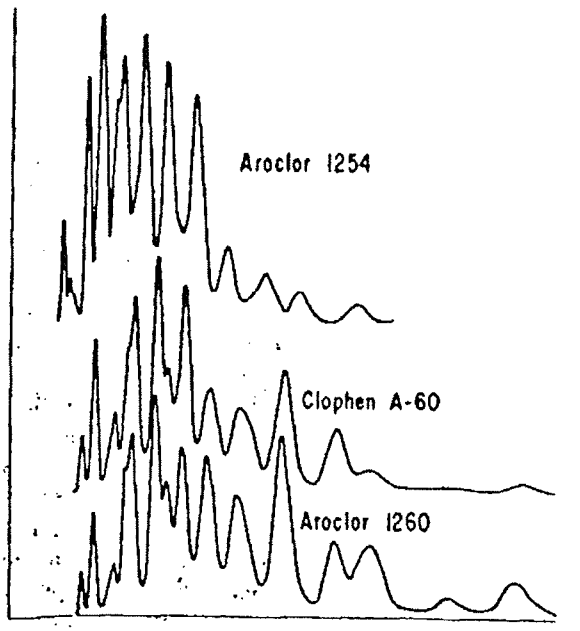

FIGURE 8. Comparison of gas chromatograms of Aroclor 1254, Chlophen A-60 (Germany) and Aroclor 1260.

Figure 8 shows a comparison between the response of Aroclor 1260 and Clophen A-60 from Germany (2).

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All the gas chromatograms shown above were produced utilizing an electron capture detector. This is an extremely good detector in many respects; however, care must be used in attempting to interpret data from it. First, it is not specific for any given type of compounds. It is generally sensitive to halogenated compounds, but many nonhalogenated ones also respond. Equally important, the electron capture response of halogenated compounds is not always in relation to the amount of chlorine. For instance, the response to carbon tetrachloride is 2,000 times greater than to ethylene dichloride—that is four chlorines in one case, and two in the other. This is an extreme example, but it points out the need for caution in interpreting response from unknown peaks. That is, a peak may or may not represent a halogenated compound, and it may represent a large or small amount. To be certain that any peak is a chlorinated compound, confirmatory tests are required. Using electron capture, amounts can only be estimated by comparison to the response of a known amount of the exact same compound.

The parameters of operating an electron capture detector also influence the responses. For instance, the voltage applied to the detector affects the relative responses between peaks.

Table 1 shows the relative responses of some individual tetrachlorobiphenyls to an electron capture detector (3).

Table 1. Retention time and electron-capture detector response of chlorobiphenyls

Compound	Relative retention time (p,p'-DDE = 1.00)	Relative response per ng ± standard deviation (p,p'-DDE = 1.00)
2,2',4,4'-tetrachlorobiphenyl	0.51	0.206±0.003
3,3',4,4'-tetrachlorobiphenyl	0.96	0.770±0.027
2,2',6,6'-tetrachlorobiphenyl	0.33	0.0403±0.0016
3,3',5,5'-tetrachlorobiphenyl	0.70	0.625±0.045
2,3,4,5-tetrachlorobiphenyl	0.69	0.715±0.010
2,3,5,6-tetrachlorobiphenyl	0.51	0.505±0.010
2,3,4,5,6-pentachlorobiphenyl	1.00	1.30±0.017

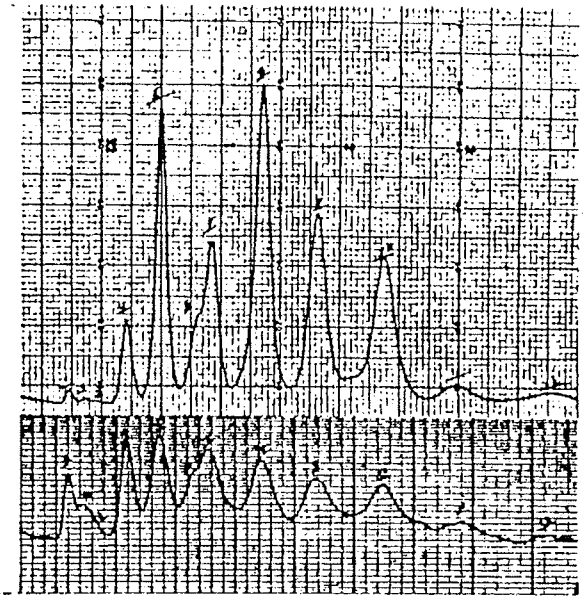

FIGURE 9. A comparison of response of 1254 utilizing different detectors. The upper curve is electron capture; lower curve is electrolytic conductivity detector.

Figure 9 shows the relative response from 1254 utilizing the same gas chromatographic conditions but different detectors; the upper curve is electron

Table 2. Physical properties of chlorobiphenyls

Compound	Melting point, C	Boiling point, C (mm Hg)
*2-chloro-	34	267–268; 154 (12)
3-chloro-	89	284–285
*4-chloro-	76	125 (14)
*2,2'-dichloro-	61	
3,3'-dichloro-	29	322–324; 320–326
*4,4'-dichloro-	148	315–319
3,5-dichloro-	36	166 (10)
2,5-dichloro-		171 (15); 182 (30)
3,4-dichloro-	49	195–200 (15)
2,3-dichloro-		172 (30)
*2,4'-dichloro-	44	
3,4,3',4'-tetrachloro-	172	230 (50)
3,4,2',5'-tetrachloro-	103	
2,6,2',6'-tetrachloro-	198	
*2,5,3',5'-tetrachloro-	162	
2,4,2',4'-tetrachloro-	83	
*2,5,2',5'-tetrachloro-	85	
2,4,5,3',4'-pentachloro-	179	195–220 (10)
2,3,4,5,2',4',5'-heptachloro-		240–280 (20)

* Present in commercial mixtures.
Reference (except *)—Hubbard.

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capture, and the lower is the electrolytic conductivity detector. The latter is more nearly responsive to the amount of chlorine; therefore, it should more nearly represent the relative amount of the various components.

Use of a microcoulometric detector may also provide a better quantitative estimate. Coulometry should yield results which would permit an exact measure of the chlorine. However, the currently available microcoulometric equipment does not give strictly quantitative response. Both microcoulometry and electrolytic conductivity are more specific for halogen-containing compounds than the electron capture detector, and accordingly they can be used as a means to be more certain that the products detected contain chlorine.

Not enough is known about the chemistry of the individual components and their relation to the whole industrial product.

Some of the individual compounds are shown in Table 2 (1). It can be seen that the physical properties vary quite widely. Note the melting points of all the compounds listed; they are solids at room temperature. Yet when the commercial products are manufactured by chlorination of biphenyl, the mixtures formed vary from liquid to soft, sticky noncrystalline products with a chlorine content of 60%, low-melting resinous

Table 3. The retention indices, chlorine numbers and structures of the major PCB constituents in Aroclor 1254. (4)

Peak No.	R.I.	Chlorine No.[a]	NMR determined structure	Alternative predictions	
				Structure	R.I.[b]
22	1994	4	2,5-2',5'	2-2',3',5'	1996
23	2010	4		2-2',4',5'	2012
				2,4-2',5'	2010
24	2022	4	2,3-2',5'	3-2',4',6'	2021
				2,4-2',4'	2027
		(5)		2,6-2',3',6'	2017
29	2089	5	2,5-2',3',6'	2,6-2',3',5'	2092
32	2119	5	2,3-2',3',6'		
33	2136	4	2,5-3',4'		
36	2159	5		2,5-2',3',5'	2159
39	2175	5	2,5-2',4',5'	2,4-2',3',5'	2175
				3,5-2',4',6'	2175
		(6)		2,6-2',3',4',6'	(2175)
				2,3,6-2',3',6'	2172
41	2191	5	2,4-2',4',5'	2,3-2',3',5'	2189
42	2203	5	2,3-2',4',5'		
43	2207	5	2,5-2',3',4'		
44	2228	5	3,4-2',3',6'	2,4-2',3',4'	2226
45	2238	—		2,3-2',3',4'	2240
				2,6-3',4',5'	2240
				3,4-3',5'	2238
				2,3,5-2',4',6'	2240
48	2264	6	2,3,6-2',4',5'	2,4-2',3',4',6'	(2263)
50	2299	6	2,3,4-2',3',6'		
52	2321	5	3,4-2',4',5'		
55	2356	5	3,4-2',3',4'		
56	2356	6	2,4,5-2',4',5'	2,5-2',3',4',5'	(2360)
				3,5-2',3',4',6'	(2357)
59	2390	6	2,3,4-2',4',5'	2,3-2',3',4',5'	(3291)
				2,4,6-3',4',5'	2388
		(7)		2,3,5-2',3',5',6'	(2390)

[a] Cl No. between brackets; Chlorine number of minor constituent not associated with accurate R.I.
[b] R.I. between brackets: Predicted R.I. using Table VIII of Ref 4a.

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products containing between 60 and 65% chlorine, and partly crystalline or crystalline products of much higher melting points containing more than 65% chlorine. Thus, the commercially-made mixtures have at least some physical properties that are quite different from any of the individual constituents. This is probably one of the very important factors in the industrial utility of the products. The same phenomenon probably has a bearing on the survival of many of the compounds through a host of possible conditions through the ecosystems. So it is remarkable that the end products in food, which we will see later, look as much like the original industrial product as they do.

A number of workers have identified the predominant number of chlorine atoms in the major components of some of the peaks. Others have made significant approaches to determine or predict the placement of the chlorines on the biphenyl rings (4), (5), (6).

Sissons and Welti (4) used a SCOT column and a flame ionization detector to obtain the graph shown in Fig. 10 from 1254. Note that there are 69 identifiable peaks. They did further purification, and then by utilizing MS, NMR, and factors such as retention time, they identified the major peaks of 1254 as shown in Table 3.

Note that the retention times do not necessarily relate to number of chlorines. These researchers also made use of retention times and other information to predict the composition of a large number of the other peaks. To attempt to be certain of the structure of the minor peaks would be tremendously complicated. It takes a significant amount of a chemical to make NMR analysis. It would be of great value to have available simple biological tests that would give a clue as to which peaks, major or minor, would be of enough significance to warrant full-scale chemical- and biological-effect investigations.

If it were found that all peaks contributed essentially equally to the biological effects (and there are certainly many biological effects), then there would be no need to perform a great amount of chemistry. On the other hand, if we determine that they vary in biological effects as widely as their propensity to capture electrons, then we would have to devise means to and measure these individual chemicals.

It is known that PCBs can be dechlorinated and oxidized by photochemical effects (7). I look forward to the discussions by Dr. Hutzinger on this subject. I want only to point out that these reactions may complicate the process of evaluation of both chemistry and toxicology. If the dechlorination is simply a reversal of chlorination, so that the higher chlorinated products revert to exactly the same compound in the lower chlorinated products, then the problem is not more complicated. If new and different chemicals form, then the problem is greater.

If there is significant photo–oxidation through the food chain, then we have the potential for chlorophenols and even the potential for the formation of polychlorodibenzofurans as further chemicals to evaluate. I hope that we hear discussion on these chemical problems during this conference.

Man has been exposed to residues of PCBs in his food in many forms; that is, from the original industrial product introduced accidentally into his food to cases where these compounds have been subjected to almost all conditions of food chain reactions, photochemical reactions, etc. Some of these conditions are:

A. PCB directly into food
   in rice oil (Japan)
B. PCB in paperboard
   transfers to food
C. PCB into animal food
   we eat animal tissue
D. PCB into environment
   into fish
   we eat fish
E. PCB into environment
   into fish
   fish into animal feed
   we eat animal tissue

The first example is that of PCB accidentally put into rice oil. The product consumed by man may have all the ingredients formed during the manufacturing process of the PCBs, plus any reaction that may be induced by food processing.

In the second example the food will have only those components which migrate from the packaging material to the food, either through the vapor phase or by contact, or both. If any polar compounds are present, they may not migrate so

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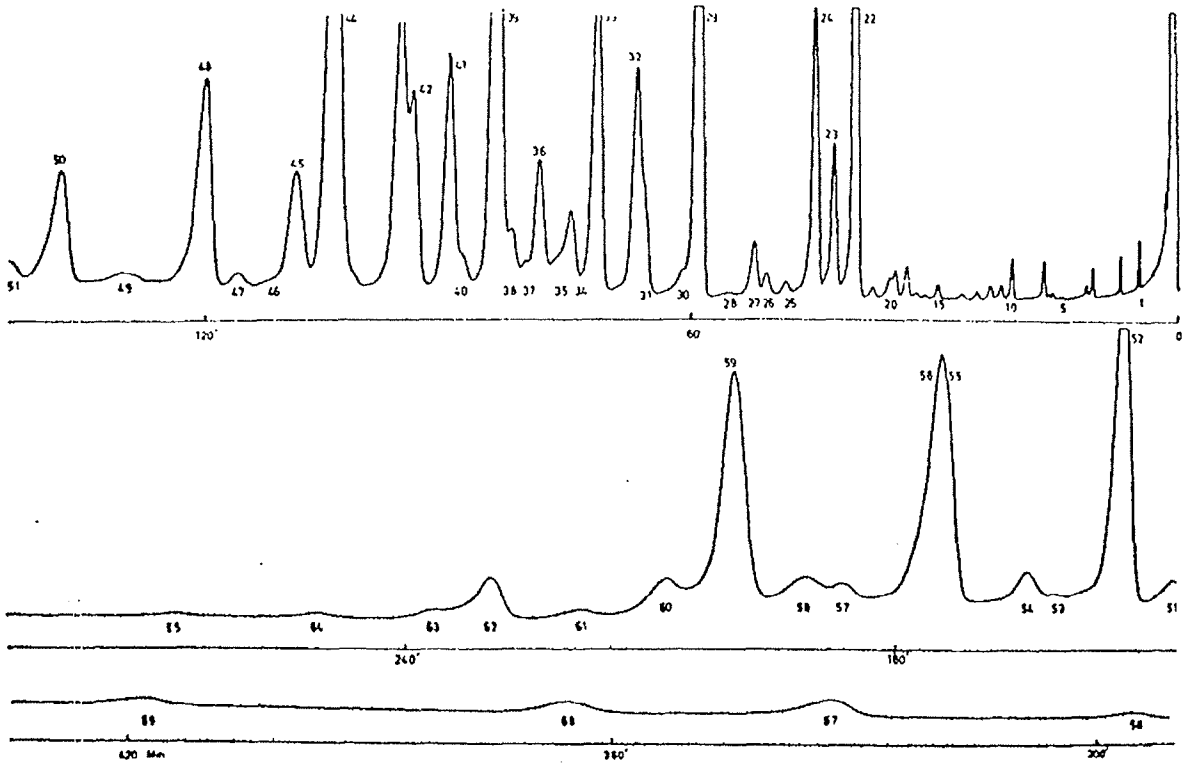

FIGURE 10. Aroclor 1254 solution chromatographed on an Apiezon L SCOT column at 205° employing a flame ionization detector (4).

readily. If the packaging material contains fat or wax, some of the higher chlorine compounds may not transfer as readily as the lower ones. However, there is the mutual effect of all on each, discussed earlier, which makes it difficult to predict. Some controlled experiments should be done.

In the third situation the whole industrial product contaminates the animal feed, and we eat the animal tissue. The animal appears to modify the product, because the gas chromatograms from tissue extracts do not look exactly like the industrial product.

In the fourth example the product or products have gotten into the environment, then into fish or other marine products, and we eat the fish or marine product. Here, there could be an accumulation of a number of different Aroclors or similar products, each of which has been subjected to various biochemical reactions through the food chain and to various other effects such as photochemical reactions. When we eat the fish, we eat the PCBs or metabolites, the end products of whatever reactions may have taken place.

And in the last example as in the previous one, the fish is fed to a domestic food animal—we eat the animal tissue.

The residues that we find in food products may look a good deal like the original industrial products or like a mixture of those products.

Figure 11 illustrates some food products, turkey and chicken tissues and cows' milk, which have been found to contain PCBs.

Figure 12 shows that the turkey sample had a product which looked most nearly like 1260. However, it can be seen that the proportion of peaks is different, and also a couple of peaks are missing.

Figure 13 shows that the chicken sample looked like 1248; yet there are some quite important differences.

Figure 14 shows some gas chromatograms from 1260 and the residues in muscle tissue from swine fed the 1260. Note that there are differences; they are not as great as in the case of the turkey tissue.

Figure 15 gives the results from a sample of coho salmon from Lake Michigan. A is before separation of pesticides from PCB, B is PCB after

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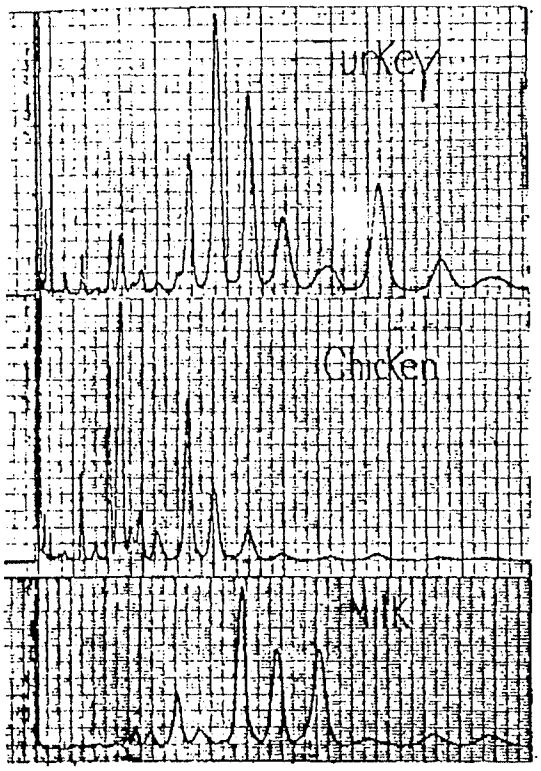

FIGURE 11. Gas chromatograms of PCBs from some food products.

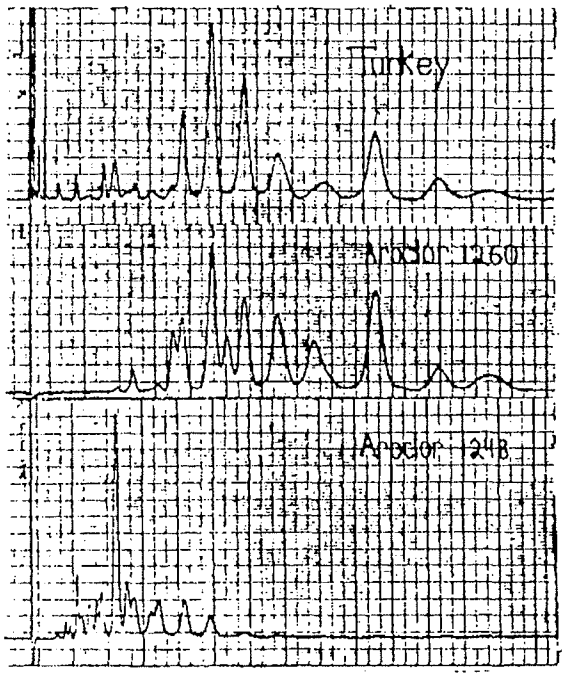

FIGURE 12. A comparison of gas chromatograms of PCB from turkey and Aroclor 1248 and 1260.

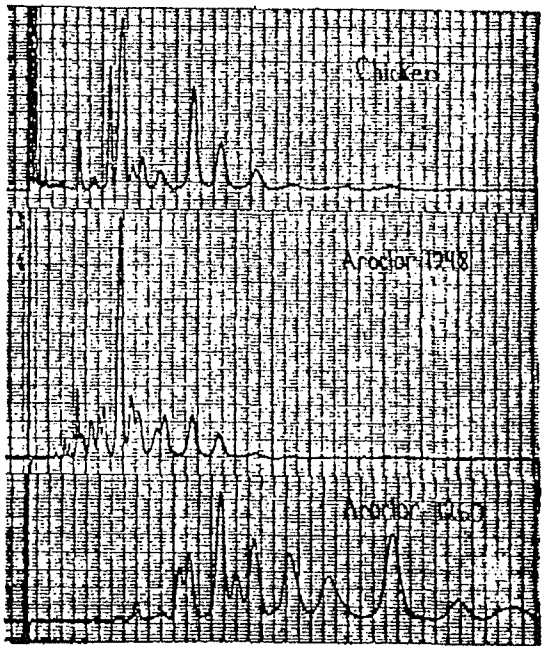

FIGURE 13. A comparison of gas chromatograms from chicken and Aroclor 1248 and 1260.

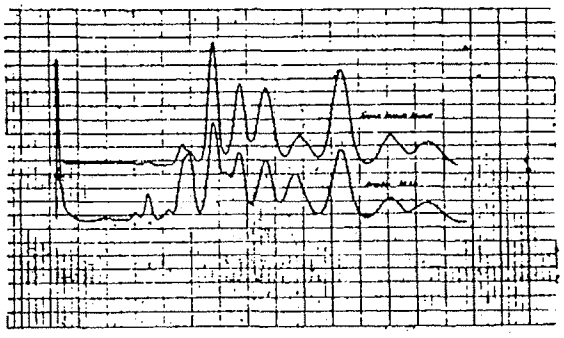

FIGURE 14. A comparison of gas chromatograms from Aroclor 1260 and residues in muscle tissue from swine fed Aroclor 1260.

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FIGURE 15. Gas chromatograms of sample from salmon from Lake Michigan. A is before separation of pesticides from PCB, B is PCB after separation and C is DDT pesticides.

separation, and C shows the DDT pesticides present. The PCBs here look most like 1254.

Many of the samples of wildlife reported by most workers yield chromatograms which look like 1254.

Figure 16 shows the residue in rat blood following the feeding of 1254. The peak, which disappeared, is a pentachloro compound. The material in the rat blood looks more like 1254 than does the material from coho salmon.

Veith (8) made a study of the PCBs in the Milwaukee River and out into Lake Michigan. He found that the lower chlorine content products disappeared more rapidly than the higher chlorinated ones. It may be that the residues in food products, which come from a long food chain, do

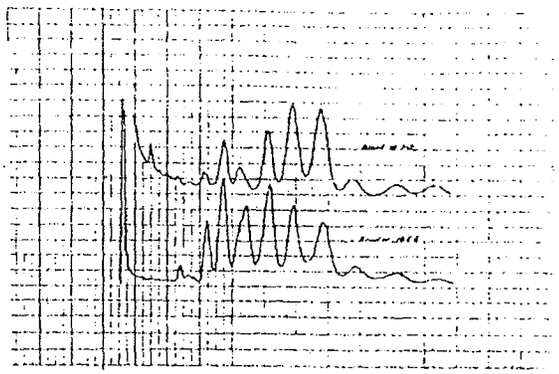

FIGURE 16. Comparison of gas chromatogram of Aroclor 1254 residue in rat blood following feeding of Aroclor 1254.

not originate entirely from the industrial product which is similar to the residue but may be the result of a concentration of the higher chlorinated units originally in the lower chlorine content products, the lower chlorinated units of which have largely disappeared.

So even though these industrial products are generally stable and persistent, they are altered by microorganisms, in animal systems, by light, and probably in plant systems. Some units disappear and some concentrate. We need to develop some short term in vitro and in vivo bioassay that can be used to give clues for more thorough and detailed examination. The chemist has tools and techniques to obtain almost any degree of sophistication required by the biologist.

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Photochemical Degradation of Chlorobiphenyls (PCBs)*

by O. Hutzinger and S. Safe† and V. Zitko**

Polychlorinated biphenyls (PCBs) are stable towards oxidation, hydrolysis and other chemical reactions which conceivably occur in the environment. Although there is little direct evidence available, it is generally assumed that PCBs, particularly those with a high chlorine content, are also quite resistant to metabolic change.

Over the past years photochemical degradation was shown to be a possible major route of environmental breakdown for a number of pesticides (1–4). Until relatively recently (5), however, simple chloroaromatic compounds have not received much attention. The limited interest of photochemists in compounds of this type can perhaps be explained by the commonly held view that there is generally little cleavage of the C–Cl bond in chloroaromatic compounds (6).

We have previously shown (7) that 2,2',4,4',6,6'-hexachlorobiphenyl, when irradiated at 3100 Å in hexane, photolyzes rather readily to give products which are formed by loss of chlorine, rearrangement and condensation. We now wish to present a first report of our studies on the photochemical stability of a number of chlorobiphenyl isomers in hexane as well as on the breakdown of chlorobiphenyls under natural and simulated "natural conditions", with particular emphasis on polar products.

Considerable experience in laboratory irradiations of pesticides has accumulated (1, 3, 8) and an elaborate "weathering chamber", which also allows controlled irradiation, has been described (9). "Natural conditions", however, are difficult to simulate for water-insoluble compounds such as the PCBs. Even in sunlight, irradiation experiments in solution are artificial since "unnatural" organic solvents have to be used. Irradiations of chlorobiphenyls in thin films, in the gas phase (both in the presence of water or water vapor) or in aqueous suspension are likely to be closer to the natural environmental conditions to which PCBs are actually exposed.

Equipment and Methods

Gas chromatographic (glc) separations were carried out with a Hewlett Packard model 5750 chromatograph using a flame ionization detector and an 8 ft × ¼ in stainless steel column containing 3% SE-30 on acid washed Chromosorb W (60/80 mesh). Temperature was isothermal or programmed as indicated. Quantitative data were obtained with a Packard A7901 gas chromatograph equipped with a 6 ft × ½ in 4% SE-30 column and an electron capture detector using the conditions previously described (10).

Combined glc mass spectral data were recorded with a Hewlett Packard model 5750 instrument, using a 6 ft × ⅛ in glass column, coupled to a DuPont/CEC 21-491 mass spectrometer via a single stage jet separator.

A DuPont/CEC 21-110B mass spectrometer equipped with a direct introduction-constant temperature probe (11) was used for obtaining spectra of individual samples.

The infrared (ir) spectra (thin films) were recorded on a Perkin Elmer 237 spectrophotometer.

Thin-layer chromatography (tlc) was carried out on precoated Merck silica thin layer plates (F-254) and plates (100×20 cm) coated with

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* Issued as NRCC No. 12259.

† Atlantic Regional Laboratory, National Research Council of Canada, Halifax, Nova Scotia.

** Environment Canada, Fisheries Services, St. Andrews, New Brunswick.

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Merck silica gel (F-254; 0.9 mm thickness). Solvents used were: hexane and (A) 1,1,1-trichloroethane-hexane (5:1), (B) hexane–acetone (3:1), (C) benzene-ethyl acetate (12:1), (D) benzene–chloroform (2:3). The chlorobiphenyls were synthesized as described before (12,13). Aroclor 1254 was a gift of Monsanto Co.

Sources of ultraviolet light for the irradiation experiments were as follows:

(i) Sun: direct sunlight at Halifax, N.S. during the period July to September 1971.

(ii) Photochemical reactor: Rayonet (the Southern N.E. Ultraviolet Co.) using 16 R.P.R. 3100 lamps (3100 Å). The temperature was maintained at 14±2°.

(iii) Blacklight: four General Electric F40BL fluorescent lamps mounted with reflective backing ca. 15 cm above the samples. The temperature at this distance was 30–33°.

(iv) Sunlamp: General Electric 275 W "Sunlamp", one per dish at ca. 25 cm distance (temp. 35–40°). For irradiation of chlorobiphenylwater vapor, four lamps per 500 ml quartz tube were mounted horizontally at ca. 10 cm. distance.

Experiments and Results

Stabilities of Chlorobiphenyls at 3100 Å in Hexane Solution

Time study of 2,2',5,5'-tetrachlorobiphenyl decomposition—2,2',5,5'-Tetrachlorobiphenyl (50 mg) in hexane (300 ml) was irradiated in the Rayonet reactor with N_2 bubbling through the solution. 50 ml Aliquots were removed from the reaction vessel at 2, 4.5, 17, 41 and 89 h. Good quantitative data could not be obtained for the 89 h sample, probably because much of the material had polymerized. Chromatograms observed after injecting equal amounts of these aliquots and using a flame ionization (F.I.) detector are shown in Fig. 1. Percentages of starting material remaining after different irradiation times are also presented in Fig. 1. These data were obtained by measuring the area under the peak with retention time identical to the starting material

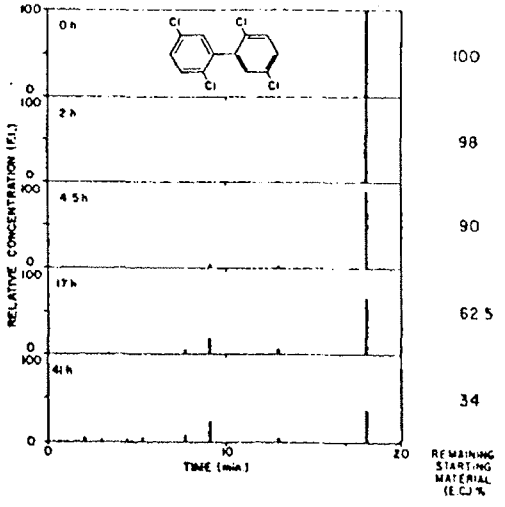

FIGURE 1. Irradiation of 2,2',5,5'-tetrachlorobiphenyl for different times; chromatograms and percent starting material remaining.

using an electron capture (E.C.) detector. Mass spectral and glc data for the 41 h sample are given in Fig. 2.

Comparative stability of chlorobiphenyl isomers—A 0.1% solution (3 ml) of the chlorobiphenyl hexane was flushed for 3 min with nitrogen, stoppered and irradiated in the Rayonet reactor for 24 h. The volume of the solution was removed from the quartz reaction cell and, after rinsing with hexane and evaporation of some of the solvent, adjusted to 3 ml. Equal amounts of the irradiated solution and the original solution

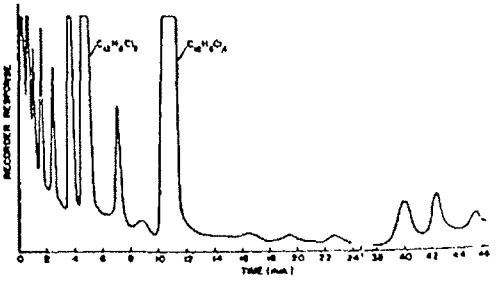

FIGURE 2. Chromatogram and mass spectral data for the 41 h sample. Oven temperature 150° isothermal, at 36 min beginning of programming (4°/min). A number of further peaks were observed after 46 min.

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Table 1. Relative Photochemical Labilities of some Chlorobiphenyl Isomers

Compound	Starting material remaining after 24 h at 3100 Å in Hexane
Tetrachlorobiphenyls	%
3,3′,4,4′-	32
2,2′,6,6′-	29
2,2′,5,5′-	33
2,2′,4,4′,5,5′- Hexachlorobiphenyl	3.8
2,2′,3,3′,4,4′,5,5′- Octachlorobiphenyl	<1

were injected into the gas chromatograph, and the areas under the peak corresponding to the starting material compared (Table 1). No immediate correlation can be seen between steric hindrance of *o*-chlorine substitution or uv spectra (14) and photochemical lability, although chlorobiphenyls with high chlorine content seem to degrade more rapidly.

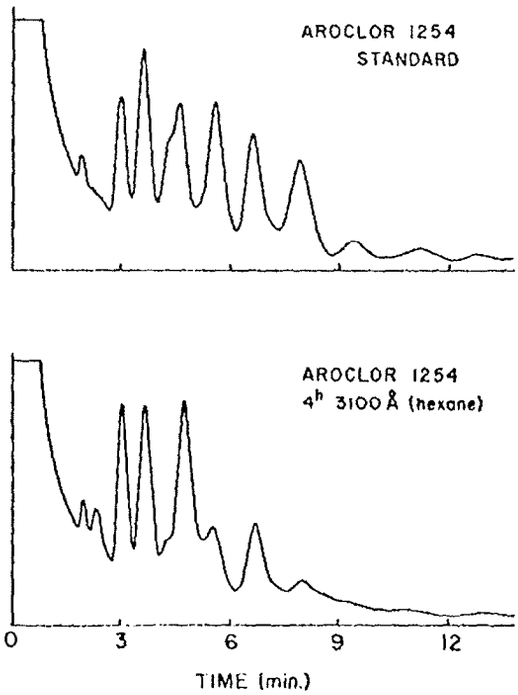
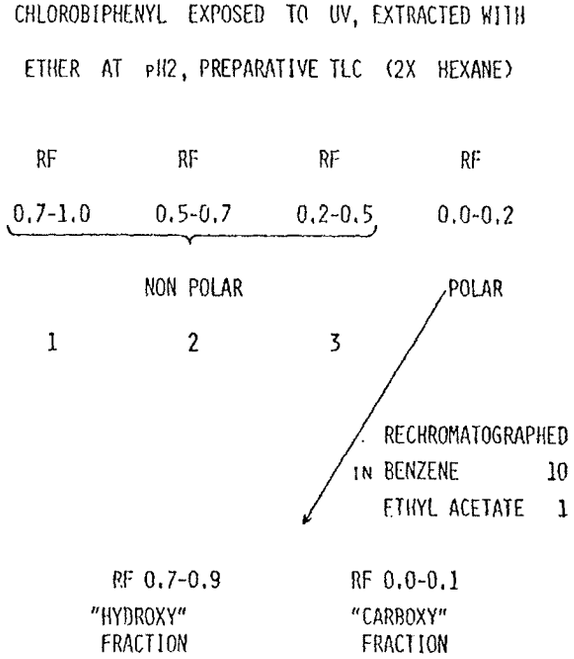


FIGURE 3. Chromatogram of Aroclor 1254 before and after irradiation. Glc conditions of (10).

Stability of Aroclor 1254—Aroclor 1254 (20 mg) in hexane (3 ml) was irradiated for 4 h at 3100 Å in the presence of air. Examination of the irradiated mixture and standard Aroclor 1254 by glc shows gross differences with tendencies toward shorter retention times (Fig. 3).

Irradiations in Sunlight; Under Simulated "Natural" Conditions and in Solvent Mixtures Containing Water

Aroclor 1254 I; Aqueous dioxane suspension at pH 9—A suspension of Aroclor 1254 (2 g) in a mixture of dioxane (150 ml) and water (350 ml) containing sodium bicarbonate (0.5 g) was irradiated in the Rayonet reactor for 24 h with air bubbling through the mixture. After this period another portion of sodium bicarbonate (0.5 g) was added, and the irradiation continued further for 24 h. The mixture was worked up as shown in Scheme 1. Preliminary mass spectral data for the crude nonpolar fractions one, two and three indicated the presence of only chlorobiphenyls since no ions containing oxygen corresponding to hydroxychlorobiphenyls or chlorodibenzofurans were detected. The mass spectrum of the crude



SCHEME 1. Separation-scheme for irradiated chlorobiphenyls.

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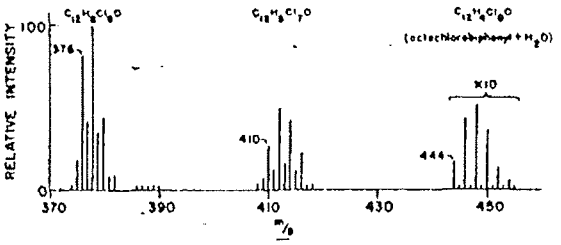

FIGURE 4. Partial mass spectrum (70 eV; probe temperature 35°) of "hydroxy" fraction from Aroclor 1254 irradiation experiment I. The chlorine isotope pattern for the Cl_4 and Cl_5 compounds are distorted due to impurities or fragment ions.

"hydroxy" fraction, part of which is shown in Fig. 4, indicated the presence of compounds formed by the addition of water to chlorobiphenyls. Hydroxy and carbonyl stretching frequencies in the infrared spectrum of the crude "carboxy" fraction (Fig. 5) are indicative of the type of compounds present in this mixture.

Aroclor 1254 II; Irradiation as thin film—Aroclor 1254 (0.5 g) was dissolved in benzene (3 ml), and this solution spread over a circular pyrex dish (diameter: 35 cm). The benzene was allowed to evaporate slowly in a way to ensure formation of a uniform Aroclor 1254 film. Water (3 ml) was added, and the dish was covered with plastic film. Water was frequently replaced during the Blacklight one-week irradiation. The irradiated product was fractionated according to Scheme 1. Preliminary mass spectra of the crude nonpolar fractions 1 and 2 (in this experiment no material

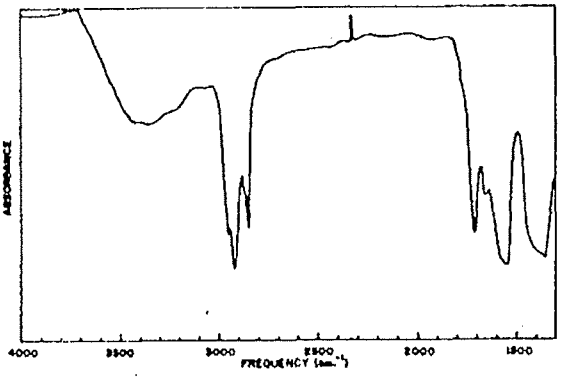

FIGURE 5. Partial infrared spectrum (thin film) of "carboxy" fraction from Aroclor 1254 irradiation experiment I.

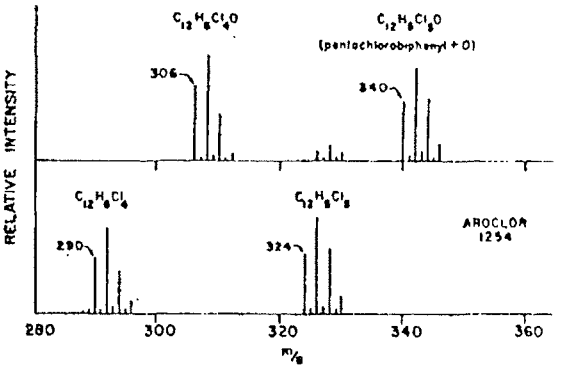

FIGURE 6. Partial mass spectra (70 eV; probe temperature 35°) of "hydroxy" fraction from Aroclor 1254 irradiation experiment II (top)) and standard Aroclor 1254 (bottom; ions <340 not shown).

corresponding to 3 was detected) showed only the presence of chlorobiphenyls. A mass spectrum of the crude "hydroxy" fraction (Fig. 6) showed ions which can be most easily explained as resulting from hydroxychlorobiphenyls. The ir spectrum of the crude "carboxy" fraction was almost identical to that from experiment I (Fig. 5).

Irradiations of 2,2′,5,5′-tetrachlorobiphenyl—Some information on the types of products formed from 2,2′,5,5′-tetrachlorobiphenyl on irradiation under a variety of conditions is given in Table 2. Samples were fractionated as

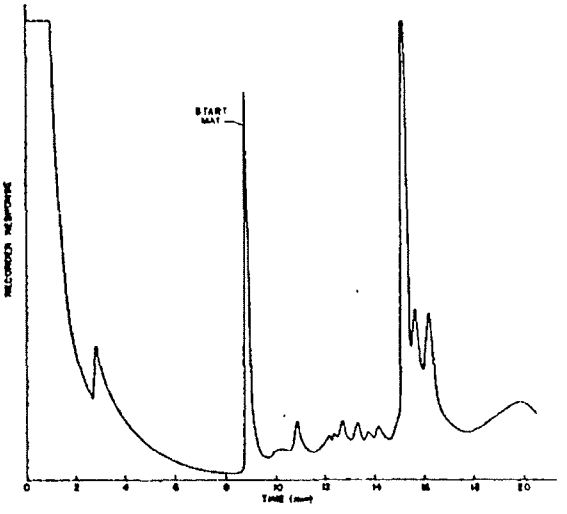

FIGURE 7. Chromatogram for 2,2′,5,5′-tetrachlorobiphenyl irradiation experiment II. Oven temperature programmed from 150° at 10°/min until 310 . A number of further peaks were observed after 20 min.

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Table 2. Photochemical Decomposition of 2,2′,5,5′-Tetrachlorobiphenyl.

Experiment number	Irradiation conditions	UV source	Time	Types of products formed[a,b]			
				"Polar" products		"Nonpolar" products	
						Retention time[c]	
				"Hydroxy-"	"Carboxy-"	Short	Long
1	Aqueous suspension (+ emulsifier)	sun	2 mo	−	+	(+)	+
2	Thin film	sun	2 mo	(+)	+	(+)[d]	+[d]
3	Thin film	sun lamp	24 h	(+)	+	(+)	+
4	Thin film	black light	1 wk	(+)	+	(+)	+
5	Dioxan-2N NaOH solution	reactor	16 h	−	+	+	+
6	Aqueous dioxane solution + $FeCl_3$	reactor	16 h	(+)	+	+	+
7	Vapor (refluxing with water)	sun lamp	16 h	−	+	(+)	+

[a] + = present; (+) = present in small quantities; − = not detected.
[b] For further description of products, etc. see text.
[c] Glc retention time shorter or longer than starting material (2,2′,5,5′-tetrachlorobiphenyl).
[d] See Fig. 7.

described in Scheme 1. The nonpolar fraction was analyzed by glc (e.g. Fig. 7).

"Carboxy"-type compounds were indicated in all experiments by the chromatographic properties of a portion of the reaction products which were similar to the corresponding fractions from the Aroclor 1254 irradiation experiments I and II (i.e., $R_f < 0.1$ in solvents A–C; high R_f in aqueous methanol mixtures).

The presence of "hydroxy"-type compounds was indicated by:

(i) chromatographic comparison of the appropriate fractions with authentic hydroxy- and dihydroxybiphenyls in solvent systems A, (R_f 0.3–0.8); B, (R_f 0.3–0.7) and C, (R_f 0.4–0.8), and

(ii) by reacting the "polar" fractions with dansyl chloride (1-dimethylaminonaphthalene-5-sulfonyl chloride) and comparing the chromatographic properties (solvent D; R_f ca. 0.75 for monohydroxy and ca. 0.4 for dihydroxy compounds) and in situ fluorescence excitation (350 nm) and emission (525 nm) wavelength with those of dansyl derivatives of authentic hydroxy and dihydroxybiphenyls (see reference 15 for the general method).

Discussion

The photolysis of chlorobiphenyls reveals a number of degradative reactions which occur on irradiation in sunlight and a number of laboratory conditions. In hexane, the most prominent reaction was the progressive reductive dechlorination of these compounds as well as formation of polymeric materials. Irradiation of Aroclor 1254 in hydroxylic solvents at pH 9 yields compounds corresponding to the addition of water to the PCB molecules and a more polar carboxylic acid fraction. The same sample irradiated as a thin film does not yield the water-addition products, but mass spectrometry reveals the presence of new hydroxylated species. This experiment also gives a more polar "carboxylic" fraction. Data for a number of different irradiations of 2,2′,5,5′-tetrachlorobiphenyl indicate that dechlorination, formation of polymers, and carboxylic products as well as hydroxylation, does occur.

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The present photolytic work thus reveals decomposition of PCBs by both oxidative and reductive pathways.

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Rates and Routes of Transport of PCBs in the Environment

by Ian C. T. Nisbet* and Adel F. Sarofim†

Polychlorinated biphenyls (PCBs) are one member of a class of chlorinated organic compounds which give rise to concern, because of their wide dispersal and persistence in the environment and tendency to accumulate in food chains, with possible adverse effects on animals at the top of the food webs, including man. In the past, attention has been concentrated on chlorinated hydrocarbon pesticides, such as DDT, dieldrin, heptachlor and HCH (hexachlorocyclohexane). More recently attention has been focused on PCBs and on chlorinated dibenzodioxins. Likely candidates for future attention are hexachlorobenzene, chlorinated dibenzofurans, and chlorinated phenols. In the past, such compounds have generally been studied individually. As the number of compounds giving rise to concern increases, there is an urgent need to establish uniform monitoring schemes and generalized models describing environmental transport and bioaccumulation which will be applicable to all compounds with these properties. Models such as the global monitoring scheme outlined by the SCEP study (1), and the global transport model outlined recently by Woodwell et al. (2), are needed in order to help identify sources of environmental contamination, to establish acceptable levels of discharge, and to estimate the effectiveness of different control strategies.

This paper summarizes the fragmentary knowledge available about production, uses, and losses of PCBs, and attempts to define the major routes of transport and reservoirs of PCBs in the environment. It discusses only production and uses within North America, and distribution of PCB residues in North America and adjacent seas (the eastern half of the North Pacific Ocean and the western half of the North Atlantic). It is expected that the picture will be generally similar for other areas where PCBs have been used extensively (i.e., industrialized regions, primarily in the North Temperate Zone), with minor differences depending on local patterns of use and disposal. However, references to studies made in Europe and Japan are made in this paper where they help to fill gaps in the North American picture.

Although we attempt to cover all the important environmental aspects of the use and distribution of PCBs, much of the discussion in this paper is extremely speculative. One of its main purposes is to point out the most important gaps in knowledge which must be filled before firm estimates can be made of present and future levels in the environment.

Production and Use in North America

The sole manufacturer of PCBs in North America is the Monsanto Company. Commercial mixtures are sold under the trade name Aroclor and are distinguished by numbers, in which the first two digits 12 specify polychlorinated biphenyls and the last two digits the approximate percentage of chlorine in the mixture (3). In November, 1971, Monsanto released figures for U.S. domestic sales of Aroclors during the period 1963–70, summarized here in Figs. 1 and 2, where they are divided according to category of use and grade of Aroclor.

Prior to Monsanto's voluntary reduction of sales in September, 1970, approximately 60% of

* Research Department, Massachusetts Audubon Society, Lincoln, Massachusetts 01773.

† Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139.

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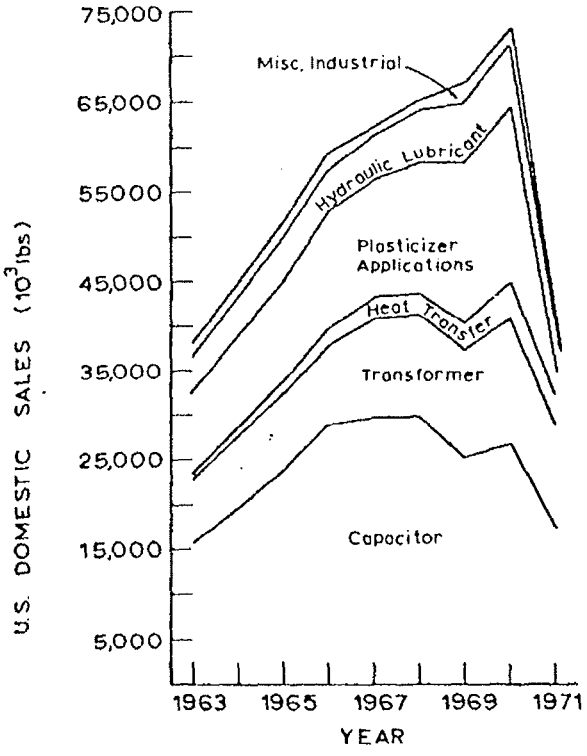

FIGURE 1. U.S. domestic sales of PCBs by category.

sales were for closed-system electrical and heat transfer uses, 25% for plasticizer applications, 10% for hydraulic fluids and lubricants, and less than 5% for miscellaneous applications such as surface coatings, adhesives, printing inks, and pesticide extenders. Exports by Monsanto have averaged 13% of domestic sales over the period 1963 to 1970. Imports are thought to be small, comprised primarily of plasticizers in resins and adhesives, transformer oils and capacitor fluid in electrical equipment. Exports to Canada should be of the order of 7% of U.S. sales and are included in this model of North America.

Monsanto has restricted sales of Aroclors based on consideration of either the possibility of contamination of food products or its inability to control or monitor possible losses into the environment. The fraction of sales for use in confined systems, primarily electrical applications, will increase to approximately 90 percent in 1971 and to 100% in 1972. The higher chlorinated Aroclors, 1254 and above, comprised approximately 30% of sales prior to 1971. Their share of the reduced sales in 1971 is expected to fall to 22%. Aroclor 1242 is being replaced with Aroclor 1016 having a similar composition but with isomers containing 5 or more chlorine atoms removed.

The breakdown by grade of the current uses of PCB is: electrical capacitors, mainly Aroclor 1016 with limited usage of Aroclor 1221 and Aroclor 1254; electrical transformers, Aroclors 1242, 1254 and 1260; vacuum pumps, Aroclor 1248 and 1254; and gas transmission turbines, Aroclor 1221 and 1242. The breakdown by PCB grade of former uses include Aroclors 1232, 1242, 1248, 1254, and 1260 for hydraulic fluids; Aroclor 1242 for heat transfer systems; Aroclors 1248, 1254, 1260, 1262, and 1268 for plasticizers in synthetic resins; Aroclors 1221, 1232, 1242, 1248 and 1254 for adhesives; Aroclors 1221, 1232, 1242, 1248, 1254 and 1268 for plasticizers in rubbers; Aroclors 1242, 1254 and 1268 for wax extenders; Aroclors 1254 and 1260 for dedusting agents; Aroclor 1254 for pesticide extenders, inks, lubricants, and cutting oils; and Aroclor 1242 for carbonless reproducing paper.

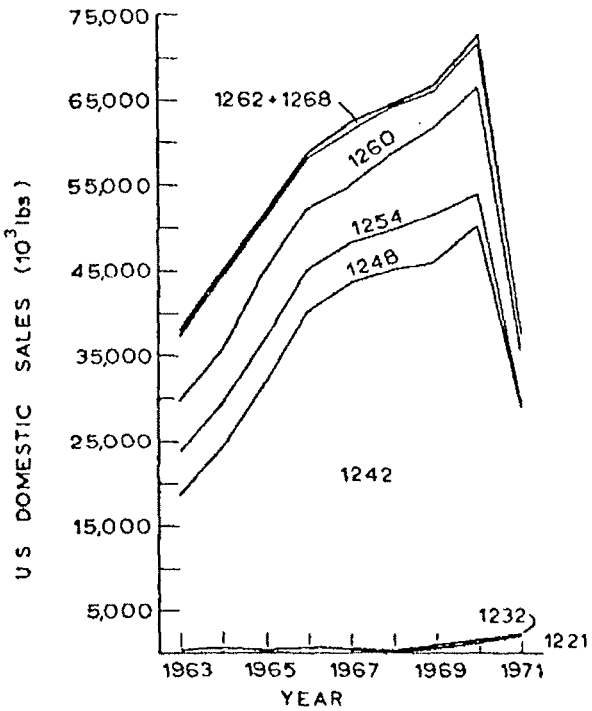

FIGURE 2. U.S. domestic sales by PCB grade.

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Data on production outside the U.S. are not available. It has been estimated (4) that the Japanese production is 26 million pounds per year, 40–50% for capacitors, 15% for transformer oil, 10–15% for heat transfer fluid, 5% for plasticizers, 15% for carbonless duplicating paper, and 5—10% for export.

Routes into the Environment

As in the case of most industrial chemicals, loss figures for PCBs are practically non-existent. Possible routes into the environment include: (1) leaks from sealed transformers and heat exchangers; (2) leaks of PCB-containing fluids from hydraulic systems which are only partially sealed; (3) spills and losses in the manufacturing either of PCBs or PCB-containing fluids; (4) vaporization or leaching from PCB-containing formulations; (5) disposal of waste PCBs or PCB-containing fluids.

Examples of losses by each of these proposed routes include leaks from faulty heat exchangers in the contaminated Japanese rice oil (5) and contaminated chicken feed (Holly Farms) incidents; leakage of hydraulic fluid from an air compressor in Escambia Bay, Florida (6); the indirect evidence of possible losses during manufacture provided by the high level of PCBs in catfish in waters near Anniston, one of the two sites at which PCBs are manufactured in the U.S. (7); the leaching of PCBs from silos by sileage (7) and the use of waste electrical insulator containing PCBs as solvent in herbicide treatment of power rights-of-way near Martinsburgh, West Virginia (7). Other unreported disposal of scrap undoubtedly occurs into sewers (8). Statistical information on such losses is not available but could be generated by an accounting of PCB inventories and disposal by major users. To meet the problem of scrap disposal, Monsanto has set up a disposal system with a capacity of 10 million pounds per year for their customers. Within a year of announcement of the service, 500,000 pounds of waste PCBs had accumulated at the disposal site, where it was held in storage, pending the completion of an incinerator (9).

Another waste disposal problem is that of PCB-containing products including the PCB-impregnated paper in capacitors, caulking compounds, and carbonless duplicating paper. It has been postulated that the PCBs may be vaporized during incineration. Based on studies into the incineration of PCBs and pesticide residues, municipal incinerators meeting design guidelines of a residence time of 2 seconds at 2000°F are expected not to be a significant source of PCBs (9, 10). Poorly operated commercial and municipal incinerators, small domestic and apartment incinerators, and open-burning dumps may, however, be major sources of emission, but data are not presently available. Another problem is that of leaching from dumps. Again, data are scarce, but analysis (11) of stagnant water close to a sanitary landfill indicated levels below the detection limit of 4 ppb.

Quantitative Estimates of Rates of Loss into the Environment

It is unlikely that reliable quantitative estimates of past losses of PCBs into the environment will ever become available. However, in order to assess the biological significance of the PCBs now in the environment, it is necessary to make some numerical estimates, however rough. The following calculations are intended to provide order-of-magnitude estimates of rates of loss. Figure 3 shows qualitatively some of the many possible routes of loss into the environment.

Estimates of the rates of loss and disposal of Aroclors in 1970 will be based on rough guesses of the useful service life in different applications. Transformers are fairly permanent installations, and it is therefore estimated that only 10% of sales of transformer fluid is to replace oil that was scrapped and that the remaining 90% is for new units. The useful life of capacitors, especially those used in small items such as fluorescent light ballasts, is expected to be under a decade, and it is therefore estimated that the rate at which the capacitors are discarded, primarily into landfill dumps, is equal to half the rate of production. (Figure 1 indicates a doubling period for sales of about 10 years.) A similar figure is assumed for the scrapping of heat exchangers, but it is expected that the replacement of fluid decomposed under extreme thermal conditions would have led, in the past, to additional direct losses, primarily into sewers. It is estimated that the rate of vaporization of plasticizer amounts to 10 to 20% of

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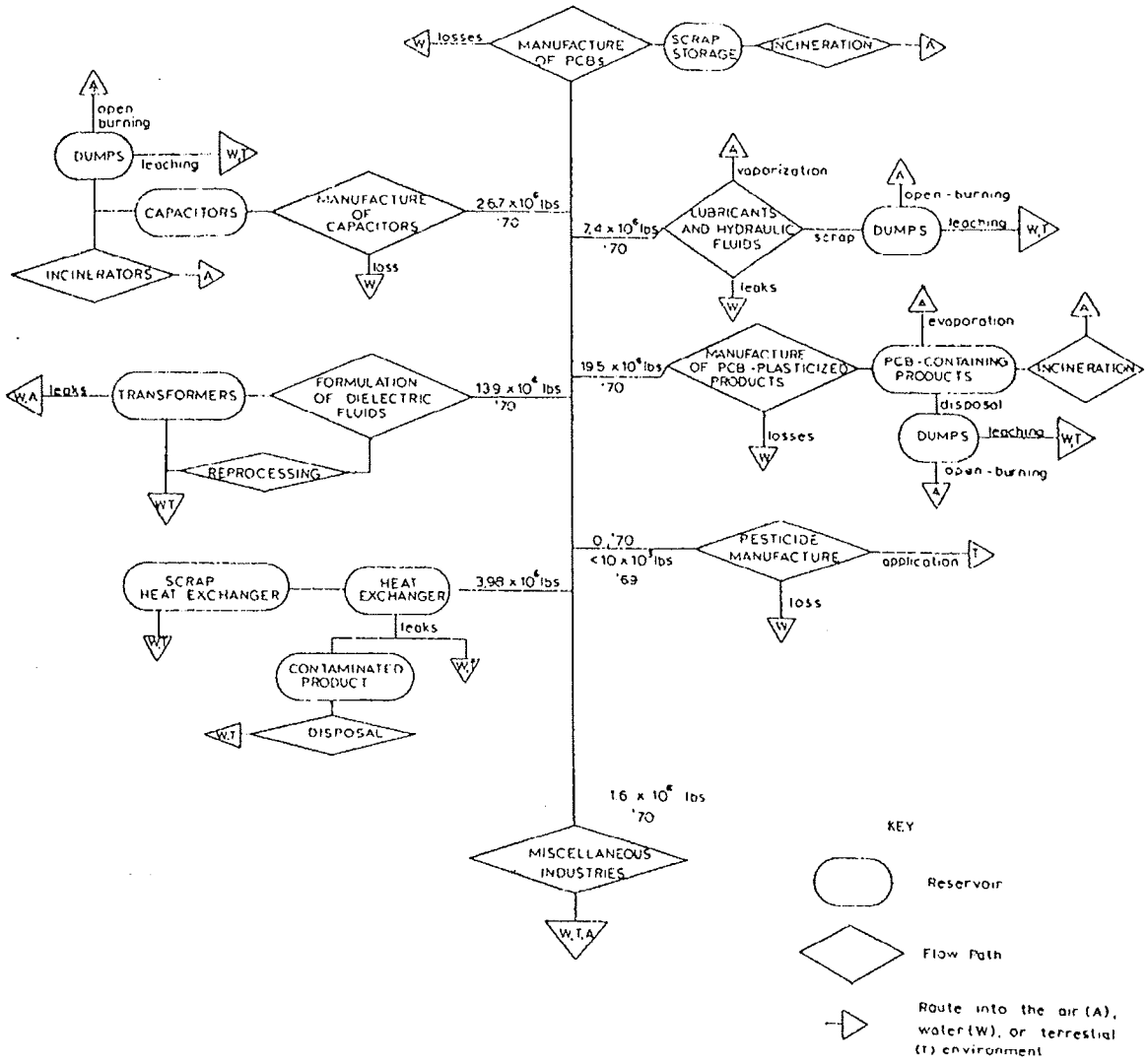

FIGURE 3. Possible routes of PCBs into the environment.

sales (12). Since many plastic objects have relatively short useful lives the rate of disposal of plasticizers into dumps is assumed to equal the residual 80 to 90% of sales. Hydraulic fluids and lubricants are rarely re-used, and it is therefore assumed that a major fraction of these fluids used for this purpose, together with those going into the miscellaneous applications, were scrapped at rates approximately equal to those of corresponding sales.

On the basis of these gross estimates, it appears that only about 20% of the 1970 sales in North America, some 7×10^3 tons, represented a net increase in the amount of PCBs in service, in transformers, heat exchangers and capacitors. The remainder is assumed to have been discharged into the environment, $1\text{–}2\times10^3$ tons by evaporation of plasticizers; $4\text{–}5\times10^3$ tons by leaks and disposal of hydraulic fluid and lubricant plus small amounts by disposal of heat transfer and trans-

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Table 1. Gross Estimates of Rates of Input and Accumulation of PCBs in North America in 1970.

Category of input	Rates (tons/year)	PCB Grade
Vaporization of plasticizers	$1\text{–}2\times10^3$	Mainly 1248 to 1260
Vaporization during open-burning	4×10^3	Mainly 1242
Leaks and disposal of industrial fluids	$4\text{–}5\times10^3$	1242 to 1260
Destroyed by incineration and open burning	3×10^3	Mainly 1242
Disposal in dumps and landfills	1.8×10^4	1242–1260
Accumulation in service	7×10^3	1242–1254

Reservoir	Accumulation (tons)	
	PCBs	ΣDDT
Soil (excluding dumps)	1.5×10^4	3×10^4
Oceans (adjacent to North America)	1.5×10^4	10^4
Fresh water (dissolved or in suspension)	10^3	$\sim10^3$
Fresh water sediment	2×10^4	?
Biota	$<10^3$	$<10^3$

former oils, and 22×10^3 tons by disposal in incinerators, dumps and sanitary landfills. Of the latter, we estimate (13) that 10 to 20% (3×10^3 tons) were destroyed by burning, and 2% (4×10^2 tons) were vaporized, mainly by open burning of wire scrap, auto components, and material in dumps.

Routes into the Environment

The total rate of loss of PCBs is thus estimated to have been of the order of 1.5 to 2×10^3 tons/year into the atmosphere, 4 to 5×10^3 tons/year into fresh and coastal waters, and 1.8×10^4 tons/year into dumps and landfills. The input into soils via the use of Aroclors as pesticide extenders is believed to have been small, less than 10 tons/year, on the basis of reports of purchases for this purpose. The unauthorized and unrecorded use of scrap PCBs as pesticide extenders is difficult to estimate but has probably been small. Direct discharge into the oceans, e.g. by dumping of hydraulic fluids and lubricants from ships, has probably been of relatively small magnitude, but may have been locally significant (49).

Most of the PCBs discharged into the atmosphere will have been Aroclor 1248 to 1260 vaporized from plastic resins, augmented primarily by 1242 vaporized from burning dumps. The discharge into waters will be heavily weighted by the Aroclors used as hydraulic fluids and lubricants and is therefore likely to have included a mixture of Aroclors 1242 to 1260. The residual in dumps will have a large fraction of the Aroclor 1242 production.

Rates of Transport within the Environment

The modes of transport of the PCBs within the environment are complex. Vaporized PCBs will be partially adsorbed on particulates, transported with the prevailing winds, and deposited on land or water by particle sedimentation or rain-out. PCBs introduced into water streams may be adsorbed by the waterborne particulates or the benthos; the adsorbed PCBs will diffuse into the bottom sediment, redissolve in the water stream or be entrained with sediment eroded from the bottom surface.

The problem is further complicated by the assimilation, transport and degradation of PCBs by the biota. A major fraction of the PCBs discarded in dumps is encapsulated in sealed containers or plastic resins. The rate of loss from these will therefore be low until the confining material is degraded and the PCBs released. The PCBs will then slowly diffuse through the surrounding soil. In principle, the rates of transport may be calculated from knowledge of the physicochemical properties of the PCBs and the pertinent data on atmospheric conditions, particulate transport, hydraulic dispersion, bottom sediment transport, and biological degradation rates. At present, the data are too incomplete and the interactions between the different elements in the environment too complex to attempt a formulation of but the crudest of transport models. The similarity between the properties of the PCBs and DDT, summarized in Table 2, permits rough estimation of some of the otherwise unknown routes and rates of transport of PCBs from the corresponding information on DDT. In Fig. 4, a generalized model for the distribution and transport of the PCBs is outlined, without any indication of the transport down spatial gradients within the differ-

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Table 2. Comparative Physical Properties of PCBs and DDT (Ref. 9, 23, 84, 85).

Molecular weight		Aroclor 1242	Aroclor 1248	Aroclor 1254	Aroclor 1260	DDT
	Range[a]	154–358	222–358	290–392	324–460	352
	Average	262	288	324	370	352
% Chlorine		42	48	54	60	50
Solubility[b] in H_2O (at 20°C ppb)		200	100	50	25 Est.	0.7
Vapor pressure[b] at 38°C (mm, Hg)		10^{-3}	3.7×10^{-4}	6×10^{-5}	2×10^{-7}	2×10^{-5c}
Vapor pressure at 20°C (mm. Hg)		10^{-4c}	3×10^{-5c}	3.6×10^{-6c}		1.5×10^{-7}

[a] Based on constituents present in amounts of 1 percent or more.
[b] The high values of the solubilities and vapor pressures of the PCBs are a consequence of the heavy weighting given the lower chlorinated species.
[c] Extrapolated.

ent compartments of the environment. An attempt is made in the following sections to estimate fluxes between and within each compartment.

Air Transport

The estimated emission of 1500 to 2500 tons a year of PCBs, mainly by vaporization and open burning, is expected to be concentrated in urban areas. By analogy to DDT (17, 18) it is expected that most of the airborne PCBs will be adsorbed on particles. Since roughly three quarters of total suspended particulates (TSP) in urban areas are of non-agricultural origin (19), and the emission of TSP within cities is of the order of 2×10^7 tons per year (20), this gives rise to a rough estimate of PCB level on urban particulates of 50 to 80 ppm.

The half-life of particulates in the air will depend greatly on the size of particles to which the PCBs are attached and the extent of atmospheric precipitation. (19) It is estimated that most of the vaporized PCBs will be deposited within 2 or 3 days, mostly onto the land mass and coastal waters surrounding urban areas. The small amounts of PCB attached to fine particulates will reside in the atmosphere for extended periods and be transported to remote areas.

Based on the above assumptions, the rate of terrestrial net input of PCBs by serial fallout in North America will be a little less than the rate of vaporization, about 1000 to 2000 tons/year. The vapor pressure of Aroclor 1254, the dominant airborne contaminant, is close to that of DDE (21), and it is estimated, by analogy to DDE (17) that the likely half-life of PCBs in soil is of the order of five years. The PCBs removed from the soil

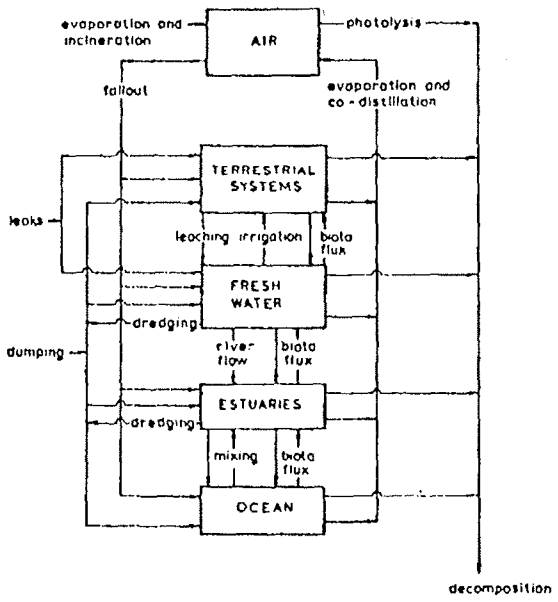

FIGURE 4. Environmental transport model.

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will become adsorbed to particulates, mostly of relatively short range, and be redeposited and vaporized one or more times until they reach the coast. By analogy to DDE (1, 2), the redistribution of PCBs by this mechanism is expected to have resulted in the redeposition into the ocean of one quarter of the PCBs that originally fall out in the terrestrial environment. The deposition pattern will be influenced by the size distribution of particles to which the PCBs are attached, with a major fraction ending in coastal waters and a small amount being transported into remote regions.

It is expected that a negligible fraction of the PCBs reaching the oceans will be revaporized; their concentration will be too low.

Water Transport

On account of the low water solubility and high specific gravity of PCBs, it is expected that most of the PCBs discharged into the environment will be resting as sludges or adsorbed in the sediment at the bottom of rivers or lakes near their point of discharge, and that transport in streams will be primarily by means of waterborne particles. Evidence for this postulate is indirect, consisting mainly of the strong adsorption of PCBs by surfaces (22), the observation of PCB concentrations as high as five times the solubility limit (6), and the evidence that chlorinated hydrocarbon pesticides are removed from contaminated lakes by adsorption in sediments and codeposition with algal bloom (24).

The transport of PCBs within rivers is as described previously, by solution and readsorption in the sediment and by sediment transport. The data on partition coefficient between sediments and water and on diffusivity in bottom sediment are not available so that the transport models (25) developed for rivers cannot yet be applied. The only measurements reported on PCBs in ground sediment are for Escambia Bay (6, 26). The PCB concentration in the sediment 10 miles downstream from a discontinued source of PCB loss was found to remain constant over a year suggesting that the PCBs are strongly adsorbed to sediment.

The amount of PCBs transported to the ocean in solution or suspension in river water is estimated as 200 tons/year (49), a small amount compared to the estimated input into rivers. Irrigation waters amount to about $\frac{1}{15}$ of the total river flow and are expected to carry a correspondingly lower amount, about 15 tons/year, of PCBs to the terrestrial environment.

Dredging and Dumping

Dredging of inland rivers and harbors may lead to significant transfer of PCBs from contaminated sediments, especially when the dredge spoils are dumped at sea. According to the Council on Environmental Quality (27), some 13×10^6 tons of polluted dredged spoils are dumped at sea annually off the United States, 90% off the Gulf and Atlantic coasts. A high figure for PCB levels in polluted sediments is 30 ppm, the highest level recorded in the Escambia Bay incident away from the immediate vicinity of the discharge (26). If all the polluted dredge spoils were contaminated to this level, the total rate of transfer of PCBs to the oceans would be around 400 tons/year, mostly into the Atlantic Ocean and Gulf of Mexico. Based on the general level of contamination a more reasonable estimate of rate of transfer would be only 20 tons/year.

Biota Flux

The total quantity of PCBs stored in migratory animals is very small (see below), and their movements cannot account for a significant fraction of environmental transfer of PCBs. The movements with the greatest local significance are probably the migrations of seabirds from the northern to the southern hemisphere; these might account for the movement of about a ton/year (28). Human fishing is estimated to remove only 1–2 tons/year from the eastern North Pacific, the western North Atlantic and the Great Lakes (30).

Separation and Transformation of PCB Isomers in the Environment

There are three processes by which the compositions of PCB mixtures may change after release into the environment.

Fractionation

In general, the water solubilities and vapor pressures of PCB isomers decrease with increasing

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Table 3. Percent Loss in Area of Seven Chromatogram Peaks of Aroclor 1254 After Steam Heating for 25 and 60 Minutes. [From Ref. 23]

Peak	25 Minutes	60 Minutes
1	66	83
2	41	74
3	22	73
4	40	54
5	14	51
6	0	15
7	0	33

chlorine content, although the decreases are not uniform (Table 2) (31). Hence the processes of evaporation, co-distillation and dissolution in water are expected to fractionate mixtures of PCBs, the lower isomers being much more mobile. This has been confirmed for co-distillation by the results of Freed (21, 23) (Table 3), which show that, after 60-min steam heating, the ratio of the first to the sixth of the seven major chromatographic fractions of Aroclor 1254 was reduced by a factor of 5. Thus environmental transport mechanisms involving these processes are expected to reduce the proportions of the lower isomers in environmental samples near to the point of release, and to augment them in samples from remoter areas.

Photolysis and Chemical Decomposition

Photolytic decomposition of certain PCB isomers has been reported by Risebrough et al. (32, 33), and Safe and Hutzinger (34, 35). The results of the latter suggest that in natural sunlight some higher isomers are more easily broken down than the lower isomers (35). Hence photolysis is expected to reduce the proportions of at least some of the higher isomers. Photolytic dechlorination is also expected to give rise to lower isomers, including some which may not be present in commercial mixtures (35).

Because of their high stability in industrial use, we assume that other forms of chemical (non-biological) decomposition of PCBs are very slow in the environment.

Metabolism and Excretion

Published studies of the dynamics of PCBs in rats and birds (36–39) indicate that the higher isomers are generally taken up and/or retained more efficiently. In each case the proportions of pentachloro- and lower isomers found in the animals' tissues were generally lower than those in the Aroclor mixture to which they had been exposed, but there appears to have been little differentiation of the higher isomers except for one hexachloro-isomer (36–38). It is not clear in each case whether the deficiencies of the lower isomers resulted from metabolism or differential excretion, but it is likely that both occurred. Because of their higher solubility in water (see above), the lower isomers would be expected to be excreted more easily than the higher isomers. In one study on rats, loss of PCBs from the body was markedly retarded by administration of carbon tetrachloride (36), suggesting interference with metabolism in the liver. In another study with rats, hexachloro-isomers were found in disproportionate amounts in the urine (37), suggesting that the tetra- and pentachloro-isomers were metabolized and the hexachloro-isomers excreted in part, while the heptachloro-isomers were differentially retained. Two studies of metabolism of PCBs in fish (40, 41) gave inconsistent results but showed no marked tendency for the higher isomers to accumulate differentially.

Evidence from Environmental Samples

Veith (42) has found that the proportion of higher isomers increases downstream in some Wisconsin rivers and that certain isomers characteristic of lower Aroclors disappear. Since the lower isomers are unlikely to be differentially retained in sediments (see above), this suggests that they are decomposed rapidly in the river environment. Evidence of biodegradation of pesticides under anaerobic conditions (43) suggests the possibility that the degradation of the missing lower isomers may occur primarily by microbial metabolism in the bottom sediment. We recommend that higher priority be given to this aspect of decomposition of PCBs.

A number of writers have reported that PCB samples extracted from animals generally match Aroclors 1254 or 1260, but that the lowest isomers are sometimes relatively deficient or missing, especially in animals high in food chains (38, 44). Since substantial quantities of Aroclors 1242 and

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Table 4. Relative Peak Heights in Gas-Liquid Chromatograms of Extracts from Sewage Sludges. [From Ref. 45]

Relative retention time	Sludge A	Aroclor 1254	Sludge B	Aroclor 1260
0.69	38	30	39	3
0.81	63	72	24	14
1.00	51	59	17	4
1.21	104	110	51	30
1.45	100	100	100	100
1.71	92	92	66	69
2.00	32	26	70	70
2.34	19	12	53	55
2.83	21	19	84	87
3.33	10	8	29	30
3.70	5	4	25	23

1248 must have been released into the environment (see above), these observations suggest that a large proportion of the lower isomers (those with 4 or fewer chlorine atoms) is missing from the animal samples. However, few critical data have been published. In one set of data from sewage sludge (45) (Table 4), none of the lower isomers is clearly deficient. In published chromatograms for birds (38, 46, 47) (Table 5), only the lowest isomers (tetrachlorobiphenyls) and one higher isomer were markedly reduced in comparison to Aroclor 1254. In human adipose tissue (48), however, pentachloro- and hexachloro-isomers were reduced or missing.

The higher isomer reduced in the bird samples (38, 46) is probably that reported as deficient in samples from the Gulf of California and Quebec and found to be degraded by ultraviolet light (32, 33). With this one exception, we conclude tentatively that higher isomers (pentachlorobiphenyls and higher) are not significantly differentiated as they pass through food chains up to fish and birds. Mammals appear to be able to excrete and/or metabolize penta- and hexachloro-isomers.

The lack of the lower isomers in samples from relatively contaminated areas could be accounted for either by metabolism or by their greater mobility. However, Risebrough and Berger (33) found that lower isomers were relatively deficient also in samples from fish in a remote lake in northern Canada. This suggests that differential metabolism is the primary mechanism in the environmental differentiation of isomers, outweighing the effects of differential photolysis and differential mobility, which would be expected to have reduced the proportion of higher isomers in remote samples.

Conclusions and Comments on Separation of PCB Isomers in the Environment

We conclude that most PCB isomers with four or fewer chlorine atoms have been degraded in the environment, possibly by microbial action. Decomposition of penta- and hexachloro-isomers appears to occur in birds and mammals, but this will have affected only a negligible fraction of the PCBs in the environment. Accordingly, we assume (3) that some 75% of the Aroclor 1242 released into the environment, 60% of Aroclor 1248, 20% of Aroclor 1254 and 5% of Aroclor 1260, have disappeared.

The above discussion has been confined to a general comparison of lower and higher isomers, because of the scarcity of data on relative proportions of individual isomers in environmental samples. In fact, the physical and chemical properties and biodegradability of PCB isomers depend on the positions of substitution as well as the total chlorine content. More precise data on the

Table 5. Relative Peak Areas (Peak 11 = 100) for Chromatogram of Extract of Eagle Carcass and Aroclor 1254. [From Ref. 47]

Peak No.	Relative retention rime[a]	Eagle carcass	Aroclor 1254
1	0.39	7.5	6.8
2	0.49	34.8	100.0
3	0.53	55.0	42.3
4	0.58	15.7	21.0
5	0.70	68.6	191.5
6	0.79	28.0	32.2
7	0.83	111.9	127.5
8	0.97	68.7	66.5
9	1.04	91.6	105.2
10	1.23	92.5	139.5
11	1.46	100.0	100.0
12	1.72	62.2	75.5
13	2.01	28.7	23.6
14	2.32	18.4	42.6
15	2.86	32.6	38.8
16	3.38	15.4	22.2

[a] p,p'-DDE = 1.00

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exact quantities of individual isomers in environmental samples would permit more precise conclusions about the sources and routes of contamination. We urge that numerical characterization of individual peaks should be published whenever possible.

Cumulative Input into the Environment

From an extrapolation of the sales curve in Fig. 1 to zero production in 1930, it is estimated that the cumulative sales in North America over the period 1930–1970 were 5×10^5 tons. Assuming that the proportions of sales for different uses were similar throughout the period, the cumulative losses may be estimated as about 3×10^4 tons into the air, 6×10^4 tons into fresh and coastal waters, and 3×10^5 tons into dumps and landfills. Using the results of the previous section, it may be estimated that roughly one-third of the PCBs released into the air and one-half of those released into water have now been degraded. It is difficult to estimate the extent of degradation in dumps, because some of the PCBs there may still be in sealed containers, but we assume in any case that leaching from dumps into fresh waters has so far been negligible.

Of the PCBs released into the air, we have assumed that most would have been adsorbed onto particulates soon after volatilization and would therefore have fallen out relatively close to the source. By analogy with DDT residues, PCBs in soil would be expected to have a half-life of the order of 5 years (2, 17). Again using the analogy with DDT and its metabolites, we may estimate that roughly one-quarter (1) of the total (i.e., 5×10^3 tons) will have been transferred into the sea, mainly into the Atlantic Ocean, and the remainder (1.5×10^4 tons) distributed over terrestrial North America, with a negligible fraction having fallen into fresh waters. Other inputs to the sea are difficult to estimate but have probably been of the order of 10^4 tons (49). The remainder (2×10^4 tons) is assumed to have accumulated in lakes and rivers.

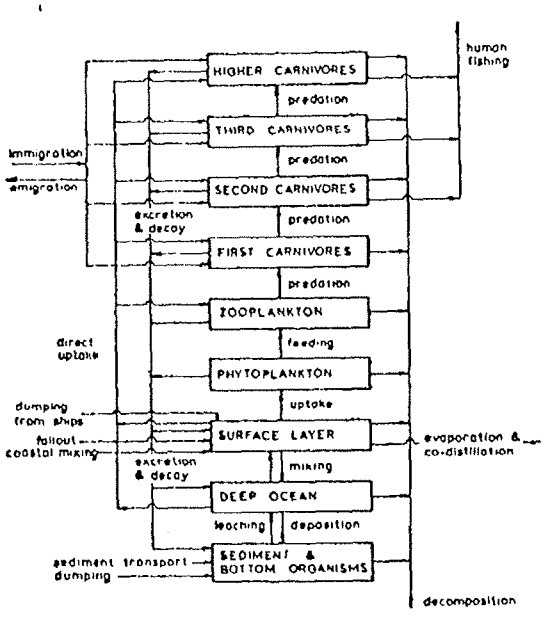

FIGURE 5. Ocean sub-model.

Table 6. Range of concentrations of PCBs in samples from the Irish Sea and Firth of Clyde, Scotland October 1969 and subsequently [Ref. 58]. All figures are in ppm except those for seawater.

	Sample size	PCB	ΣDDT[a]
Seawater	53	<10 ppt	<1–5 ppt
Zooplankton	7	0.01–0.03	0.01
Mussels	c.200	0.05–0.5	0.01
Norway lobster	33	0.01–0.1	0.01–0.1
Herring	154	0.01–2.0	0.01–1.0
Whiting: muscle	18	0.01–0.4	0.01
liver	15	1.0–7.0	1.0–2.0
Cod: muscle	5	0.3–1.8	0.4–0.52
liver	5	4.5–50.	2.3–12.
Other fish	83	0.1–1.0	0.04–1.0
Razorbill livers	7	2.–44.	5.–13.
Guillemot livers:			
birds found dead	49	2.–880.	1.7–26.
birds shot	5	0–2.	0.1–0.8

[a] These figures, as given in the original source, include dieldrin as well as DDT and its metabolites, but dieldrin comprised only 4% of the total in the case of the guillemots.

PCB Levels and Σ DDT/PCB Ratios in the Environment

In this section we compare the above estimates of cumulative input into the environment with measurements of PCB levels in physical and biological samples. The purposes of this comparison are: (1) to use the observed distribution of PCBs in the environment to check the consistency of our

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Table 7. PCB levels in Swedish marine organisms 1965–68 [From Ref. 44]. Figures given are the mean (ppm in extractable fat), with range and sample size in parentheses; ΣDDT is the total of DDT and its metabolites.

	Baltic Sea				Stockholm Archipelago			
	Mean	Range	N	ΣDDT/PCB	Mean	Range	N	ΣDDT/PCB
Mussel	4.3	(1.9–8.6)	(40)	1.4	5.2	(3.4–7.0)	(15)	0.6
Herring	6.8	(0.5–23)	(18)	2.5	5.1	(3.3–8.5)	(4)	1.5
Seal	35.	(16–44)	(3)	3.4	30.	(16–56)	(3)	5.7
Guillemot eggs	250.	(140–360)	(9)	2.3		—		
Heron					9400.		(1)	1.5
White-tailed Eagle:								
breast muscle					14000.	(8400–17000)	(4)	1.8
brain					910.	(490–1500)	(3)	2.1
eggs					540.	(250–800)	(5)	1.9

models of environmental transport; (2) to define the types and locations of input to the environment which are responsible for the most serious contamination of the biota.

Uptake and Biological Magnification of PCBs in Food Chains

A large fraction of the reported measurements of PCB concentrations in environmental samples have been from animals, especially fish and birds high in marine food chains. Figure 5 outlines schematically a model for the transport of PCBs within a simple marine ecosystem. Laboratory experiments (6, 41, 55) indicate that aquatic invertebrates and fish can accumulate PCBs to levels between 3×10^3 and 7×10^4 times higher than those in the ambient water. It is not yet clear whether concentrations of PCBs are consistently increased as they pass up food chains from invertebrates to fish and from fish to fish (57). However, PCB levels are clearly magnified within food chains involving birds and mammals by a factor on the order of 10 to 100 at each step (32, 44, 56, 57, 58). Hence, in the long food chains characteristic of marine systems (59), the levels in the top predators may be 10^7 times higher than those in the ambient water (Tables 6 and 7). In extreme cases, such as the starved guillemots in Table 6, or the eagle fat in Table 7, the concentration factors may be as high as 10^8 or 10^9. Because of the extreme variability observed both within and between samples, it is difficult to use measured levels in animals as a direct measure of levels in the environment. However, it is possible to compare levels in the same species or in ecologically equivalent species to define spatial gradients in contamination (29, 44).

ΣDDT/PCB Ratios

Another approach (32, 56) utilizes the similarity in properties between the persistent PCBs and the persistent metabolites of DDT, primarily DDE. (In the remainder of this section, DDT and its metabolites DDE and DDD are grouped together as ΣDDT). Experiments suggest that PCBs and ΣDDT are accumulated by aquatic animals to an extremely similar degree from ambient water (41), and measurements in the same areas indicate that the ratios ΣDDT/PCB are very similar in animals from different levels in the food chain (Tables 6 and 7) (32, 56, 75). Hence the ratio ΣDDT/PCB in environmental samples, even in animals at the top of food chains, can be used to infer the ratio in the substrate, at least to order of magnitude (32, 56). Then the extensive information about the environmental distribution and transport of ΣDDT (2, 17) can be used to make inferences about the distribution and transport of PCBs.

Air

The only numerical measurements available of PCB levels in air are some estimates of levels in total suspended particulates (TSP) in four U.S. cities between 1968 and 1970 (E.P.A., unpublished data). The mean level of PCBs was about 50 ppm, with levels in one city substantially

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higher. This is at least consistent with our earlier estimate based on rates of transport in the environment.

Risebrough et al. (60) were unable to detect PCBs in a sample of marine air off the California coast. This suggests that long-range (inter-continental) transport of PCBs in air may be negligible.

Land

We have estimated the cumulative net input into terrestrial North America as of the order of 1.5×10^4 tons. For comparison, the total quantity of ΣDDT remaining in terrestrial North America is of the order of 3×10^5 tons, as estimated by two methods: total application minus estimated total losses to the sea (1); mean levels of 2–3 ppm in a wide variety of treated soils, and lesser amounts in untreated soils (2, 17, 61). Hence the continental mean ΣDDT/PCB ratio should be of the order of 20. However, PCBs are expected to be distributed primarily around urban areas and downwind from them, whereas ΣDDT is still concentrated in the agricultural and forested areas where it was applied (61).

A nation-wide monitoring scheme using starlings (*Sturnus vulgaris*) and woodcocks (*Philohela minor*) is in progress. Early results show mean PCB levels in pooled samples of fat from woodcock wings from eastern states in the range 4–7 ppm (62). ΣDDT levels from these pools are not yet available, but fat from woodcocks in Canada contains 6–130 ppm, depending on the local history of application (29). Woodcocks feed on earthworms and should be a good indicator for soil concentrations.

Other data from terrestrial birds may be biased towards low ΣDDT/PCB ratios, because the birds were obtained in industrialized regions. The median ΣDDT/PCB ratio in a small sample of terrestrial birds from California was 4 (32). Terrestrial birds of prey in five European countries have very high levels of PCBs, with ΣDDT/PCB ratios in the range 0.3–5 (63–67).

In human food, market basket surveys in the United States suggest a ΣDDT/PCB ratio of about 4 (68). Ratios in human milk (69, 70) and human blood plasma (71) are in the same range. However, except for sporadic instances of contamination, most of the human intake of PCBs appears to be in fish (68), whereas ΣDDT is much more widely distributed in the diet (72). If the fish and shellfish portions of the diet are excluded, the ΣDDT/PCB ratio would probably be greater than 10. Similar data have been reported from Sweden, where the ΣDDT/PCB ratio in total diet samples and in human milk is about 7 (73).

Fresh Waters

Measurements of PCB levels in water are available only for relatively polluted rivers and bays and are summarized in note (50). Table 8 summarizes PCB levels and ΣDDT/PCB ratios in freshwater fish and birds. PCB levels are both absolutely and relatively high in industrial rivers and in the Great Lakes; within the Great Lakes there is a gradient in ΣDDT/PCB ratios from west to east. Levels of PCBs are lower in the three samples from lakes in less industrialized areas listed at the bottom of Table 8. These data are consistent with our conclusion that most PCBs in fresh water systems result from local discharges by industries, but that there is also widespread aerial fallout of small quantities.

Based on our assumptions of mean concentrations in fresh waters (50), we estimate that the water of the Great Lakes contains a quantity of PCBs of the order of 100 tons; the total quantity

Table 8. PCB Levels and ΣDDT/PCB ratios in North American Fresh Water Vertebrates.

Area	Mean PCB level in fish (ppm)	ΣDDT/PCB ratio in fish and/or fish-eating birds	Reference
U.S.: industrialized rivers	1–213	0.005–0.35	55
Lake Ontario	19	0.08–0.2	55, 74
Lake Michigan	20	0.4–1	55
Lake Huron (Georgian Bay)	—	0.8	75
Lake Superior	—	2.4	75
Ontario: Lake Nipigon	0.1	1–4	75
U.S. & Canada: Prairie Lakes	<0.1	2	76
N. Quebec: Lake Minto	0.1	1	33

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in other lakes would be negligible. The quantity carried in river water would be only of the order of 3 tons. It is difficult to estimate the total quantity in the biota, but since the Great Lakes' fisheries probably remove less than a ton of PCBs per year (30), the total quantity in fish is probably only of the order of 10 tons. These quantities are negligible in comparison to the estimated cumulative input of 2×10^4 tons after allowing for degradation. Hence we conclude that almost all the PCBs that have been released into fresh waters are adsorbed onto bottom sediments. There are no data to indicate the rate at which they are being released into water and into the biota or being biodegraded.

It is expected that PCB levels in ground water will be negligible, since any PCBs in water percolating through the soil should be adsorbed onto soil particles (77).

The Sea

According to our estimates, the total input of PCBs into the seas around North America has been of the order of 1.5×10^4 tons, mostly into the Atlantic Ocean, in part by localized discharge and in part by aerial fallout. For comparison, the total input of ΣDDT into the oceans has been estimated as of the order of 5×10^5 tons (1), of which a disproportionate quantity, probably more than 10^5 tons, would have fallen into the North Atlantic Ocean, because of the heavy use of DDT in North America (17). Thus, on these arguments, the total quantity of ΣDDT in the oceans would exceed that of PCBs by several times, especially in the eastern North Pacific. However, ΣDDT has been introduced over large areas (1, 2), so that it should be more uniformly distributed than the PCBs, the discharges of which are localized near industrialized coasts.

Table 9. PCB Levels and ΣDDT/PCB ratios in Marine Vertebrates.

Area	Mean PCB level in fish (ppm)	ΣDDT/PCB ratio in fish, fish-eating birds and/or mammals	Reference
Long Island Sound	1.2	0.08–0.18	78
Bay of Fundy	0.5	0.4	29, 79, 80
Atlantic Ocean	0.1	0.2–0.5	57
Puget Sound	0.16	1.1	56
San Francisco Bay	0.1–1.2	1–3	32, 56
Californian Coast	0.02–1	5	32, 56
Gulf of California	—	10	32
Gulf of Panama	—	1–2	32
Pacific Ocean (Galapagos & Hawaii)	0.03	>10	56

Available measurements of PCB levels and ΣDDT/PCB ratios, summarized in Table 9, are generally consistent with these predictions, although the pattern is less clear than that in Table 8, in part because of the wide variability between species (cf. Tables 6 and 7). The highest PCB levels and the lowest ΣDDT/PCB ratios have generally been in areas close to industrial activity. At least in the Pacific, PCB levels decrease and ΣDDT/PCB ratios increase with increasing distance offshore. Data on marine birds summarized by Keith and Gruchy (29) show that these offshore gradients exist in both the Atlantic and Pacific, and show further that PCB levels in the Atlantic seabirds are 5–10 times higher than those in the same or ecologically equivalent species in the Pacific. The correlation of high PCB levels with industrial activity has been demonstrated most clearly in Europe, where it has been observed along the coasts of Sweden (65, 81), the Netherlands (38) and Great Britain (82).

The only major discrepancy from this general picture is the observation (57) of extremely low ΣDDT/PCB ratios (of the order of 0.01) in zooplankton over a wide area in the North Atlantic Ocean. ΣDDT/PCB ratios in fish in the same area were of the order of 0.2 to 0.5. It is almost inconceivable that aerial fallout of PCBs could have exceeded that of ΣDDT by such large factors over such a large area; hence to explain the discrepancy, it is necessary to assume either selective removal of ΣDDT or a local source of PCBs. A likely local source is dumping or leakage from ships. PCBs introduced into the ocean in this way would frequently be mixed with oil, would tend to mix with surface slicks (83), and hence would be peculiarly subject to uptake by plankton. Further study is necessary to confirm this suggested explanation or otherwise explain the anomalous low ΣDDT/PCB ratio in the Atlantic Ocean.

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Assuming the standing crop of fish in the eastern North Pacific to be of the order of 10^8 tons, and that in the western North Atlantic to be of the order of 5×10^7 tons (derived from 59), we may estimate the corresponding loads of PCBs to be of the order of 10 and 15 tons respectively. Assuming the standing crop of plankton to be five times larger than that of the fish (1, 2), the corresponding load of PCBs might be of the order of 100 tons. In the case of ΣDDT, it has been estimated that the total load in marine plants may be several times larger than that in marine animals (2); this may be true for PCBs also, but we know of no measurements to support it. In any case it seems unlikely that more than a few $\times10^2$ tons of PCBs are stored in the marine biota of the area considered, a very small fraction of the total quantity introduced. This fraction may be significantly greater than the corresponding fraction for ΣDDT (1), because of the extraordinarily high levels of PCBs in the zooplankton.

We know of no measurements of PCB levels in sea water. However, as in the case of fresh water, it seems likely that most of the PCBs would be attached to sediments or to floating particles. Observations on sediments (6, 26) are in accord with this.

As for ΣDDT, the deep oceans represent an ultimate sink for PCB residues. There is no information from which to estimate the rate of transfer of either to the deep oceans.

Summary and Conclusions

According to the estimates made in this paper, the PCBs released into the North American environment in the past are now concentrated in three major compartments in the environment: (a) buried in landfill dumps (roughly 3×10^5 tons, without allowing for degradation); (b) attached to sediments in rivers and the Great Lakes (roughly 2×10^4 tons); (c) attached to sediments on the continental shelf (roughly 10^4 tons). A further substantial quantity (of the order of 2×10^4 tons) has been widely distributed over the land and sea by aerial fallout and by disposal from ships. All the numerical estimates are expected to be valid to order of magnitude only.

Transfer of PCBs within the environment is expected to take place by the following main routes: (a) volatilization, aerial transport on particulates, and fallout; (b) leaching from dumps; (c) sediment transport in rivers and in the shallow sea; (d) sedimentation in the ocean. Uptake and transport by the biota is probably a quantitatively unimportant route of transfer of PCBs but is of major biological significance. Virtually no evidence is available on the rate of transport by any of these routes, but it is expected that all are very slow. In particular, PCBs in sediments in rivers and lakes are likely to move downstream and augment those in the shallow sea for a long period into the future.

As a result of Monsanto's restrictions on distribution, several inputs into the environment are likely to have been sharply reduced. As existing products containing PCBs are scrapped, the remaining inputs into the North American environment are expected to decline gradually over a period of the order of ten years.

As in the case of DDT and its metabolites, the total quantities of PCBs accumulated by the biota are an extremely small fraction (less than one percent) of those in the environment. This raises the possibility that the dissemination of relatively small fractions of the PCB production into sensitive areas may be primarily responsible for locally high levels of contamination of the biota. Evaluation of the long-term effects of the accumulation of PCBs and of the change in use patterns and production will require the development of environmental transport models more sophisticated than those currently in use, together with the requisite data.

The following aspects of environmental transport of PCBs should have high priority for future research:

1. *Properties and Rates.* Data are needed on the solubilities and vapor pressures of individual isomers; on the partition coefficients between water and sediments, water and fats; on the rate of photochemical decomposition and aerobic and anaerobic biodegradation; diffusion coefficients in sediment.
2. *Routes.* The magnitude of leaching from dumps and aerial transport of PCBs must be established by direct measurements of concentrations in ground waters, airborne

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particulates, and rainfall. Data are needed on the magnitude of industrial losses in the past.

3. *Transport Models.* Data are needed on the transport of PCBs in contaminated sediments in rivers and shallow seas, including the dynamics of uptake from shallow sediment and losses to the ocean floor.
4. *Levels.* Additional data, including quantitative evaluation of the fractionation of isomers, are needed for fresh waters, soils, terrestrial plants and animals.
5. *Bioaccumulation model.* Inasmuch as the period since the first commercial application of PCBs is less than the lifetime of a number of species, including man, and the use and discharge pattern has been variable, there is a need to project the long-range impact of the PCBs on the different elements of the ecosystem.

Emphasis should be on coordinated approaches to sampling and modelling rather than a statistical accumulation of data.

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13. Approximately 190×10^6 tons of municipal waste and an equivalent amount of industrial solid waste are disposed of annually in the U.S.A., mostly in dumps. Some 4.7×10^6 tons of industrial solid waste are dumped at sea (27). Of the remainder, 18×10^6 tons are burned in municipal incinerators, 14×10^6 tons in commercial incinerators, and 14×10^6 tons in open-burning dumps (14). Emission of PCBs from these sources may be gauged indirectly from measurements of the emission of combustible matter and polynuclear aromatics. From measurements reported by A. D.

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Little (15) and Hein and Engdahl (16) it is estimated that 0.25 percent of solid waste is emitted by municipal incinerators and up to 20 percent by open burning. If it is assumed that the fraction of PCBs in the organic matter evolved from these sources is equal to the fraction of PCBs in the solid waste, the rate of emission of PCBs will be of the order of two tons per year from municipal incinerators and 200 tons/year from open burning dumps. Commercial incinerators and burning of automobile components each contribute a total quantity of polynuclear aromatic compounds (a rough gauge of incomplete burning) similar to that emitted by open burning (14). The estimate of 200 tons/year of PCBs from the burning of commercial waste and auto components is based on the relative magnitude of combustible pollutants from these different sources, weighted by the estimate of the fraction of total PCB production disposed of in open dumps. These estimates are highly speculative but are considered sufficient to discount the burning of wastes as the major source of PCB emissions.

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Accumulation of Polychlorinated Biphenyls in Ecosystems

by Robert W. Risebrough and Brock de Lappe*

Chlorinated biphenyl compounds have become ubiquitous components of the global environment. They were first detected in species of Swedish wildlife (1, 2, 3) and were subsequently found in seals from the North Atlantic (4), in a bird of prey in Great Britain (5), in fish and birds from the Netherlands (6), and in both terrestrial and marine species of wildlife in western North America (7). In the Arctic they are present in the polar bears (*Thalarctos maritimus*) of Hudson Bay (8) and in fresh-water fish of tundra lakes that receive no pollutants save those that are present in the fallout from the atmosphere (9). In the Southern Hemisphere, they were found in all bird species analyzed that inhabit waters north of the Antarctic Convergence but could not be detected in a number of samples of wildlife resident in Antarctica south of the Convergence (10). In this paper some of the available residue data are summarized within the framework of a preliminary formulation of a mass balance equation for the global distribution of polychlorinated biphenyls.

Commercial manufacture of PCBs was begun in the United States in 1929 (11), and environmental input may be assumed to have begun at that time. Current levels of PCBs in major sinks, such as the oceans, are evidently a function of many parameters, including the proportion of the total amount of PCBs produced over the past forty years that has so far entered the environment, pathways of dissemination, rates of degradation to other compounds, partition coefficients between water and lipid fractions of organisms and the rates of permanent deposition in the sediments. The determination of accumulation rates in major sinks and the prediction of levels to be expected in the future, given estimates of global production and of environmental input, constitute a challenging problem in environmental science.

A mass balance equation that would predict future levels of PCBs in the sea might profitably be modeled on the approach recently used by Weiss et al. (12) in considering the amount of mercury introduced to the sea as a result of the activities of man. Like PCBs, mercury in its various chemical forms is not essential to organisms but may be accumulated by them. Both groups of compounds move through the environment by water and air transport and are mobilized from pools that exist in a variety of industrial products, in addition to the pool of mercury in the earth's crust. The transfer of mercury from the continents to the oceans in river runoff was estimated to be at most 2.8×10^9 grams per year, considerably less than the estimated rate of exchange between the continents and the atmosphere, between 2.5×10^{10} and 1.5×10^{11} grams per year. The latter figures were obtained from measurements of mercury in rainwater of the 1930's and in uncontaminated glacial ice. The amount of mercury produced by man per year is currently on the order of 10^{10} grams, of which one third was estimated to enter the atmosphere. Other sources of atmospheric mercury include the burning of fossil fuels, cement production and mining operations, but the integrated input resulting from man's activities in industry and agriculture was less than that released into the atmosphere by natural processes. The doubling of the mercury content in dated layers of snow from a Greenland

* Bodega Marine Laboratory, University of California, P.O. Box 247, Bodega Bay, California 94923.

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ice sheet over the past several decades was attributed to an increased disturbance of sedimentary rocks as a result of human disturbance (12).

Such an approach yielded the prediction that the current mercury content of marine organisms is at most no more than double that present in pre-technological times (12).

The only synthetic pollutant for which a comparable mass balance approach has been formulated is the insecticide DDT, together with its derivatives (13). Over the past fifteen years about 6×10^{10} grams per year of DDT have been manufactured in the United States, of which a considerable fraction is exported. The total global production per year was estimated to be in the order of 10^{11} grams (13), of which all may be assumed to be released into the environment. Analyses of DDT concentrations in river waters indicated that only a small fraction of the DDT applied to the land would reach the sea by river transport. The few data available for DDT concentrations in precipitation indicated, however, that as much as one quarter of the global production and use could be transported to the sea through the atmosphere (13, 14). Yet the total amount of DDT compounds estimated to be in pelagic marine organisms was only 10^8 grams. Evidently additional measurements of DDT in marine precipitation and in the water and organisms of the mixed layer are required before the mass balance approach can be used to predict future levels of DDT compounds in the oceans.

The ubiquity of the PCB compounds and their relatively high concentrations in marine organisms of certain areas require that an approach now be made toward the formulation of a mass balance equation in the global environment, as a necessary step in the implementation of a program that would prevent the buildup of unacceptable levels.

At the request of a number of environmental scientists concerned about the possibility that PCB levels would continue to increase in the sea, the Monsanto Company has recently released the total production figures for their Aroclor PCB products from 1960 through 1970, with estimated figures for production in 1971. Total U.S. production of the combined total of all PCB products rose steadily between 1960 and 1970, from approximately 2×10^{10} grams in 1960 to approximately 4×10^{10} grams in 1970. Estimated production figures for 1971, however, show a sharp drop from those of 1970 and are equivalent to 1960 levels. Approximately half of this amount is used in "closed system" applications as electrical insulating liquids. The domestic sales for all other uses, including heat transfer systems, hydraulics and lubricants, plasticizers, etc., rose from approximately 5×10^9 grams in 1960 to about 1.5×10^{10} grams in 1970, but estimated sales in 1971 were far below the 1960 figures. A considerable fraction of PCBs used in the "open system" applications can be expected eventually to enter the environment. U.S. export sales showed approximately no change between 1960 and 1965 and were in the order of 2×10^9 grams per year but have since risen to about 5×10^9 grams (15). Of the other industrialized countries that produce chlorinated biphenyl compounds, notably France, Germany, Italy, Japan, and the Soviet Union, production figures are apparently readily available only from Japan where PCBs are manufactured by two companies, the Kanegafuchi Chemical Industry and the Mitsubishi-Monsanto Chemical Company, with combined production figures on the order of 10^{10} grams per year, somewhat less than the estimated 1971 total U.S. production of 2.0×10^{10} grams. In 1970, 60% of the PCBs produced in Japan were used for insulating fluids of capacitors and transformers, 15–20% for the solvent in carbonless reproducing paper, 10% for heat-transfer media, and 5–10% as plasticizers and for export (16).

The figures made public by Monsanto were for total PCB production only. The chlorine composition of the PCBs detected in marine systems may vary widely between that in waste water outfalls and in the tissues of fish-eating birds (17, 18). It is therefore essential to have production figures for each class of PCBs in order to understand PCB movements into and through marine ecosystems. These have been presented in the previous paper.

Combined U.S. and Japanese figures for 1970 were, therefore, on the order of 5×10^{10} grams. A reasonable estimate for global production figures, including the PCBs manufactured in the Soviet Union and Western Europe, is therefore on the order of 10^{11} grams per year, equivalent to those of DDT.

Few data are yet available that permit calcu-

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lations of the amounts of PCBs transferred to the sea. Holden (19) found that about one ton of PCBs per year was entering the estuary of the Clyde as a component of crude sewage sludge from the Glasgow district. Approximately a ton of PCBs was also entering the estuary of the Thames as a component of sewage sludge from London. Comparable amounts of PCBs were found to be entering the Pacific Ocean from each of several waste water outfalls in California (17). The evidence for atmospheric dissemination of PCBs derives from their virtually universal distribution in the global environment, including resident Arctic species (9) and from the detection of PCB peaks in all samples of rain water obtained over a year at seven widely distributed sites in Great Britain (14). These PCBs were not quantitated. Permanent snow fields provide a historical record of PCB deposition with time. The possibility therefore exists for measuring the total amount of PCBs transferred from the atmosphere to snow fields receiving fallout from the major wind systems.

Measurements of PCB concentrations in the mixed layer of the ocean or in deeper waters have not yet been made. Concentrations in collections of zooplankton from the Atlantic, however, are surprisingly high. Samples from eleven stations over the Continental Shelf and Slope areas east of the northeastern United States contained a median concentration of 57 ppm PCBs, with a range from 2 to 260 ppm, in the extractable lipids, defined as all materials extracted in a Soxhlet apparatus with 2:1 hexane:acetone. Median dry and wet weight concentrations were on the order of 2 and 0.2 ppm respectively. Comparable values were obtained in samples from two stations near Bermuda and from two stations in the South Atlantic in the wind path from the industrialized areas of Brazil. Concentrations of PCBs in two samples from the eastern South Atlantic were lower by an order of magnitude (20). Because of wide differences in the total mass of zooplankton per unit volume of water, the values obtained in analyzing zooplankton samples are an imperfect indication of the local burden of PCBs. Moreover, partition coefficients between PCB concentrations in sea water and in zooplankton might be expected to depend on the nature of the lipids and other nonpolar substances in which PCBs accumulate.

Quantitation procedures for PCBs have not yet been standardized. In many waste water and biological samples, however, the profile of PCB peaks closely matches one of the commercial preparations, and accurate quantitation can then be made using the height of any one of the prominent peaks. The Atlantic zooplankton and fish samples from Long Island Sound (21) had a PCB composition that closely matched that of Aroclor 1254. Other environmental samples, particularly birds (10, 18), frequently have a PCB composition that is intermediate between 54 and 60 per cent chlorine. An arbitrary decision must therefore be made which may affect the values obtained by a factor as much as 2 or more. Most of the PCB values reported in papers from our laboratory have been obtained by comparing the total area of all PCB peaks emerging after p,p'-DDE to the total areas of the same peaks in chromatograms of the Aroclor 1254 standard. These values may be somewhat too high. Samples may contain a lower proportion of the PCB compounds with fewer chlorine atoms than does the 1254 standard. Also, the biphenyl molecules with more chlorine atoms have a greater ability to capture electrons and give, in general, proportionately greater responses in the electron capture detector per molecule of biphenyl. Thus, two eggs of the Common Tern, *Sterna hirundo*, from the colony on Great Gull Island, Long Island Sound, were quantitated for PCBs using two different methods. The areas of PCB peaks emerging after DDE were first compared with the 1254 standard and concentrations of 308 and 214 ppm in the egg lipids were calculated, respectively. When areas were compared with the total areas of the same peaks in chromatograms of the Aroclor 1260 standard, the PCB values obtained were lower by a factor of 2 and were, respectively, 145 and 98 ppm. The column used in this instance was 5% QF-1 on Chromosorb W, 80–100 mesh, acid washed, HMCS treated. Under the conditions employed, the integrated areas of all peaks of chromatograms of equal amounts of Aroclor 1242, 1254, and 1260, respectively, increased in the ratio 1.00:1.68:2.46 (22).

The different quantitation procedures used by various laboratories may be expected to yield, therefore, somewhat different estimates of PCB concentrations. It is reasonable to assume, how-

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Table 1. PCB in Marine Fish and in Fish from an Arctic Tundra Lake.

Locality, reference	Species	ppm, fresh weight	ppm, extracted lipid or fat
Tokyo Bay	Sea bass *Lateolabrax japonica*	1.7–3.4	120
Nova Scotia Banks (23)	Atlantic salmon *Salmo salar*	0.45	—
	Atlantic herring *Clupea harengus*	0.32–0.54	—
	Atlantic mackerel *Scomber scombrus*	0.35	—
	Cod *Gadus morhua*	0.02	—
Swedish West Coast (2)	Cod *Gadus morhua*	0.02	7.3
	Fish oil	—	0.7
Baltic Sea (2)	Atlantic herring *Clupea harengus*	0.27	6.8
	Cod *Gadus morhua*	0.03	11
	Atlantic salmon *Salmo salar*	0.30	2.9
	Fish oil	—	3.5
North Atlantic (24)	Flying Fish *Cypselurus exsiliens*	0.001–0.007	0.4–0.5
	Dolphin *Coryphaena hippurus*	0.01	10
	Shark (liver) *Carcharhinus longimanus*	0.3	0.6
	Mesopelagic Fish *Chauliodus danae* 800–900 meters	0.01–0.06	1.5–7.3
Long Island Sound (21)	Atlantic round herring *Etrumeus teres*	1.2	30
	Atlantic silverside *Menidia menidia*	3.2	52
	Atlantic mackerel *Scomber scombrus*	1.2	79
California Coastal Waters (25)	Northern anchovy *Engraulis mordax*	1.0	—
	Jack mackerel *Trachurus symmetricus*	0.02	—
	Bluefin tuna *Thunnus thynnus*	0.04	—
Peruvian Coastal Waters (26)	Sardina peruana *Sardinops sagax*	0.01	0.6
	Caballa *Scomber japonicus*	0.02	2.0
	Bonito *Sarda sarda*	0.01	0.5
	Tiburón azul *Prionace glauca*	0.08	11.4
Lake Minto Ungava, Quebec (9)	Lake trout *Salvelinus namaycush*	0.04–0.6	1.1–12.0

(—) = Concentrations in parts per million.

ever, in comparing these results, that differences of an order of magnitude would yield useful information on geographical distribution.

In Table 1 are summarized some of the reported PCB residue data in fish from representative areas of the oceans and, for comparison, PCB values obtained in lake trout, *Salvelinus namaycush*, from a remote Arctic lake. Among these, highest concentrations were in the fish from Tokyo Bay and from Long Island Sound (Table 1). These bodies of water can be expected to receive significant amounts of PCBs from urban sewage outfalls. A comparison of the residues in three species, the Atlantic salmon, *Salmo salar*, the Atlantic herring, *Clupea harengus*, and cod, *Gadus morhua*, from the Nova Scotia banks and from Swedish waters shows that both areas have comparable levels of PCB contamination. That PCBs have penetrated below the mixed surface layer of the North Atlantic is shown by their presence in fish characteristic of deeper waters obtained at depths between 800 and 900 meters. PCBs were also detected in the fish from the coastal waters of Peru. The levels in lake trout from Lake Minto in the Arctic region of Quebec were higher than expected. Lakes in this area are shallow with rocky bottoms, almost no vegetation, and very low amounts of organic matter. It is interesting that levels in the Atlantic herring, a fatty fish, from both the Nova Scotia banks and the Baltic

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Table 2. PCB residues in petrels and shearwaters. Mean arithmetic concentrations in parts per million.

Species, reference	Breeding area	Non-breeding area	Tissue (N)	ppm, fresh weight	ppm, liquid lipid
Ashy Petrel (27) *Oceanodroma homochroa*	California coastal waters	California coastal waters	Whole body (9)	21	200 (120–320)
			Egg (6)	37	240 (130–370)
Leach's Petrel (28) *Oceanodroma leuchorhoa*	Baja California, Pacific Coast	Eastern Pacific	Egg (1)	—	350
Black Petrel (7) *Loomelania melania*	Gulf of California	—	Whole body (8)	1.0	—
Least Petrel (7) *Helocyptena microsoma*	Gulf of California	—	Whole body (3)	0.4	—
Fulmar (7) *Fulmarus glacialis*	Alaska	Eastern Pacific	Whole body (3)	2.3	—
Pink-footed Shearwater (7) *Puffinus creatopus*	Chile	Western Mexico, northern Pacific	Whole body (1)	0.4	—
Sooty Shearwater (7) *Puffinus griseus*	New Zealand	Northern Pacific	Whole body (2)	1.1	—
Slender-billed Shearwater *Puffinus tenuirostris* (7)	Australia	Northern Pacific	Whole body (1)	2.1	—
Wilson's Petrel (10) *Oceanites oceanicus*	Hallett Station, Antarctica	Australian seas	Whole body (10)	2.1	11.0
	Palmer Station, Antarctica	North Atlantic	Whole body (9)	33	190
Giant Petrel (10) *Macronectes giganteus*	Palmer Station, Antarctica	Southern oceans	Egg (3)	0.2	3.5
Snow Petrel (10) *Pagodroma nivea*	Hallett Station, Antarctica	Antarctic ice-pack	Whole body (10)	0.08	0.3

were considerably higher, by an order of magnitude, than levels in the cod on a fresh weight basis, although the cod occupies a higher position in the food web. On a fat basis, however, residues in both species in the Baltic were comparable. These data suggest that amounts and kinds of lipids may affect the retention of PCBs, modifying the trophic accumulation predicted by the classical food chain concentration theory. Consistent with this hypothesis are the higher PCB residues measured in extractable lipids of the North Atlantic plankton than in the lipids of fish from the same area.

Table 2 includes a representative selection of PCB residues so far measured in species of petrels and shearwaters. These birds are strictly oceanic, rarely approaching land except to breed on islands or undisturbed coastal areas. They do not dive for their food as do the fish-eating species but rather feed upon plankton and other organic material obtained at the surface of the sea. Within a given marine ecosystem, their burdens of both PCB and DDT residues may be higher than those in fish-eating species such as the Brown Pelican, *Pelecanus occidentalis*, or the Osprey, *Pandion haliaetus* (7). Many species range widely, breeding in one hemisphere and spending the remainder of the year in the other. As expected, lowest levels were found in the Snow Petrel, *Pagodroma nivea*, a species resident in the Antarctic pack-ice region. High residues were found in tissues of the Wilson's Petrel, *Oceanites oceanicus*, from the population that breeds on the Antarctic Peninsula and migrates to the North Atlantic during the southern winter months. Comparable levels were found in the Ashy Petrel, *Oceanodroma homochroa*, a species that remains largely or entirely within the California coastal waters. High PCB concentrations were also found in an egg of a Leach's Petrel, *Oceanodroma leuchorhoa*, obtained on the Islas Coronados, Baja California, Mexico, in the vicinity of San Diego, California (Table 2).

Table 3 summarizes the PCB data obtained from analysis of eggs of the Brown Pelican over a

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Table 3. PCB residues in eggs of the Brown Pelican, Pelecanus occidentalis, from North and South America. Mean arithmetic concentrations in parts per million of the egg lipid (30).

Breeding area	Sample size	ppm PCB, lipid
Peru	5	14
Panama	6	4
Venezuela	4	5
Jamaica	4	19
Florida, 4 colonies	87	71
Gulf of California	4	4
San Benitos, Western Baja California	10	39
San Martin, Western Baja California	6	72
Los Coronados	28	266
Anacapa Island, California	65	210

substantial portion of its range in both North and South America. This species feeds only upon marine fish obtained in coastal waters. As expected, levels in the South American populations are substantially lower than those in North America. Concentrations in Florida tend to be about three times lower than those in California. This species does not occur along the coast of the heavily industrialized areas of the northeast United States, but in these areas the Osprey, another fish-eating species, may contain PCB residues five to ten times higher than those in the Florida populations of Brown Pelicans (29). The PCB or associated compounds are a possible cause of embryonic mortality observed in the Osprey populations of these areas (29).

The major difficulty in the design of a monitoring program to determine whether PCB residues are increasing or declining in a given sink is the lack of a statistical design that would establish how many individual specimens should be analyzed in order to determine the parameters of the pollutant distribution at a desired confidence level. Nor has it been established which species of organisms are most suitable for such monitoring programs. The distribution of residues of chlorinated hydrocarbon insecticides and their derivatives within a population is frequently non-Gaussian, although transformation of residue concentrations to their logarithms yields a distribution that is sufficiently Gaussian for the use of established statistical methods (31, 32). Confidence intervals, therefore, can be established for such parameters as the means of logarithms of residue concentrations. The confidence interval however, cannot be transformed back to the original distribution. There apparently are no satisfactory statistical methods that would determine the confidence intervals about the arithmetic or geometric mean of measured concentrations (32). Moreover, one hundred or more analyses of individual specimens might be necessary to determine the precision of the logarithm of the geometrical mean within a range equivalent to 10% of the value of the geometric mean (31, 32).

The two major research problems that are emerging from the occurrence of PCBs in the environment appear to be first, the determination of the accumulation rates in sinks, so that the concentrations found there might be related to input levels, and second, the determination of the effects of PCBs or their derivatives upon organisms and ecosystems at each contamination level. It might be hoped that a fraction of the considerable sums of money that would be spent at both national and international levels to "monitor" the environment will be diverted to programs designed to answer the significant questions rather than to assemble tables of random numbers, for which there is no pressing need.

Acknowledgments

Research was supported by Grant NSF-GX-28743 from the International Decade of Ocean Exploration Program, National Science Foundation.

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Contamination of the Bay of Fundy—Gulf of Maine area with Polychlorinated Biphenyls, Polychlorinated Terphenyls, Chlorinated Dibenzodioxins, and Dibenzofurans

by V. Zitko*, O. Hutzinger†, and P. M. K. Choi**

The procedure used to determine polychlorinated biphenyls (PCBs), polychlorinated terphenyls (PCTs), chlorinated dibenzodioxins, and chlorinated dibenzofurans is outlined in Table 1. It is based on the cleanup-chromatography of Holden and Marsden (1), modified as described (2). Most PCBs are eluted in fraction I. Fraction II contains some PCBs, p,p'-DDE, and PCTs, whereas heptachlor exposide, dieldrin, endrin, p,p'-DDD, and p,p'-DDT are eluted in fraction III. Fractions I and II are further chromatographed on alumina, essentially according to Porter and Burke (3), to determine chlorinated dibenzodioxins and dibenzofurans.

PCTs, hexa- and octachlorodibenzodioxin, and octachlorodibenzofuran were quantified by gas chromatography on 3% OV-210 on Chromosorb WAW 60/80 in a 6 ft × 4 mm column at 200°C. A 4% SE-30 column was used to quantify PCBs, chlorinated hydrocarbon pesticides, 2,3,7,8-tetrachlorodibenzodioxin, di-, tri-, and tetrachlorodibenzofuran.

Recovery of PCBs and chlorinated hydrocarbon pesticides in this procedure was generally better than 95% (2). Recovery of PCTs was 90%. Results of the analyses of commercial fishmeal, spiked with chlorinated dibenzodioxins and dibenzofurans are presented in Table 2.

PCTs were found only in eggs and fatty tissues of herring gulls in levels of 0.1 and 1.4 μg/g wet weight, expressed as Aroclor 5460, respectively. This indicates that PCTs are not as widely spread in the environment as PCBs.

Only a limited number of samples were analyzed for chlorinated dibenzodioxins and dibenzofurans. The samples included muscle and liver of white shark (*Carcharodon carcharias*), eggs of double-crested cormorants (*Phalacrocorax auritus*), and herring gulls (*Larus argentatus*), commercial herring oil, and groundfish herring fishmeal. No residues of chlorinated dibenzodioxins and dibenzofurans were found.

Detection limits of the individual compounds are estimated in Table 3.

The absence of chlorinated dibenzodioxins and dibenzofurans from aquatic animals is encouraging. However, these compounds are extremely toxic, and a more detailed investigation is necessary. The analytical technique should be further improved to achieve lower detection limits.

The levels of PCBs and chlorinated hydrocarbon pesticides found in fishes, double-crested

* Environment Canada, Fisheries Research Board, Biological Station, St. Andrews, New Brunswick.
† Atlantic Regional Laboratory, National Research Council of Canada, Halifax, Nova Scotia.
** McMaster University, Hamilton, Ontario, Canada.

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Table 1. Procedure for determining PCBs, PCTs, chlorinated dibenzodioxins, and chlorinated dibenzofurans.

Chromatography on alumina (800°C 4 h, 5% H_2O)		
Chromatography on Silicar^R (130°C overnight, 3% H_2O)	Fraction I I (20 ml 2% CH_2Cl_2 in hexane)	PCB, C_6Cl_6
Fraction I (10 ml hexane)	Fraction I II (10 ml 20% CH_2Cl_2 in hexane)	HCDD faster isomer, TCDD, OCDD, OCDF
Chromatography on alumina (130°C overnight)		
	Fraction II I (20 ml 2% CH_2Cl_2 in hexane)	p,p'-DDE
Fraction II (20 ml hexane)		
	Fraction II II (10 ml 20% CH_2Cl_2 in hexane)	HCDD slower isomer, TCDD, CDF
Fraction III (10 ml 10% ether in hexane)		

TCDD = 2,3,7,8-tetrachlorodibenzodioxin.
HCDD, OCDD = hexa-, octachlorodibenzodioxin.
CDF = mixture of di-, tri-, and tetrachlorodibenzofuran.
OCDF = octachlorodibenzofuran.

cormorants, and herring gulls from the Bay of Fundy-Gulf of Maine area are summarized in Table 4. The concentration of PCBs is generally higher than that reported for the same or similar species from other areas, such as the Baltic Sea (4), California coastal waters (5), Britain (6), and Western Canada (7).

It is interesting to note that the levels of PCBs found in fishes from the Bay of Fundy–Gulf of Maine area are about one order of magnitude higher than those in fishes from the Nova Scotia banks (2). Determination of PCB residues in Atlantic salmon (*Salmo salar*) also indicates that elevated levels of PCBs are associated with coastal areas. Thus muscle of salmon caught off Greenland contains 0.20 μg/g of PCBs, whereas in specimens landed in Canada PCB levels are from 0.45 to 0.62 μg/g on wet weight basis.

In all samples the peak pattern of PCB residues resembles quite closely that of Aroclor 1254, and the results are expressed as Aroclor 1254. However, fish from lower and intermediate levels of the food web contain relatively lower amounts of hexachlorobiphenyls (4th, 5th, and 6th major Aroclor 1254 peak) and higher amounts of tetra- and

Table 2. Analysis of spiked fishmeal samples, μg/g wet weight.

Sample	TCDD*		HCDD*		OCDD*	
	Added	Found	Added	Found	Added	Found
1	1.18	0.96	1.08	1.15	1.30	1.29
2	0.59	0.64	1.08	1.04	0.65	0.57
3	0.29	0.33	0.26	0.31	0.32	0.50

	CDF*		OCDF*	
	Added	Found	Added	Found
4	1.21	1.41	0.91	0.96
5	0.60	0.62	0.45	0.38
6	0.30	0.28	0.23	0.14

* See Table 1.

Table 3. Detection limits of chlorinated dibenzodioxins and dibenzofurans

Compound	Detection limit, μg/g wet weight
TCDD*	0.04
CDF*	0.02
HCDD*	0.02
OCDD*	0.01
OCDF*	0.01

* See Table 1.

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Table 4. PCB and chlorinated hydrocarbon pesticides in aquatic animals from the Bay of Fundy–Gulf of Maine area.

Species	Tissue	PCBs (as Aroclor 1254)	µg/g wet weight p,p'-DDE	Other
Herring *Clupea harengus*	whole fish	0.34	0.06	
Mackerel *Scomber scombrus*	muscle	0.35	0.07	
Plaice *Hippoglossoides platessoides*	muscle	0.38	0.01	
White hake *Urophycis tenuis*	muscle	0.44	0.03	
Ocean perch *Sebastes marinus*	muscle	0.32	0.03	
Cod *Gadus morhua*	muscle	0.55	0.04	
Sea raven *Hemitripterus americanus*	muscle	0.21	0.08	DDT 0.24
	viscera	0.73	0.30	
Basking shark *Cetorhinus maximus*	muscle	0.07	—	
	liver	1.07		
White shark *Carcharodon carcharias*	muscle	0.77	0.48	
	liver	218	335	DDT 63 DDD 43
Bluefin tuna *Thunnus thynnus*	muscle	1.54	0.15	
Herring oil		3.55	2.27	DDT 0.37
Fishmeal		0.54	0.19	
Double-crested cormorant *Phalacrocorax auritus*	eggs	43.5[a]	29.4[a]	
		17.2[a]	8.6[a]	
	muscle	3.38	8.40	
	liver	2.13	4.16	
	Subcut. fat	38	164	
	Abdom. fat	52	162	
Herring gull *Larus argentatus*	eggs	12.6[a]	5.67[a]	
		5.54[a]	2.83[a]	
	muscle liver	5.06	2.07	
		6.50	2.08	
	Subcut. fat	75	26	

[a] Different nesting colonies.

pentachlorobiphenyls (1st and 2nd major Aroclor 1254 peak) than high trophic level white and silky shark (*Carcharodon carcharias* and *Carcharhinus falciformis*, respectively) and aquatic birds. Atlantic salmon parr fed Aroclor 1254-contaminated diet in the laboratory also contain relatively higher amounts of tetra- and pentachlorobiphenyls. This may indicate that fish more efficiently absorb the lower chlorinated biphenyls. On the other hand, these compounds may be metabolized and excreted, so that a preferential accumulation of higher chlorinated biphenyls occurs in the food chains. The fractions of hexachlorobiphenyls at different trophic levels are summarized in Table 5.

The toxicological significance of environmental levels of PCBs and their rate of accumulation in the environment are not known. In spite of this, PCBs and other industrial halogenated hydrocarbons should be considered potentially harmful,

Table 5. Fraction of hexachlorobiphenyls at different trophic levels.

Sample	Fraction of hexachlorobiphenyls in terms of Aroclor 1254 peak heights,[a] $\frac{4+5+6}{1+2+4+5+6}$
Aroclor 1254	0.56
Fish, wild	0.38
Atlantic salmon parr on Aroclor 1254-contaminated diet	0.41
Silky shark	0.74
White shark	0.71
Eggs of double-crested cormorants and herring gulls	0.72

[a] Numbers refer to the major Aroclor 1254 peaks.

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and their release into the environment should be limited as much as possible.

Acknowledgments

We thank our colleagues at the Biological Station, St. Andrews, New Brunswick, Canada, for samples of fish and Dr. D. Firestone, Food and Drug Administration, Washington, D.C., U.S.A., for samples of chlorinated dibenzodioxins and dibenzofurans. We thank Mrs. Madelyn M. Irwin for typing the manuscript.

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Recent Fluctuations of Chlorobiphenyls (PCBs) in the Green Bay, Wisconsin, Region*

by Gilman D. Veith, Ph.D.†

Introduction

During the past six years, Wisconsin and Michigan have maintained a major stocking program for trout and salmon in Lake Michigan in an effort to re-establish a sport fisheries, and to control nuisance fish, such as the alewife. In 1969, Veith and Lee (1) found these large predators contained chlorobiphenyls (PCBs) at concentrations ranging from 10 to 25 μg/gm (fresh weight—as Aroclor 1254). Furthermore, the coho salmon eggs contained 12 to 17 μg/gm PCBs (fresh weight) which, based on the results of Johansson et al. (2) suggest that the PCBs may contribute to the poor reproductive success of the stocked fish in Lake Michigan. The seizure of fish from Lake Michigan, because of the presence of chlorinated pesticides and PCBs at concentrations greater than that regarded as safe for human consumption, has demonstrated that the continuous input of trace quantities of these chemicals may jeopardize major food resources for man, if proper management of hazardous chemicals is not practiced.

There are numerous examples of the aqueous transport of PCBs originating from industrial and sanitary wastes (3, 4). Veith and Lee (5) reported the presence of PCBs in sanitary wastes of all cities and villages studied in the Milwaukee River (Wisconsin) watershed. Dube (6) has found similar results in a study of PCBs in municipal wastes from southeastern Wisconsin. All of these data were obtained prior to or shortly after the voluntary partial ban on PCB sales by Monsanto Company. This paper presents the preliminary data from studies of the levels and sources of PCBs in rivers discharging into Lake Michigan—Green Bay in northeastern Wisconsin.

Green Bay (Figure 1) is an 120-mile long elongated bay in the northwest corner of Lake Michigan. The major rivers tributary to lower Green Bay are the Fox (mean discharge of 11×10^6 m^3 day^{-1}), which enters at the southwest end of the Bay, and the Oconto (2×10^6 m^3 day^{-1}), Peshtigo (2×10^6 m^3 day^{-1}) and the Menominee (9×10^6 m^3 day^{-1}), all of which enter along the northern shore of the Bay (7). The Fox and Menominee Rivers receive the wastes from the cities of Green Bay and Marinette (Wisconsin)—Menominee (Michigan), respectively. The Oconto River receives the wastes from Oconto Falls and Oconto, and wastes from the city of Peshtigo are discharged into the Peshtigo River. The Big Suamico and Pensaukee Rivers primarily drain agricultural and forested land and do not receive any major municipal wastes above the respective sampling sites.

Experimental Procedures

Field Sampling

The procedures used in this study have been discussed in detail elsewhere (5). The collection of water was conducted with all-glass systems to

* Contribution of the Water Chemistry Program, Department of Civil and Environmental Engineering, University of Wisconsin, Madison, 53706. This work was supported under the University of Wisconsin Sea Grant Program, the Department of Civil and Environmental Engineering, and the Engineering Experiment Station.

† Assistant Professor of Civil and Environmental Engineering, University of Wisconsin, Madison, Wisconsin 53706.

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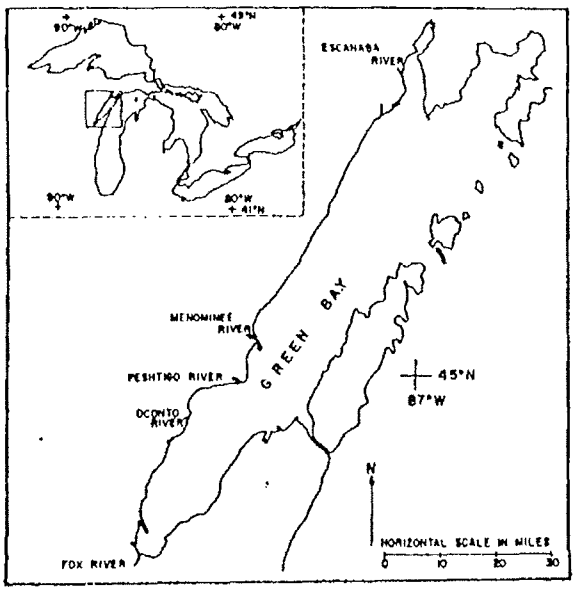

FIGURE 1. Location of Green Bay, Lake Michigan and major rivers tributary to the Bay.

preclude possible interaction of the sample with rubber, plastic, or polyvinyl chloride surfaces. Samples were collected from the river by submerging a weighted, glass carboy (20 l) at a depth of 0.2 m in the center of the main channel. Preservatives were not added, as samples were cooled and extracted within 24 hours of collection.

Extraction

Water samples (20 l) were batch-extracted twice with hexane in 2 l separatory funnels. The hexane portions were combined, dried with anhydrous Na_2SO_4 and concentrated to 15 ml for cleanup in a gentle air stream.

Liquid Chromatography

The cleanup of extracts for gas chromatographic analysis was conducted with liquid chromatography on Florisil, as described by Reynolds (8) and Hughes et al. (9). The media (Kensington Scientific) was extracted in a Soxhlet extractor for 24 hours with an azeotrope of hexane and acetone (1:59) to remove organic impurities. The azeotrope was evaporated from the Florisil at 105°C, and the Florisil was heated to 650°C for 2.5 hours for activation.

The Florisil column for preliminary cleanup was prepared by vibrating 19 g of Florisil into a 25 mm o.d. glass column, which was fitted with a glass frit and Teflon stopcock. The column was topped with 10 g anhydrous sodium sulfate to prevent deactivation of the Florisil by traces of water in the extract. The extracts were placed on the column and eluted with hexane (200 ml) to recover the PCBs, if present. With some samples it was necessary to rechromatograph the hexane eluate from the 25 mm o.d. Florisil on a smaller diameter Florisil column (9 mm i.d.) to isolate the PCBs from organic interferences. If necessary the PCBs were separated from the DDT group of pesticides through chromatography on silicic acid (10, 11).

Instrumentation

The analyses of water extracts were conducted on an Aerograph 1745–20 gas chromatograph equipped with concentric tube electron capture detectors (^3H, 250 mC) and a 50:50 effluent splitter for simultaneous analysis with electron capture and flame ionization detectors. Analytical GLC columns consisted of 2.0 m × 1.8 mm glass coils which were packed with either OV-101 (3 percent), OV-101/XE-60 (3:3 percent), or OV-101/QF-1 (3:4.5 percent), coated onto Gas Chrom Q (720/140 mesh). The carrier gas (purified N_2) was maintained at 21 ml min^{-1}; and the injector, column, and detector temperatures were 250°C, 180°C, and 220°C, respectively.

Analysis

The commercially prepared PCBs in the U.S.A. (Monsanto Company, St. Louis, Mo.) exist as seven complex mixtures under the name "Aroclor", which range in chlorine content from 21 to 62 percent. When the PCB mixtures are chromatographed with GLC, the mixtures of isomers produce both resolved and superimposed peaks and are somewhat characterized by the GLC fingerprint presented as relative peak heights and retention times. Because of the complexity of the mixtures, determinations have been quantitatively defined by comparing the area of a sample chromatogram to the area of a known quantity of the commercial mixture which most closely resemble that of the sample.

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Table 1. Variations of Chlorobiphenyls in Rivers in the Green Bay Study Area.

River	Concentration (μg/l as Aroclor 1254)[a]			
	12/29/70	5/21/71	7/20/71	8/6/71
Peshtigo River, Peshtigo	0.31	0.38	<0.01	<0.01
Oconto River, Oconto	0.45(3)	0.16	<0.01	<0.01(3)
Pensaukee River, Pensaukee	<0.01	<0.01	<0.01(1)	<0.01(1)
Big Suamico, Suamico	<0.01	<0.01	<0.01(1)	<0.01(1)
Fox River, Green Bay	0.18[b] (3)	0.26	0.16	0.15

[a] Mean of two analyses unless noted otherwise.
[b] Fox River samples analyzed as Aroclor 1248. Late eluting components present near municipal waste treatment outfall.

Explicit chemical confirmations for the presence of PCBs in each sample were not possible in this study. PCBs with similar retention volumes and relative peak heights were confirmed in the fish from the Milwaukee River using i.r. and mass spectrometry (5). The analyses do not preclude the possible presence of other chemicals which may have similar chemical properties, such as the chloronaphthalenes.

The recoveries of PCBs from water samples which were "spiked" with Aroclor 1254 and 1260 were approximately 80 to 85 percent. Although the relative error at 1 μg/l was about ±8 percent, the relative error in replicate analyses of river waters at the 0.1 μg/l level may increase to 15 to 20 percent.

Results and Discussion

The results of the monitoring of five rivers in the Green Bay area for PCBs during 1971 are presented in Table 1. The concentration of PCBs in the Pensaukee and Big Suamico Rivers remained below the determinable limit of 0.01 μg/l throughout the period of the study. The PCBs in the Peshtigo River below the city of Peshtigo were approximately 0.3 to 0.4 μg/l (as Aroclor 1254) until May 21, 1971, but decreased to below the determinable limit by July 20, 1971. PCBs in the Oconto River below the city of Oconto were present at a concentration of 0.45 μg/l (as Aroclor 1254) on December 29, 1970, but decreased during the spring of 1971 and were also below the determinable limit on the July 20 sampling date. In contrast, no trends in the concentrations of PCBs in the Fox River were observed, and the levels were found to be above 0.1 μg/l (as Aroclor 1248) throughout the study. It should be noted that the chromatograms of the PCB mixtures in the Oconto and Peshtigo Rivers were most similar to that of Aroclor 1254, whereas the mixtures in the Fox River were predominately Aroclor 1248, with minor components similar to those in Aroclor 1254 sometimes apparent.

The initial purpose in monitoring the tributaries of Green Bay was to relate the composition and concentration of PCBs in the major tributaries to those in the water from the Bay and to examine possible changes in the composition of PCBs in the sediments. The data obtained from the initial monitoring, which shows that the PCB concentrations in two of the major tributaries decreased sharply to below the determinable limit in 1971, suggests that the self-imposed restrictions on the sale of PCBs by Monsanto Company in 1970 and 1971 may be having an immediate effect in some river systems. Continued monitoring of the Fox River will be conducted to determine if the levels of PCBs arising from this highly industrial area will also diminish in the near future.

Although the analyses of water from Green Bay indicated the concentrations of PCBs varied from 0.07 μg/l (as Aroclor 1248) at Long Tail Point near the city of Green Bay to 0.04 g/l approximately 35 miles northeast of the city near Sturgeon Bay, the variations introduced by windmixing and the inability to measure small concentration differences below the 0.05 μg/l level may make it impossible to determine if the decline in PCBs in tributaries will be reflected in the water from the open Bay in the near future. Consequently,

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the sampling program has been expanded to include fish and other organisms of the Bay which contain μg/gm quantities of the PCBs.

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DSW 025083

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Polychlorinated Biphenyl Residues in Milk of Environmentally and Experimentally Contaminated Cows

by G. F. Fries*

Polychlorinated biphenyl (PCB)† residues have been found in the milk of cows. In some instances the residue levels exceeded the FDA guideline 0.2 ppm in milk (equivalent to 5.0 ppm in milk, fat), and the milk was removed from the market. The major source of PCB residues in milk is Aroclor 1254 that has been used in coatings for concrete silos. Aroclor 1254 is unaltered in silos, and most of the contamination is adjacent to the walls (1, 2).

We have observed a number of farms with PCB-treated silos and have fed Aroclor 1254 to cows under controlled conditions. This paper summarizes our major findings.

Nature and Occurrence of Residues

The PCB recovered from silage is identical to standard Aroclor 1254 (1). However, many of the components with short gas chromatographic retention times do not occur in milk residues (Fig. 1). The components with short retention times are apparently less chlorinated than those with long retention times. Our observations in cows are similar to observations in other species (3).

A complication in analyzing environmental samples is a possibility of interference by DDT and some of its related compounds. We dehydrochlorinated the samples to remove possible interferences of DDD and DDT. DDE did not interfere, because those components of Aroclor 1254 with the same retention time as DDE did not occur in significant amounts in milk (Fig. 1).

We obtained milk tank samples from six farms with PCB-contaminated silos. The PCB levels in milk fat are summarized in Table 1. In all cases PCB concentration in milk fat exceeded the FDA guideline, at least part of the time, when silage was fed. When the silage was not fed, levels of PCB in milk fat were always lower than the guidelines.

The farms represent a variety of feeding and management conditions. The results suggest that any dairyman with a contaminated silo will probably exceed the guideline at least some of the time during the year. While a few higher levels have been reported for herds removed from the market, most were within the range of our observations. The number of farms with PCB-contaminated silos is not known. However, PCB contamination of milk is probably more prevalent than the regulatory record would suggest.

Comparison of DDE and PCB

We have observed a herd that was simultaneously contaminated with high levels of DDE and Aroclor 1254 from a silo. By a fortuitous set of circumstances, the cows had been removed from the sources of both contaminations at about the same time. A detailed description of this work has been prepared (4).

* United States Department of Agriculture, Animal Science Research Division, ARS, Beltsville, Maryland 20705.

† In this paper the term PCB is interchangeable with Aroclor 1254 or, in the case of milk and body fat, residues derived from Aroclor 1254.

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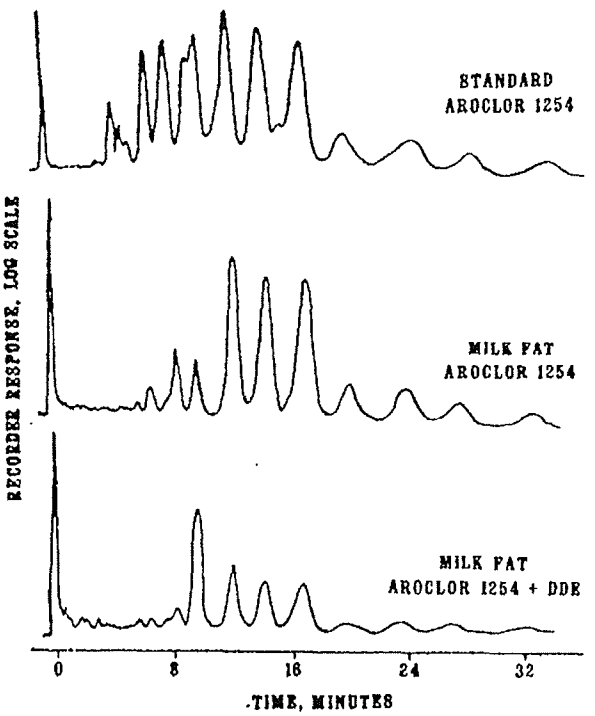

FIGURE 1. Gas chromatograms of Aroclor 1254 standard and residues in milk. The 6 ft. × ¼ in. o.d. column was packed with 10% DC-200 on Gaschrom-Q. A 95% argon–5% methane mixture was used as carrier gas and the column temperature was 200°C.

The average concentration of PCBs in milk fat was 19.3 μg/g when the silage feeding stopped. This concentration declined to 10.3 μg/g within three weeks. We continued to sample the individual cows at three-week intervals for approximately four months. After three weeks the decline in concentration was linear, when plotted on semilog graph paper, suggesting first order kinetics.

Table 1. PCB concentrations in milk fat from farms with contaminated silos[a]

Farm	11/10	12/7	1/21	3/24	5/11
			(μg/g)		
1	9.6	8.8	6.7	N.S.	7.0
2	7.5	5.7	6.7	6.6	*3.6*
3	N.S.[b]	*1.5*	7.4	5.0	*2.1*
4	N.S.	*2.0*	*1.4*	*1.4*	6.0
5	*3.5*	*2.7*	6.1	10.3	10.5
6	N.S.	*1.0*	6.2	5.2	*2.9*

[a] Silage was not being fed when italicized samples were obtained.
[b] Not Sampled.

Table 2. Average rates of decline of DDE and PCB concentration in milk fat of cows in various stages of lactation.[a]

Group	Cows	DDE	PCB
	(No.)	(day^{-1})	(day^{-1})
Mid lactation	16	0.0145	0.0147
Early lactation	9	0.0107	0.0106
Late lactation	6	0.0147	0.0140
All	31	0.0134	0.0134

[a] The values are for the constant, k, in the linear form of the first-order equation: $\ln C = \ln C_0 - kt$ where C is the milk fat concentration, C_0 is the initial concentration, and t is time in days. Fitted by least squares.

First-order rate constants were calculated for both DDE and PCBs for the individual cows. A summary is presented in Table 2. The overall average of the rate constants for PCB was identical to that for DDE. The average DDE rate constant was similar to the rate that we have previously reported (5). There was a tendency for cows in early lactation to have lower rate constants than the other groups of cows.

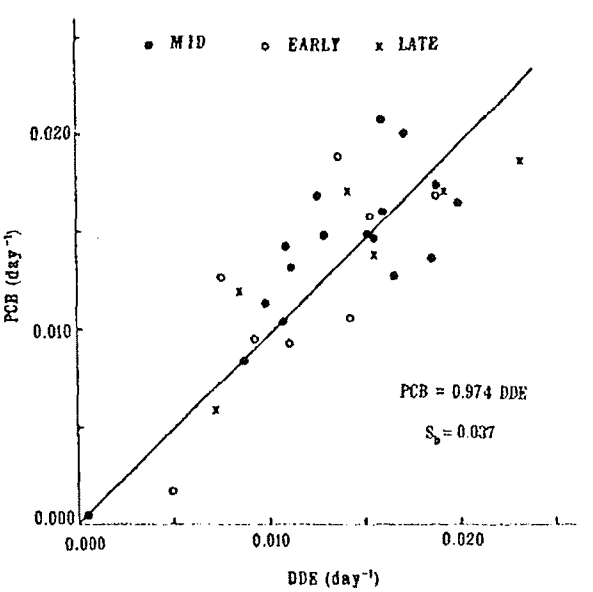

FIGURE 2. Relationship of the rates of decline of DDE and PCB concentration in milk fat of individual cow. Mid, early, and late are stages of lactation (4).

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Table 3. Effect of phenobarbital and phenobarbital with activated carbon on the concentration of DDE and PCB in milk fat.[a]

Treatment	Cows (No.)	Concentration Initial (μg/g)	Concentration Final (μg/g)	Final Initial	S.D.
			DDE		
Control	5	11.6	5.2	0.45	±0.05
Phenobarbital	10	9.5	4.3	0.45	±0.15
Phenobarbital+ Carbon	6	8.0	3.7	0.46	±0.20
			PCB		
Control	5	19.3	7.4	0.38	±0.08
Phenobarbital	10	17.7	7.0	0.40	±0.11
Phenobarbital+ Carbon	6	22.3	8.5	0.37	±0.14

[a] Observation period was 6 weeks. Phenobarbital was fed at 5 grams per day for the first three weeks and activated carbon at one kilogram per day for the 6 weeks.

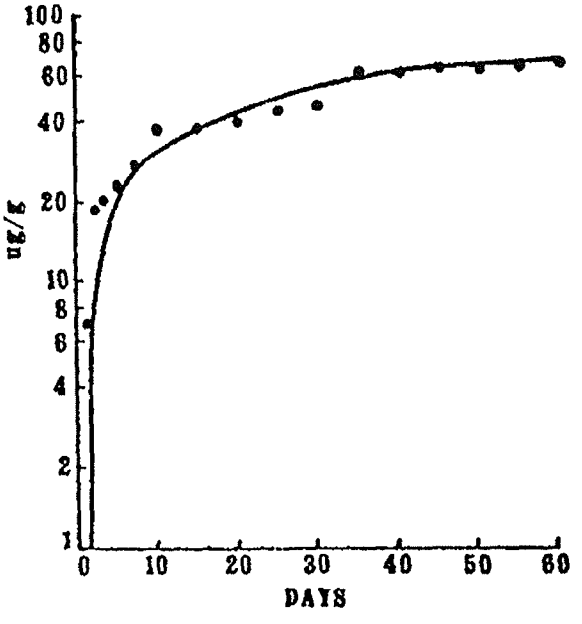

FIGURE 3. Concentration of PCB in milk fat of cows fed 200 mg/day Aroclor 1254 for 60 days. Each point is an average of nine cows.

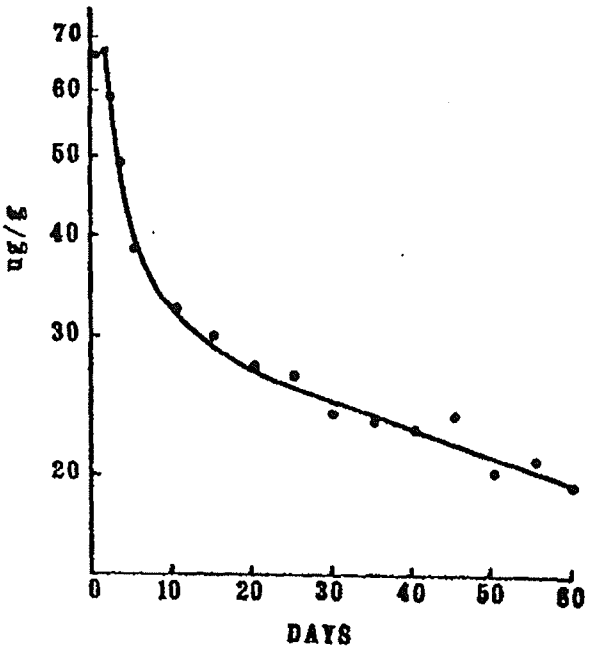

FIGURE 4. Concentration of PCB in milk fat after feeding Aroclor 1254 stopped. Each point is an average of nine cows.

Relationship of the rate constants for DDE and PCBs within individual cows is presented in Fig. 2. The correlation ($r = 0.82$) between the rate constants of the two compounds was significant ($P<0.01$). The usual linear regression was calculated, and the intercept did not differ significantly from zero (6). Therefore, the regression in Fig. 2 was recalculated, forcing the intercept through zero. The regression coefficient, 0.974, was essentially unity.

In some cases there was considerable variation between the rate constants for DDE and for

Table 4. Concentration of PCB in milk fat and body fat of cows fed 200 mg/day PCB for 60 days.

Time	Milk fat	Body fat
(days)	(μg/g)	(μg/g)
30	45.6±8.4[a]	21.9±8.5
60	66.7±8.4	44.0±12.2
90	24.0±3.0	40.3±6.4
120	19.4±2.4	28.8±6.2

[a] Standard deviation.

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Table 5. Normalized concentration of chlorinated hydrocarbon compounds in milk fat and body fat of cows after continuous feeding.[a]

Compound	Reference	Observations (No.)	Milk fat 20 days (μg/g)	Milk fat 60 days (μg/g)	Body fat 60 days (μg/g)
Aroclor 1254		9	0.20	0.33	0.20
DDE	(5)	3	0.21	0.29	0.23
	(7, 8)	12	0.27	—	—
Dieldrin	(7, 8)	12	0.19	—	—
DDD	(5)	3	0.063	0.077	0.046
p,p'-DDT[b]	(5)	3	0.036	0.049	0.033
	(7, 8)	12	0.036	—	—
	(9)	4	0.106	—	—
o,p'-DDT[b]	(9)	4	0.023	—	—

[a] Values are normalized to an intake of 1 mg/day.
[b] Values are sum of DDD and DDT.

PCBs within a given cow. The rate constants for the two compounds within a given cow were tested statistically (6), but the differences were not significant.

The usefulness of phenobarbital alone, or in combination with activated carbon, in accelerating the reduction in milk concentration of DDE and PCBs was tested using three groups of cows. Neither phenobarbital nor phenobarbital in combination with activated carbon had an effect on the relative reduction in concentration of either compound (Table 3). We have previously reported a small effect of phenobarbital on DDE, but we did not consider it of practical significance (7). The absence of an effect here is consistent with that interpretation. The failure of activated carbon, even in combination with phenobarbital, to affect the reduction of DDE and PCB concentrations is consistent with our previous interpretation of laboratory experiments (8).

Aroclor 1254 Feeding Study

We have carried out a controlled feeding study using nine Holstein cows. The cows were fed 200 mg per day Aroclor 1254 for 60 days. Milk samples were obtained periodically during the 60-day feeding period and the subsequent 60-day period. Body fat samples were obtained by biopsy at 30-day intervals.

The cows were selected to have a range in both the stage of lactation and level of milk production. However, preliminary examination of the results indicates that there was little difference in the residue levels due to these factors. Therefore, only average data for all cows are presented.

The average level of PCBs in the milk fat during the feeding period is presented in Fig. 3. The standard deviation of any given point did not exceed ±10% of the mean. The concentration of PCBs increased rapidly and approached a "steady state" at about 40 to 60 days. As expected from our field studies, the shape of the curve is similar to that for other chlorinated hydrocarbon pesticides, particularly DDE (5).

The decline in PCB concentration in the milk for the 60 days after feeding stopped is presented in Fig. 4. This curve is quite typical of previous findings with DDE and was resolved into a two-component first-order system (5). The equation for the curve normalized to an initial concentration of 1.0 μg/g is:

$$C = 0.52e^{-0.25t} + 0.48e^{-0.008t}$$

Where C is concentration, e is the base of the natural logarithms, and t is days.

The constants are similar to our previous observations for DDE (5). While the rate for the second component in this study was less than the values found in the field, it was within the range of values that one might expect.

The level of PCB in body fat at various times is presented in Table 4. For reference purposes, milk fat levels at these times are also presented.

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While the PCBs were being fed, the level in milk fat was higher than that in body fat. When feeding stopped, milk fat levels dropped below and appear to reflect body fat levels.

We have conducted a number of studies with chlorinated hydrocarbon pesticides under conditions similar to those of this PCBs study. A summary of levels in milk fat at 20 and 60 days and body fat at 60 days with normalized intakes is presented in Table 5. The levels of PCBs are similar to levels of DDE and dieldrin in corresponding samples. In contrast DDD, p,p'-DDT, and o,p'-DDT are transferred to the body fat and milk fat at much lower rates than DDE and PCBs.

Conclusions

Farms having silos that have been treated with PCB-containing paints will frequently have residues of PCBs in milk which exceed the FDA guidelines. The behavior of this residue in the cows is similar to DDE and other chlorinated hydrocarbon pesticides resistant to metabolic degradation. The only practical countermeasures appear to be to decontaminate the silos sufficiently to remove most of the PCBs or to discontinue the use of these silos completely. Limited field experience and the similarity of PCBs to DDE suggest that there are no practical procedures to minimize the transfer of PCBs from the diet to the milk or to speed up the elimination of PCBs from cows.

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Polychlorinated Biphenyls in Non-carbon Copy Paper

by Masanori Kuratsune and Yoshito Masuda*

In an effort to clarify the distribution of polychlorinated biphenyls (PCBs) in our environment, it was suggested by Dr. T. Hirayama, National Cancer Center, [Japan] that PCBs or their related compounds might be used for production of non-carbon copy paper. Three of 4 Japanese brands of the paper on the market were, therefore, collected and examined for PCBs. The paper consists of three different sheets, namely upper, middle, and lower ones. These sheets of paper were separately extracted with acetone in a Soxhlet apparatus for 3 days. The extracts were examined by gas chromatography with an electron capture detector, using 3 different liquid phases, namely 5% SE-30, 5% QF-1, and 2.5% DEGS coated on Chromosorb W AW DMCS. The gas chromatographic patterns observed were all identical with that of Kanechlor-300 (a commercial brand of PCBs) containing 43% of chlorine (Fig. 1). The infrared absorption spectra and mass-spectra of the extracts also demonstrated the presence of Kanechlor-300. The approximate concentration of PCBs was 2–6% in the upper and middle sheets and 0.02% in the lower sheets as shown in Table 1.

To estimate how much PCBs stick to fingers by handling the copy papers, a set of 96 precut sheets of the same size paper, consisting of 32 sheets of each of the upper, middle, and lower ones, was counted by turning over all the sheets one by one by fingers. After counting, the fingers were washed with n-hexane once and the resulting n-hexane washings were examined for PCBs. The amount of PCBs in the washings, calculated as Kanechlor-300, ranged from 11.40 to 52.68 μg in 5 subjects (Table 2). It was proved thus that PCBs in the paper easily stick to fingers when handled. It was also demonstrated that only one third of the PCBs stuck to fingers can be removed by ordinary hand washing with soap and water (Table 2).

In addition to the above domestic brands of [Japanese] paper, a few other samples of non-carbon copy paper were analyzed for PCBs. One of them was a brand made by one of the leading paper makers in Britain and was obtained as packed in its original container in May, 1971. Analysis showed that both upper and middle

Table 1. Amounts of PCB in carbonless copying paper.

Brand	Kind of paper	Weight of paper (g/100 cm²)	Amount of acetone extract[a] (mg/g)	Amount of PCB found[b] (mg/g)
A	Upper	0.47	117.0	64.7
	Middle	0.56	125.8	63.8
	Lower	0.51	23.4	0.24
B	Upper	0.48	40.8	30.7
	Middle	0.55	37.8	26.0
	Lower	0.51	79.8	0.28
C	Upper	0.40	59.6	31.6
	Middle	0.53	61.0	22.2
	Lower	0.50	28.2	0.20

[a] Weight of extract per weight of paper.
[b] Weight of PCB calculated as Kanechlor-300 per weight of paper.

* Department of Public Health, Faculty of Medicine, Kyushu University and Department of Analytical Chemistry, Daiichi College of Pharmaceutical Sciences, Fukuoka, Japan.

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sheets of this make contained PCBs. The second sample, which was of American make and obtained as packed in its original container in June, 1971, also contained a considerable amount of PCBs. Another sample of paper, which was sent to us for examination by a company in Thailand, however, did not contain PCBs. We were told that it is also of American make. A sample of non-carbon copy paper which was used by the U.S. Printing Office in 1967 was obtained from the American Consulate in Fukuoka and analyzed for PCBs. It also contained a large amount of PCBs.

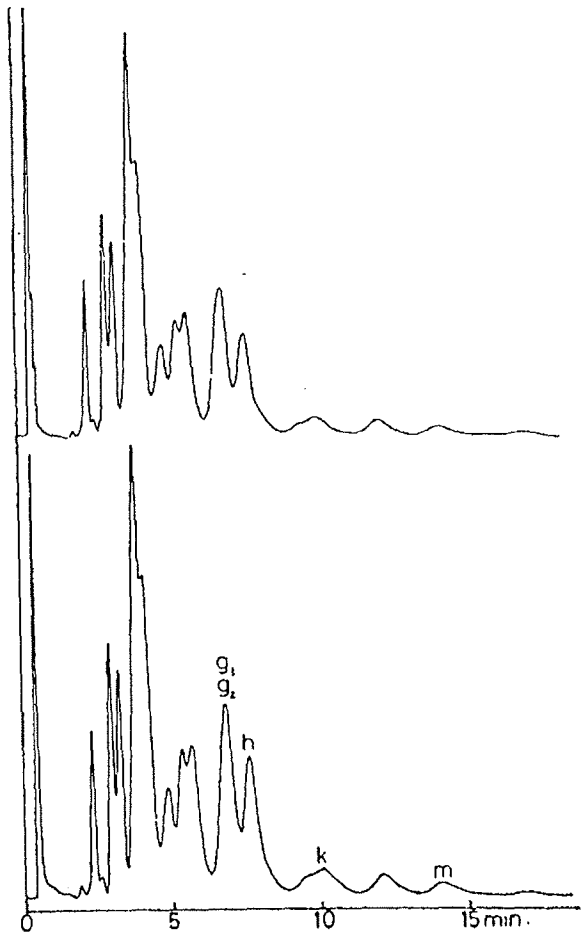

FIGURE 1. Gaschromatograms of kanechlor-300 (upper) and the acetone extract of copying paper (lower). Column: Glass column (3mm×2m) containing 5% SE-30 on chromosorb W AW DMCS. Column temperature: 195°C.

Table 2. Amounts of PCB found from fingers after counting 96 sheets of paper.

Subject	Amount of PCB[a]	
	Washed with n-hexane (μg)	Washed with n-hexane after hand washing with soap (μg)
1	29.76	16.39
2	12.60	10.47
3	11.40	3.50
4	52.68	47.15
5	44.68	25.45
Average	30.22	20.60
Standard deviation	16.60	15.11

[a] Calculated as Kanechlor-300.

Needless to say, the free use of PCBs for this kind of very popular copy paper necessarily leads to the uncontrollably heavy, direct exposure of the general population to it. In view of its chemical and pathological characteristics, we consider that the use of PCBs for copy paper should be discontinued in any country in order to prevent another occurrence of "Yusho". The Japanese makers of the paper are said to have acted quickly along this line in April, 1971. To our regret, however, there have been no control measures set up by the government against the use of PCBs and a considerable amount of the PCB-polluted papers are still widely used in Japan.

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PCB and the Paper Industry— A Progress Report

by Paul E. Trout*

The paper industry's experiences with PCBs were chronologically reviewed at a recent "PCB Workshop" sponsored by the Grocery Manufacturers of America, Inc. (1). This history goes back somewhat over a year, at which time two paperboard manufacturers were apprised of the presence of PCBs in folding cartons for packaging dried fruit.

These two manufacturers immediately began searching for the source of the PCB contamination, screening all converting materials used in making cartons from the paperboard. These materials, including printing inks, varnishes and adhesives, proved not to be the source of the contamination. Examination of the fibrous materials used in the paperboard showed the source of PCB contamination to be some of the paperstocks used in manufacturing the paperboard. Certain high quality white and colored paperstock grades, such as ledger papers, were found to contain carbonless carbon papers which were the source of the PCBs.

PCBs at that time were used as one component of the dye carrier in the carbonless carbon paper system.

The American Paper Institute, the industry association, alerted the paper industry to this source of PCB contamination. Combination paperboard manufacturers, those who use recycled fibers in paperboard manufacture, immediately adopted operating procedures to eliminate the use of carbonless carbon papers and thus to minimize the PCBs in the paperboard.

This action unquestionably was essential. Yet, in one respect, it does run counter to the national interest and the goals of the solid waste program of the Environmental Protection Agency. The combination paperboard industry now uses 70% of all paper that is recycled in the United States. Additionally, the paper industry is being implored by government agencies to recycle more wastepaper in order to help combat our mounting solid waste problem.

Continuing research by the industry also showed that virgin fiber grades of paper and paperboard sometimes contained measurable amounts of PCBs, even though no carbonless carbon papers were used in their manufacture. Since PCBs have been established to be widespread in both our air and water environment (2, 3, 4, 5), the presence of PCBs in virgin fiber products is felt to be a reflection of these background environmental levels.

The Monsanto Company, sole domestic manufacturer of PCBs, withdrew PCBs from sale for other than closed system applications as of September 1, 1970. The manufacturers of carbonless carbon papers discontinued the use of PCBs in their product on June 1, 1971. These actions, together with the paper industry's elimination of carbonless carbon papers from paperboards used for food packaging, have resulted in marked reductions in the levels of PCBs in combination paperboard. They are also expected to show decreasing environmental or background PCB levels in the future.

Under the sponsorship of The American Paper Institute (A.P.I.), research is currently in progress at Hazleton Laboratories, Inc. to study the mechanism by which PCBs migrate from paperboard to various food products and, also, to survey the levels of PCBs in United States pulps, papers and paperboards.

The former study is only well begun so I am

* Director, Environmental Control, Container Corporation of America, Oaks, Pennsylvania 19456.

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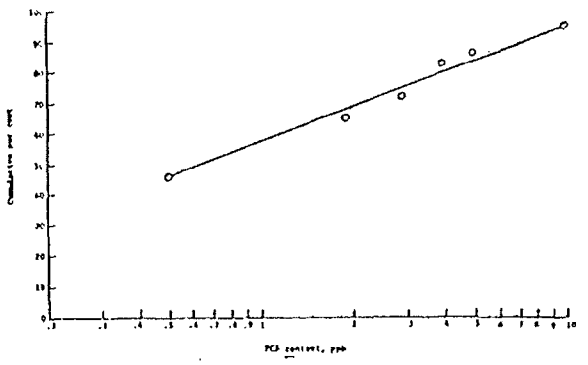

FIGURE 1. Cumulative-distribution of PCB in 676 samples of paper and paperboard.

unable to present any meaningful data on migration to you at this time. The survey study is progressing smoothly, however, with approximately 676 samples having been analyzed to date. The results of these analyses are shown as a cumulative-distribution in Fig. 1. They have not yet been classified according to paper or paperboard type, so represent a broad spectrum of virgin and reclaimed paper and paperboard materials. This cumulative distribution shows 94% of all the samples to have PCB contents less than 10 ppm; 86% less than 5 ppm; and 46% less than 0.5 ppm (not detectable).

A second survey study is being sponsored by the Boxboard Research and Development Associ-

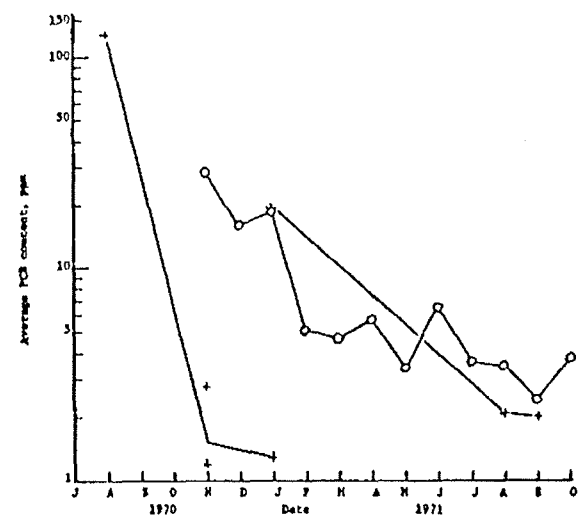

FIGURE 2. Effect of furnish control on PCB content of combination paperboard.

Table 1. Estimated PCB content of typical packaged foods.

Product	Product weight, oz.	Carton: product ratio	Calculated ppm PCB in food product for 33% transfer from cartons containing	
			5 ppm	10 ppm
Rice	42	0.048	0.08	0.16
	28	0.045	0.07	0.14
	14	0.053	0.09	0.17
Uncooked cereal	22	0.07	0.11	0.22
	11	0.096	0.16	0.32
Candy gum drops	6	0.08	0.13	0.26
Spaghetti	48	0.056	0.09	0.18
	16	0.06	0.10	0.20
	6	0.136	0.22	0.45
Raisins	15	0.052	0.09	0.17
	1.5	0.13	0.22	0.44

ation at The Institute of Paper Chemistry. This study is concerned only with recycled fiber paperboards. Preliminary data from this study indicate a mean PCB value of 3.6 ppm and an average PCB value of 6.4 ppm.

That the prompt action of the paper industry has indeed been effective in reducing PCB levels in food packaging materials can be seen from Fig. 2. Figure 2 depicts the change in PCB content with time for several grades of recycled fiber paperboard, where sufficient data were available to permit reconstruction of such a history. Similar histories could be developed for other food-packaging grades. This however would not add materially to our understanding of the problem more than is already seen from Fig. 2.

Although we do not yet have the results of the A.P.I. migration study, preliminary research by one A.P.I. member indicates that PCB migration from paperboard to high fat-content products does not exceed 33% of the total PCB in the paperboard, even in the absence of any protective barrier between the paperboard and the product. Based on this 33% migration factor and PCB levels of 5 to 10 ppm as being common in paper and paperboard, we can estimate expected levels of PCBs in products packaged directly therein.

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Such calculations are shown in Table 1 for typical foods now packaged in paperboard cartons. Since, in most instances, the ratio of carton weight to product weight is normally of the order of 1 to 20, PCB levels in food products on the market today would be expected to be generally well below one-half ppm.

This expectation is in reasonable agreement with the preliminary results of the Food and Drug Administration food survey presented by Dr. V. O. Wodicka at the previously mentioned workshop [1]. With one exception (0.6 ppm), the mean values of PCBs in fifteen food commodities ranged from 0.005 ppm to 0.4 ppm.

These preliminary data indicate that PCB levels in currently manufactured paper and paperboard food packaging materials are not such as to represent a hazard to public health. These present levels are expected to decrease further as corrective actions, that have been taken by the industries involved, become fully effective.

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DSW 025093

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Levels of Polychlorinated Biphenyls in Foods in Sweden

by Fredrik Berglund, M. D.*

PCBs have been used in industry in Sweden since 1929, initially only in electric transformers and capacitors, later also in paints, lacquers etc. The importation of PCBs to Sweden in 1970 was close to 500 tons. Approximately half of this amount was exported to other countries in electric capacitors. The use of PCBs in dyes was 65 tons in 1968 but was voluntarily diminished by industry to 35 tons in 1970. The Swedish Riksdag (parliament) has enacted the 1971 PCB Act, authorizing the government to prescribe conditions regarding importation, production, offering for sale and handling of PCBs, but detailed regulations have unfortunately not yet been published.

The high degree of persistence and lipid solubility of the PCBs have led to their accumulation in some foods of animal origin. In Sweden they now occur in the natural environment roughly in the same amounts as the chlorinated pesticides (1). The special hydrographical conditions of the Baltic favor the accumulation of PCBs in certain species and areas. The water exchange with the North Sea is very limited because of the thresholds (8 m and 18 m) and the narrowness of the Danish Sounds. The wide archipelagos at some places also make possible in some localities high concentrations of pollution, e.g., outside of Stockholm.

Table 1 briefly summarizes some of the earlier data, illustrating these points. The difference in levels of total DDT equivalents and PCBs between the Swedish west coast and the Baltic is more than 5-fold in fish oil and almost 10-fold in herring. The levels in fat are relatively high in all species shown. The role of the food chain is shown by the high levels in seal and by the extremely high levels in the white-tailed eagles from the Stockholm archipelago. Their muscle fat contained about 2.5% of total DDT metabolites and 1.4% of PCBs. The Stockholm archipelago is evidently heavily contaminated (cf below). The very high levels of DDT and of PCBs in muscle fat compared with the levels in eggs are most probably due to sampling from unrelated individuals. The muscle samples were from sick birds, whereas the eggs derived from apparently healthy birds.

There are only few data from seal. The levels of ΣDDT and of PCBs per kg fat show fair agreement between seal pup and seal milk (from the stomach of one of the pups).

The samples were analyzed by gas chromatoggraphy and electron capture detection, combined with treatments with sulphuric acid and potassium hydroxide. The principle PCB components were found to have 7–14 chlorine atoms, with an increase of those with higher chlorine content as the PCBs passed along the food chain.

Analyses of PCBs were later taken up by Westöö & Norén (2). After extraction and routine clean-up, the extracts were separated by thin-layer chromatography into two groups, one of which contained PCBs and p,p′-DDE, o,p′-DDT, o,p′-DDE. The PCBs were determined by gas chromatography of this group, before and after oxidation of the organochlorine pesticides. Some of the PCBs were analyzed by gas chromatography without decomposition of the organochlorine compounds. Recoveries averaged 96–101% for PCBs added to purified fish extracts before application to thin-layer plate (2).

* Professor and head, Food Research Department, National Food Administration, 104 01 Stockholm 60, Sweden, as of January, 1972; former head, Department of Food Hygiene, same address.

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Table 1. Average levels of organochlorine compounds in some Swedish marine organisms 1965–1968. Modified from (1).

Species	Locality	Fat content	ΣDDT mg/kg fat	mg/kg	PCB mg/kg fat	mg/kg
Herring	W. coast		1.9		0.75	
	Baltic (18)	4.4%	17	.68	6.8	.27
Fish oil	W. coast	100%	2		.7	
	Baltic	100%	16		3.5	
White tailed eagle						
muscle	Stockholm (4)	1.5%	25 000	330	14 000	190
eggs	Stockholm (5)	5.6%	1 000		540	
Seal	Gulf of Bothnia (2)	54%	120	63	13	6.8
Seal pup	Gulf of Finland (2)	60%	42	25	6.5	3.9
Seal milk	Gulf of Finland (1)	31%	36	11	4.5	1.4

Table 2. Levels of DDT, DDE, DDD and PCBs in some fish in Sweden 1967–1969. Modified from (2).

Fish	(n)	Range mg/kg	DDT n	DDE n	DDD n	PCB n
<4% fat freshwater	(87)	0–0.1	86	84	87	86
		0.1–1.0	1	3		1
<4% fat saltwater	(65)	0–0.1	57	59	65	58
		0.1–1.0	8	8		7
>4% fat saltwater	(34)	0–0.1	9	14	23	13
		0.1–1.0	24	20	11	19
		1.0–3.0	1			2

Table 3. Levels of organochlorine compounds in fish in Sweden 1967–1970. Modified from (3).

Species	Locality (No. of samples)	Fat content	ΣDDT mg/kg	PCBs mg/kg
Herring, *Clupea harengus*	Baltic (72)	3–20%	1.17± .061	0.49± .061
	S. Baltic, deep-frozen (16)	3–20%	1.40± .079	0.27± .017
Eel, *Anguilla anguilla*	Baltic (43)	25%	0.50± .038	0.28± .036
	W. coast (11)		0.26± .056	0.43± .134
Cod, *Gadus morrhua*	Baltic (30)	<2%	0.05	0.02
			(0–0.2)	(0–0.1)
Cod liver	Baltic (7)	40–70%	10–22	2–5
	W. coast (3)		2–5	2–4
Salmon, *Salmo salar*	W. coast (5)	10–15%	0.06	Trace
			(.04–.07)	
	Lakes (5)	10–15%	2.63± .72	0.58± .177
Pike, *Esox lucius*	Lakes (37)	<2%	0.06	<0.1
			(0–0.3)	(0–0.6)

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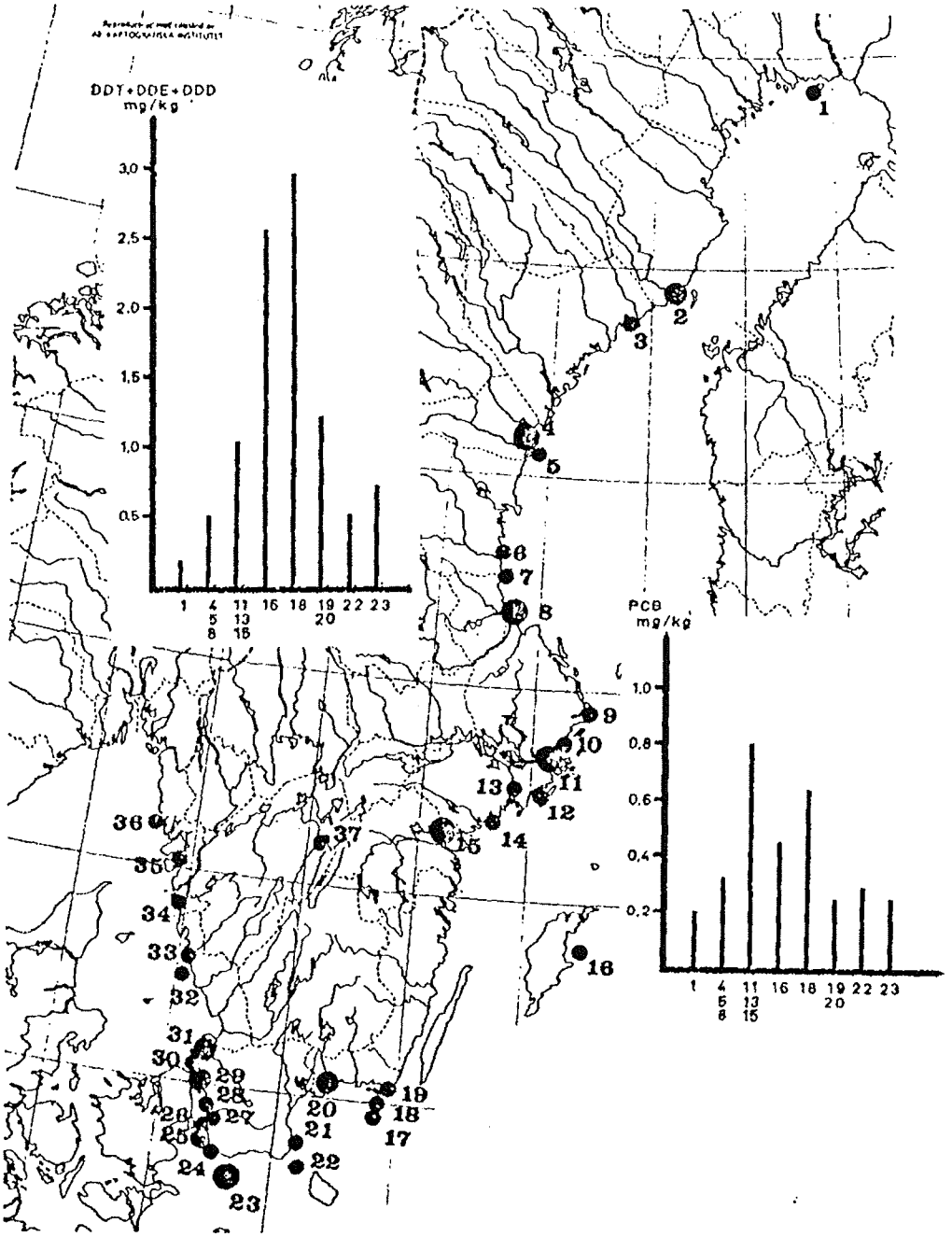

FIGURE 1. Average levels of organochlorine compounds in Baltic herring from different water areas. Modified from (3).

The preliminary survey of organochlorine compounds and PCBs in less than 200 samples of fish is summarized in Table 2. Levels of DDT, DDEs or PCBs above 0.1 mg/kg were only rarely seen in lean fish, i.e. in fish with less than 4% fat in their flesh, but occurred in more than half of the samples with more than 4% fat.

Westöö & Norén (3) later published analyses on about 1,000 samples of fish from lakes, rivers, the Baltic Sea and the Atlantic Ocean (Table 3).

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Table 4. Levels of DDT, DDE and PCBs in some foods in Sweden 1967–1969. Modified from (4).

Food	(n)	Range mg/kg	DDT n	DDE n	PCBs n
Margarine	(115)	0–0.1	115	115	115
Vegetable oils	(197)	0–0.1	196	197	197
		0.1–1.0	1		
Beef	(34)	0–0.1	34	34	34
Lamb fat	(10)	0–0.1	10	10	10
Pork	(107)	0–0.1	101	104	106
		0.1–1.0	6	3	1
Chicken	(93)	0–0.1	93	93	93
Hen	(50)	0–0.1	50	49	50
		0.1–0.3		1	
Egg white[a]	(28)	0–0.1	28	28	28
Egg yolk[a]	(185)	0–0.1	176	176	183
		0.1–1.0	8	8	2
		1.0–6.0	1	1	

[a] Six in each sample.

The levels of ΣDDT and of PCBs depended on the fat content of the fish and on geographical location.

Regular salmon, *Salmo salar*, with 10–15% fat, had an average DDT level of 0.06 mg/kg on the west coast and 2.6 mg/kg in lakes, and of PCBs only traces on the west coast but 0.6 mg/kg in the lakes. Herring and eel, both with fairly high fat contents, had high levels. The highest PCB levels in fish were found in 2 small samples of herring from the Stockholm archipelago, 2.8 and 2.5 mg/kg, and in 4 yellow eels close to Gothenburg, 1.5 mg/kg. Five chars, *Salmo salvelinus*, in Lake Vättern had an average fat content of 7% and PCB level of 1.3 mg/kg (not included in Table 3).

Cod and pike, both with less than 2% fat, usually had very low DDT and PCB levels. Cod liver, on the other hand, with 40–70% fat, had high levels of DDT and PCBs. Because of its high levels of DDT, cod liver from the Baltic was declared unfit for human consumption by the Swedish health authorities this year (1971).

Some of the geographical variation of PCB and DDT levels in herring is shown in Fig. 1. PCB levels are highest outside of Stockholm, whereas DDT is higher off Gotland (16) and Blekinge (18–20). Other PCB-contaminated fish mentioned above were the eels caught off Gothenburg (34) and the chars in Lake Vättern (37).

In other foods the levels of DDT, DDE and PCBs were much lower than in fish (4), as shown in Table 4. With few exceptions, all the samples of the foods listed contained less than 0.1 mg/kg of each compound. Egg yolk showed some high levels, but PCB levels in whole eggs would still not be expected to exceed 0.1 mg/kg.

Table 5 shows the results of analyses on cow's milk, butter, and human milk. Analyses on cow's milk showed DDT less than 0.1, DDE less than 0.3 and PCBs less than 0.5 mg/kg on a fat basis. Analyses on a much larger number of samples of butter revealed levels of DDT below 0.13, of DDE below 0.1 and of PCB below 0.1 mg/kg, also on a fat basis. The difference between cow's milk and butter in terms of DDE and PCBs is probably only a matter of sensitivity of the method—there is no reason to suspect losses of DDE and PCBs in the churning process.

In the analysis of human milk there was no need to look for "human butter", since the levels of DDT, DDE and PCBs were much higher than in cow's milk. The DDT levels in whole milk show fair agreement with those in the U.S. (5) and England (6). The PCB levels average 0.5 mg/kg fat or 16 μg/kg whole milk. This average would probably only represent the Stockholm area, since all the samples originated from a "human milk donor center" in Stockholm.

Recently Westöö et al. (7) also discovered PCBs in some samples of cereal products. The origin was found to be the packaging material. On thin-layer chromatography and on gas chromatography, the PCBs behaved as PCBs of type Clophen A 60. In several samples of one kind of imported children's food, the PCB levels exceeded 1 mg/kg. In one sample of this product, which had been stored at room temperature for two years, the level of PCB had increased to 11 mg/kg. The sale of this children's food in Sweden was stopped in July, 1971.

The main intake of PCBs via the food in Sweden thus occurs by eating fish. The average consumption of fish in Sweden 1961–1969 was 17 kg/person/year—and of crustaceans and molluscs, about 1 kg/year. In terms of fish flesh Westin (8) calculated an intake for 1965 of 35 g per person and day. Of this, about 2 g represent

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Table 5. Levels of DDT, DDE and PCBs in butter, cow's milk and human milk in Sweden 1967–1969. Calculated from (4).

Food (n)	Fat	DDT	DDE	DDT+DDE	PCBs
Cow's milk (11)	~3%	<0.1 mg/kg fat	<0.3 mg/kg fat		<0.5 mg/kg fat
Butter (154)	>81%	<0.13 mg/kg fat	<0.1 mg/kg fat		<0.1 mg/kg fat
Human milk (22)	3.25 ±0.11%	1.24±0.07 mg/kg fat	2.08±0.13 mg/kg fat	3.32±0.19 mg/kg fat	0.50±0.039 mg/kg fat
		40±3.0 µg/kg milk	67±6.0 µg/kg milk	106±8.8 µg/kg milk	16±2.5 µg/kg milk

freshwater fish, 1 g caviar, and 1 g shellfish and molluscs together. The average intake of PCBs therefore probably does not exceed 1 µg per day but may well exceed 10 µg/day in people eating herring from the Baltic 3 times a week.

A suckling baby ingests about 0.15 litre of mother's milk per kg body weight daily in the 2nd and 3rd months of life, with an average PCB level of 15 µg/kg milk. This corresponds to 2.4 µg/kg body weight per day. Thus we have a situation similar to that with DDT with the suckling baby getting the highest exposure. Also, it is known from Japan that PCBs pass the placental barrier (9).

Conclusions. Proper food processing should not produce PCB levels higher than 0.1 mg/kg food, except in fish with more than 2% fat in edible portions. Human milk has low levels of PCBs, but due to the high intake of milk, the suckling baby will experience the heaviest exposure.

To avoid further food contamination in the future, the use of PCBs should only be allowed in closed systems and under most careful supervision. Less dangerous substitutes should be looked for in industry.

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Occurrence of Polychlorinated Biphenyls in Humans*

by Harold A. Price and Robert L. Welch†

In 1967 the Human Monitoring Survey (1) was established by the Pesticides Program of the U.S. Department of Health, Education and Welfare (now, Division of Pesticide Community Studies, E.P.A.) to determine on a national scale the levels and trends of certain more commonly-used pesticide chemicals in the general population. Initially, this program was limited to the identification and measurement of chlorinated hydrocarbon residues in human adipose tissue and blood serum. The Michigan Department of Public Health has been one of several state health laboratories participating in the analysis of samples, collected by the State Services Branch of the Division of Pesticide Community Studies, from hospitals located in approximately 30 states. For the purpose of obtaining poolable data, the state laboratories use methodologies recommended by the Perrine Primate Laboratory, E.P.A. (Florida), which is responsible for methodology development, the operation of the quality control program, and serves as a repository of reference standards.

Since 1966 our laboratory has been conducting routine analysis of human adipose samples for organochlorine pesticide residues by gas chromatography, utilizing electron capture detection. In many instances, we observed numerous unidentified peaks recurring in a repeated pattern on chromatograms, some eluting much later than p,p'DDT Our attention became focused on polychlorinated biphenyls (PCBs), after the Perrine Primate Laboratory alerted laboratories involved with the human monitoring program and community studies on pesticides of the possible interference of pesticide residue analysis by these compounds. This was prompted by early reports of research workers in Europe and the United States finding PCB residues in animal and environmental media (2, 3, 4). Early confirmation of some of these compounds had been obtained with a combined gas chromatograph-mass spectrometer from extracts of fish, sea birds, conifer needles, and some samples of human depot fat (5).

In March, 1969, one of Michigan's state laboratories found 19 ppm DDT in canned coho salmon during routine residue analysis. Our residue analysis on a similar sample seemed to corroborate this finding. However, considerable interference by "artifact" peaks was evident on the chromatogram, causing difficulty in both qualitative and quantitative analysis (2, 6) of pesticide residues. To a lesser extent, similar peaks had been observed with some human adipose samples. These unknown peaks were ascertained later as polychlorinated biphenyls when compared with several Monsanto Aroclors by electron capture gas chromatography and microcoulometry.

Prior to receipt of Monsanto Aroclors, we found a readily available source of a PCB indicator, e.g., residue analysis report forms. One of us had known by previous experience that carbonless copy paper has on one side a coating of microcapsules containing an Aroclor solution of a colorless dye (16). An extract of this paper pro-

* The investigations reported herein were supported through a contract with the Division of Pesticides Community Studies, Office of Pesticides, Environmental Protection Agency, Chamblee, Georgia. The views expressed herein are those of the investigators and do not necessarily reflect the official viewpoint of the Enviromental Protection Agency.

† Michigan Department of Public Health Pesticides Laboratory, Michigan 48914.

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duced a chromatogram which matched a considerable portion of the interference observed with residues in coho salmon and human adipose tissue.

During the year following the coho salmon incident, our laboratory found substantial levels of polychlorinated biphenyls in several samples of human adipose tissue collected through the Human Monitoring Survey program. Three of these examples are given here to illustrate our findings.

Materials and Equipment

Materials and glassware used for extraction of samples and preparation cleanup of extracts are described fully elsewhere (7, 8).

A Micro-Tek Model 220 gas chromatograph (Tracor, Inc.) was used. It was equipped with two parallel-plate, tritium-source electron capture detectors, Vycor glass inserts installed at inlet ports to accommodate off-column injection, and U-shaped Pyrex glass columns (6 ft. × ¼ in. o.d.). Column packings (9) supplied by the Perrine Primate Laboratory consist of 1.5%OV-17/1.95%QF-1 (Column 1) and 4%SE-30/6%QF-1 (Column 2) on 80/100 mesh Chromosorb W HP. Operating temperatures of columns, detectors, and inlet were 200°C, 210°C, and 225°C, respectively. Carrier gas was nitrogen at flow rates of 65 ml/min through column 1 and 80 ml/min through column 2.

Reference standards of 1200 series Aroclors and organochlorine pesticides were obtained from the Pesticides Repository, Perrine Primate Laboratory, E.P.A., Perrine, Florida. Aroclors, products of Monsanto Company, are designated by numbers, such as 1260 which represents a mixture of chlorinated biphenyls having an average chlorine content of 60%.

For thin-layer chromatography, glass plates (Analtech, Inc.) precoated with aluminum oxide G, 250 microns thick, impregnated with 3.3% silver nitrate were used.

Method

The analytical scheme used for the preparation of these samples was the modified Mills procedure (8). One to three grams of tissue were extracted with hexane, partitioned with acetonitrile, and subjected to fractionation and cleanup by Florisil column chromatography. All of the PCB compounds were eluted in the first fraction (6% ethyl ether in petroleum ether), and this volume of eluate (200 ml) was reduced in a Kuderna-Danish concentrator to 10 ml. A 5-μl aliquot was injected into the gas chromatograph. The extracts were diluted as required to obtain peaks within the linear range of the detector.

For certain selected samples, the polychlorinated biphenyls were separated from the organochlorine pesticide residues by application of the method of Armour and Burke (10). The 6% Florisil eluate was chromatographed on the silicic acid-Celite column with 250 ml of petroleum ether, which eluted the PCBs. The organochlorine pesticides were recovered next after elution with 200 ml of acetonitrile-hexane-methylene chloride (1:19:80).

Results and Discussion

In Fig. 1, electron capture chromatograms of human adipose tissue extract M69305 (6% Florisil eluate) and a standard solution of Aroclor 1260 are presented to illustrate that some of the "artifact" peaks in this tissue sample match those in the PCB standard. On the 4%SE-30/6%QF-1

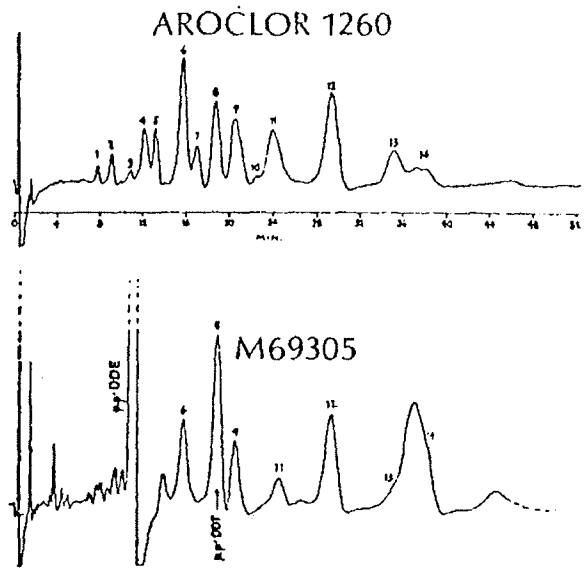

FIGURE 1. GLC-EC chromatogram of Aroclor 1260 (1.5 ng) and human adipose sample M69305. GLC conditions: 6'×¼" 4% SE-30/6% QF-1 column, oven temp. 200°C, and nitrogen flow 75 ml/min.

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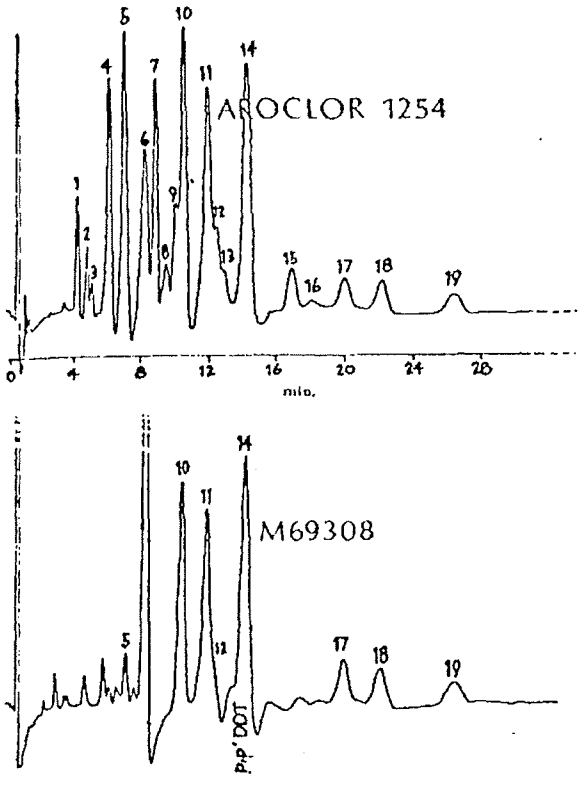

FIGURE 2. GLC-EC chromatogram of Aroclor 1254 (5.1 ng) and human adipose sample M69308. Peak 1' is 2,2',5,5'-tetrachlorobiphenyl, and peak 5 is 2,2',-4,5,5'-pentachlorobiphenyl. GLC conditions: 6'×¼" 4% SE-30/6% QF-1 column, oven temp. 200°C, and nitrogen flow 75 ml/min.

column, p,p'DDT is completely masked by Peak 8 of Aroclor 1260; however, no interference is encountered with the quantitation of p,p'DDE.

On the basis of a major peak following p,p'DDT, the level of PCBs in this sample was estimated to be seven parts per million as Aroclor 1260. For estimating PCB levels by gas chromatography, we use Peak 12, preferably on 1.5%OV-17/1.95%QF-1 column, as a reference in Aroclor 1260 which has the following retention time relative to aldrin (R_t = 1.00):

Column	Aroclor Peak	p,p'DDE	p,p'DDT
OV-17/QF-1	6.4	2.23	4.18
SE-30/QF-1	4.83	1.82	3.12

In this sample, PCBs ranging from hexachlorobiphenyls to decachlorobiphenyl were confirmed by combined gas chromatography-mass spectrometry (11).

Fig. 2 shows that tissue extract M69308 contains some components which are present in Aroclor 1254. Approximately 20 ppm PCBs as Aroclor 1254 was determined by gas chromatography. Biros (11) confirmed the presence of pentachlorobiphenyls and hexachlorobiphenyls in addition to p,p'DDE. The largest unlabeled peak following Peak 5 in sample M69308 is p,p'DDE. The level of p,p'DDT in this sample was too low for confirmation by mass spectrometry, and its peak is masked by Peak 14 of Aroclor 1254 on the SE-30/QF-1 column used in this illustration.

In the spring of 1970, we found approximately 100 ppm PCBs in a sample of subcutaneous adipose tissue (M70227) which was obtained from a hospital in southeastern Michigan. The subject was a white male, age 77, whose demise followed a pulmonary thrombo embolism.

An electron capture chromatogram of the 6% Florisil column eluate of this sample is illustrated in Fig. 3 and is compared with a chromatogram of Aroclor 1260. This extract contains late eluting isomers that are also components of Aroclor

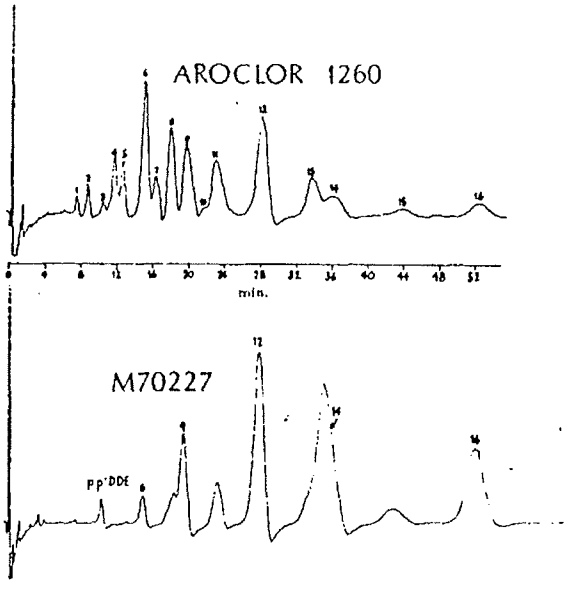

FIGURE 3. GLC-EC chromatogram of Aroclor 1260 (2.1 ng) and human adipose sample M70227 (30.5 μg; 6% Florisil fraction). GLC conditions: 6'×¼"% SE-30/6% QF-1 column, oven temp. 200°C, and nitrogen flow 80 ml/min.

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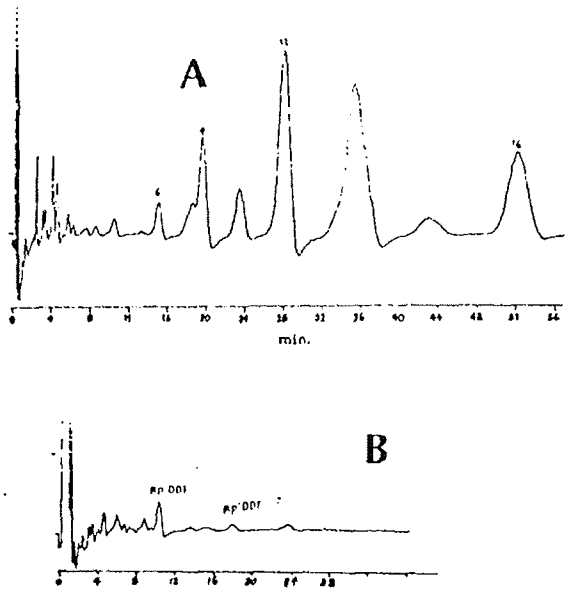

FIGURE 4. GLC-EC chromatogram showing separation of PCB (A) and organochlorine pesticides (B) by method of Armour and Burke. GLC conditions same as Fig. 3. Weight of sample injected 30.5 μg.

1268, which makes matching to any particular Aroclor difficult. Fig. 4 shows the separation of PCBs from organochlorine pesticides by the method of Armour and Burke (10), although some of the p,p'DDE eluted in Fraction A in this case. On the basis of a chromatogram of the second eluate from the silicic acid-Celite column resulting from a subsequent effort, residues of 0.37 ppm p,p'DDT and 0.81 ppm p,p'DDE were found in the fat of this sample. Using the combined gas chromatograph-mass spectrometer, Biros (12) confirmed the presence of p,p'DDE in M70227 as well as ten polychlorinated biphenyls ranging, from hexachloro- to nonachloro-compounds. These compounds are found in Aroclors 1260, 1262, and 1268.

Further examination of other tissues of the same autopsy case revealed a good correlation of PCB levels when compared on the basis of extractable lipids as demonstrated in Table 1. These data are good estimates calculated from the height of a peak corresponding to Peak 12 of Aroclor 1260 on the OV-17/QF-1 column. PCB peaks in M70227 appear to be a complex mixture of several Aroclors not usually observed in human adipose tissue.

Efforts to determine the source of PCBs in sample M70227 have not been successful. Indications are that this individual was exposed to PCBs during his lifetime. As PCBs enter a biological system, the less chlorinated isomers are either metabolized or excreted, which changes the composition of original Aroclors, so that chromatograms of these mixtures from biological samples do not match exactly those of reference standards. This has also been observed with PCBs in M70227. In fact, more than 4,000 human adipose samples examined in our laboratory have produced chromatograms that do not exactly match those of standard Aroclor solutions. We have found the distribution of PCBs in samples, such as M70227, to be uniform throughout the sample as received, indicating that these samples have not been contaminated by PCBs during handling. As for all samples, M70227 was collected from an autopsy examination through the cooperation of pathologists, according to directions specified by the State Services Branch of the Division of Pesticide Community Studies, E.P.A.

As many investigators of PCBs have found, one of the principal sources of polychlorinated biphenyls in the biological system is the food chain (5, 13, 14). Coho salmon, for example, had been a source of PCBs in food until it exceeded the action level of 5 ppm.

Another possible avenue of human exposure to polychlorinated biphenyls may be indicated by their presence in some house dust (vacuum sweepings). Our examination of house dust from residences of occupationally-exposed population of southwestern Michigan revealed that several samples contained up to 180 ppm PCBs, mainly Aroclor 1254.

Table 1. Levels of PCB's in Various Tissues Related to Sample M70227*

Tissue	% Lipid ext'd	PCB level, ppm	
		Wet weight basis	Fat basis
S. Adipose*	41.1	75	180
S. Adipose	63.9	100	155
S. Adipose	32.8	50	150
P. Adipose	44.1	50	115
Liver	0.08	2	250
Kidney	0.06	0.75	125

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Table 2. Distribution of PCB Levels in Adipose Tissue of the General Population—Analysis by Gas Chromatography

Level, ppm	Samples	Percent of total
Negative	4	2.0
Trace to <1.0	109	55.6
1.0–2.0	72	36.7
>2.0	11	5.6

As mentioned previously, levels of polychlorinated biphenyls in human tissue extracts illustrated in Figs. 1, 2, and 3 were determined by electron capture gas chromatography using a major peak after p,p'DDT (Peak 12) as a reference peak for Aroclor 1260. Since April, 1971, our laboratory has run semiquantitative analysis of polychlorinated biphenyls in samples of human adipose tissue by both electron capture gas chromatography and thin-layer chromatography. The latter based on total chlorine is an adaptation of a procedure reported by Mulhern et al., (7, 15). After gas chromatographic analysis is completed, the remaining extract is evaporated, and pesticide residues are dehydrochlorinated with ethanolic potassium hydroxide and oxidized with chromic oxide. The concentrated extract of PCBs remaining is spotted on a thin-layer plate, and the developed spots after exposure to ultraviolet light are compared with several spots of standard Aroclor 1260 at different concentrations. The limit of detection is 250 ng of Aroclor 1260. Results in Tables 2 and 3 indicate that both methods provide similar information, but thin-layer chromatography is preferred. On the basis of samples received and analyzed by our laboratory, these data show the distribution of PCB levels in adipose tissue of the general population.

Table 3. Distribution of PCB Levels in Adipose Tissue of the General Population—Analysis by Thin-Layer Chromatography

Level, ppm	Samples	Percent of total
Negative	20	10.3
Trace to <1.0	87	44.8
1.0–2.0	70	36.1
>2.0	17	8.8

Summary

According to samples of human adipose tissue received by our laboratory, 41–45% of the general population have levels of 1.0 ppm or more (wet weight) polychlorinated biphenyls with isomers from Aroclors 1254, 1260, 1262, and 1268. The presence of PCBs ranging from pentachlorobiphenyls to decachlorobiphenyl has been confirmed in selected samples, M69305, M69308, and M70227, by the use of combined gas chromatography-mass spectrometry.

Acknowledgements

We thank Dr. Anne R. Yobs of the Division of Pesticide Community Studies, Environmental Protection Agency, Chamblee, Georgia, for providing the human adipose tissue samples described in this study, and Dr. Henry F. Enos for consultation and advice. We thank also Dr. Ronald G. Webb of the Southeast Water Laboratory, Athens, Georgia, for providing samples of a pure tetrachlorobiphenyl and a pentachlorobiphenyl.

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Levels of Polychlorinated Biphenyls in Adipose Tissue of the General Population of the Nation

by Anne R. Yobs, M.D.*

Polychlorinated biphenyls (PCBs) have been reported in many different parts of the environment in this country (1-3). The purpose of this report is to present preliminary results from a monitoring program in which these materials are routinely sought and quantitated.

The Human Monitoring Survey, established in 1967 by the Pesticides Program, DHEW (now Division of Pesticide Community Studies, EPA), determines, on a continuing basis, the exposure to pesticides experienced by the general population by measuring levels of pesticide residues present in tissues or excreted in urine. Initially, technological limitations and resource restrictions limited the Survey to the identification and measurement of those chlorinated hydrocarbon residues which are stored in measurable amounts in mammalian adipose tissue, or which can be measured in blood, these residues being a reflection of past exposure. The initial program plan has been and continues to be reviewed frequently with reference to technological and/or research developments to identify other groups of pesticides and other materials of interest, which can be incorporated with slight modification of the existing program.

Samples are collected through the direct cooperation of pathologists, who are in hospital or private practice or who are in public service as city or county medical examiners. Samples are collected from both sexes in all age and racial groups. Adipose samples are collected from tissues removed for therapeutic surgery or from postmortem examinations, the latter including people who have died accidentally (trauma) or during relatively brief hospitalization. No samples are accepted from institutions for long-term care of the chronically ill patient.

All samples are analyzed by laboratories established under contract with State Health Departments or at community study projects in Michigan, Florida and Colorado. These laboratories use analytical methodologies specified by the program on the advice of technical consultants and are required to maintain acceptable levels of performance, as demonstrated in both inter- and intra-laboratory quality control programs. Technical consultation for analytical aspects of the Survey is provided by the Chemistry Branch, Perrine Primate Laboratory, EPA, under the direction of Henry F. Enos, Ph.D., Chief.

In 1969, with the increased interest in polychlorinated biphenyls, it was recognized that PCBs probably occur in several segments of the environment and that their presence would distort analytical values for certain pesticides, especially selected DDT residues. The monitoring laboratories were therefore asked to report all samples suspected of containing PCBs by gas-liquid chromatography. A reporting system was developed based on rough quantitation, as follows: negative, present in usual amounts, present in greater or lesser amounts than usual. Samples collected in late 1968 and 1969 showed that PCBs were indeed present in a significant portion

* Chief, State Services Branch, Division of Pesticide Community Studies, Office of Pesticides Programs, CP, Environmental Protection Agency, 4770 Buford Highway, Chamblee, Ga. 30341.

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of the general population of the country over a wide geographic distribution. Positive samples were reported from 14 States, including Michigan, New York, Minnesota, California, Massachusetts, Georgia, Kentucky, Illinois, North Carolina, South Dakota, Ohio, Louisiana, Delaware and Arkansas. To insure that proper identification was being made, two samples with higher levels were confirmed by combined gas liquid chromatography-mass spectroscopy (4). These two cases are discussed in another paper in this symposium (5).

Based on these preliminary findings and certain as yet unpublished information about the effect of PCBs on the accuracy of measurement of residues of DDT (Enos, H. F., personal communication), high priority was given by the Perrine Laboratory to the development and evaluation of an analytical method which would separate PCBs from pesticides permitting accurate quantitation of pesticides and confirmation of PCB levels. The resulting methodology is a further modification of the Mills-Olney-Gaither procedure in which adipose tissue is subjected to extraction by petroleum ether, acetonitrile partitioning, and Florisil cleanup. A portion of the resulting 6% ethyl ether/petroleum ether eluate, in concentrate form, is treated with KOH to effectuate dehydrochlorination of DDT and DDD to their olefins, thus eliminating the problem of separating these pesticides from the PCBs. Oxidative treatment is then applied to convert any interfering DDE to p,p'-dichlorobenzophenone which has an Rf value different from the PCBs. The PCBs are then determined by thin layer chromatography (6).

On April 15, 1971, all monitoring laboratories in the Human Monitoring Survey adopted this methodology for the analysis of all adipose samples with the lowest limit of sensitivity being 1.0 ppm. Aliquots of all samples in which measurable amounts of PCBs are found are saved for future confirmation by mass spectroscopy.

Table 1. Incidence of polychlorinated biphenyls in adipose of the general population of the nation.

	Reported		Analyzed	
	No.	%	No.	%
Total	637	100.0	688	100.0
Negative	314	49.3	235	34.2
Trace–<1.0 ppm	125	19.6	229	33.3
1–2 ppm	165	25.9	188	27.3
>2 ppm	33	5.2	36	5.2

Table 2. Descriptive statistics of samples analyzed.

		Negative and trace–<1.0 ppm		>1.0 ppm	
		No.	%	No.	%
Total number		439	100.0	198	100.0
Sex					
Male		221	50.3	127	64.1
Female		218	49.7	71	35.9
Race					
White		381	86.8	151	76.3
Nonwhite		58	13.2	47	23.7
Samples from					
Therapeutic surgery		95	21.6	29	14.6
Postmortem		344	78.4	169	85.4
Diagnostic group					
Neoplasms	140–209	77	17.5	39	19.7
Circulatory system	390–450	121	27.6	61	30.8
Gastrointestinal system	530–565	44	10.0	16	8.1
Liver & gall bladder	570–575	20	4.6	6	3.0
Pancreas	577	0	0.0	2	1.0
Urinary system	580–595	11	2.5	7	3.5
Congenital Anomalies	740–759	5	1.1	1	0.5
Accidents & violence	E numbers	16	3.6	7	3.5
All other		145	33.0	59	29.8
		439	99.9	198	99.9

Results and Discussion

Analytical results for 637 samples have b reported and are summarized in Table 1 additional samples have been analyzed but h not as yet been reported). Of the 637 sam reported, 198 (31.1%) contained measur amounts of PCBs and 125 (19.6%) conta trace amounts, with the balance being nega These samples were collected from 40 patholo in 38 cities distributed over 18 States and District of Columbia. The States are Arka Illinois, Louisiana, Oklahoma, South Da

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Tennessee, Oregon, Pennsylvania, Florida, Kansas, Maine, Georgia, North Carolina, California, Michigan, New York, Ohio and Kentucky. Positive samples came from every hospital, city and state sampled. It, therefore, appears that these materials are widely found in the population. Details of distribution by sex and race, collection procedure, and diagnostic groups are summarized in Table 2. The difference between figures for the positive and negative groups does not appear to be significant, although no statistical evaluation has been attempted because of the preliminary nature of this report. Since a statistical design has been established and is being followed for sample collection for the Human Monitoring Survey, in due time valid statistical analysis will be possible and will then be performed.

Summary

Polychlorinated biphenyls have been found in measurable amounts in 31.1% of 637 samples of human adipose tissue collected from the general population as a part of the Human Monitoring Survey. Sample collection involved 18 States and the District of Columbia. Positive samples were obtained from every State sampled.

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Polychlorinated Biphenyl Residues in the Plasma and Hair of Refuse Workers

by D. I. Hammer*, J. F. Finklea, L. E. Priester, J. E. Keil, S. H. Sandifer, K. Bridbord

Refuse workers may be exposed to polychlorinated biphenyl (PCB) residues from the burning of automobiles, tires, transformers and wire insulation. Paired plasma and scalp hair specimens were obtained from 37 refuse burners and 54 controls (lumberyard and waterworks workers).

PCB residues were dehydrochlorinated and assayed by gas chromatography with a Ni^{63} electron capture detector. Rough PCB quantitation was achieved by integrating the area under a five peak "fingerprint" associated with the PCB fractions, Arochlor 1254 and 1260. Unwashed hair specimens were all negative for PCBs. Detectable plasma PCB levels were found in 81% (32/37) of the refuse burners but only in 11% (6/54) of the controls. Median PCB levels for those with detectable amounts were similar in the two groups; refuse workers—2.6 ppb, controls—3.7 ppb. Maximum plasma PCB levels were 14.1 ppb in the refuse workers and 20.2 in the controls.

The higher frequency of measurable plasma PCB levels in refuse workers may reflect their increased PCB exposure from incinerated materials. Similar median plasma values for both groups reaffirm the role of blood as a transport rather than a storage tissue. As expected, scalp hair is of no utility in estimating PCB body burdens.

* Division of Health Effects Research, EPA National Envir. Res. Ctr., Research Triangle Park, N.C. 27709.

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Food Exposures to Polychlorinated Biphenyls

by Albert C. Kolbye, Jr., M.D.*

The PCB family of industrial chemicals has been used in a number of useful and beneficial ways for more than 40 years. PCBs have been used in capacitors, as transformer fluids, as heat transfer agents, in plasticizers and adhesives, and in sealants and printing inks. Needless to say, they have no place in the nation's food supply. The scientific community has been aware of the potential hazard of the PCBs since at least 1966. Reports in the foreign literature at that time indicated the detection of this chemical in fish and birds. However, the status of the analytical detection method for organochlorine pesticide residues in reference to PCBs was not perfected until early 1969, after it was discussed in FDA's pesticide analytical workshop and the former Division of Pesticides improved the analytical method, so that it was satisfactory for routine analysis.

We know that as a toxic substance PCBs are a potential but not immediate health hazard and that their environmental background level is not high. We do not know how long-term exposure to PCBs might affect human health, and we cannot yet explain the inconsistent presence of the chemical in certain areas of the environment. We have been faced recently with PCB adulteration of food from accidental sources and from untraceable environmental sources. The exact routes of contamination are subject to speculation. Some theories point to the industrial wastes from plants using PCB, leaching of PCB from plastic objects in waste disposal (burning at waste incineration plants or run-off from landfills into streams), and accidental use of PCB as solvents for which they are not intended (vehicles for spray preparations etc.)

Some of FDA's earliest findings of PCBs in foods occurred during the fall 1969 when PCBs were encountered in coho salmon and milk. In August, 1969, Baltimore District found an adulterant identified as PCBs in 5 of 12 milk samples from West Virginia. During a six-month followup investigation, it was learned that PCBs were commonly used as a heat transfer agent in electrical capacitors in the area from which the milk samples were collected. Further investigation revealed that the electric company had allowed a right-of-way sprayer to utilize a transformer oil base for defoliant spraying. Vegetation collected from the power line right of way was tested and found to be positive for PCBs. It was eventually concluded that the apparent misuse of the transformer fluid containing PCBs for defoliant spraying operations adjacent to dairy pasturage was responsible for the milk contamination.

As a result, in November, 1970, the FDA undertook a specific survey of the incidence of the PCBs in the milk supply.

The Monsanto Company informed FDA on July 16, 1971, that large amounts of fish meal may have been contaminated with PCBs (Aroclor 1242) during pasteurization of fish meal at the East Coast Terminal, Wilmington, North Carolina. PCBs were used as the heat exchange fluid.

The U. S. Department of Agriculture informed FDA on July 19, 1971, that Holly Farms, a large North Carolina poultry producer, had discovered the presence of PCBs in its poultry, resulting from an investigation to determine the cause of reduced hatchability in eggs. The firm's investigation

* Deputy Director, Bureau of Foods, U.S. Food and Drug Administration, Washington, D.C. 20204.

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implicated the fish meal ingredient as the causative agent.

The FDA tested the processed fish meal and confirmed that fish meal on hand was contaminated with PCBs. Continuing investigation indicated the leak began on April 30 and continued through July 16. Approximately 16,000 tons of fish meal were shipped during that period. The contamination was caused by a leak in the heat exchange equipment, which used PCBs in the heat exchange fluid. Individual fish meal samples examined contained from 14 to 30 ppm PCBs.

Fish meal, the direct subject of this contamination incident, is a minor component of poultry and fish feed. Investigations conducted by FDA and USDA focused attention on the contaminated meal as a possible source of PCB residues in poultry, eggs and commercially raised catfish. The Food and Drug Administration visited primary and subconsignees and initiated followup sampling of fish feeds, commercial fish, and eggs, when the contaminated fish meal was implicated. USDA was informed when investigation indicated eggs were being distributed to egg breakers.

The firm recalled outstanding stocks of fish meal on July 21 and approximately 1,500 tons of fish meal were returned to the plant. Sampling of poultry for PCB residues was instituted promptly by USDA who advised that no lots of broiler poultry were found exceeding the 5 ppm interim guideline for PCB residues.

Follow-up sampling on eggs was initiated on August 3, 1971. As of September 29, 1971, 224 samples of eggs were analyzed in the follow up, with 71 containing residues in excess of 0.5 ppm (range 0.6-4.2).

We seized five shipments of fish feeds in the States of Louisiana, Georgia, and Mississippi that contained from 0.6 to 4.5 ppm PCBs. Eleven samples of catfish, which had been fed the contaminated feed, contained PCB residues within the 5 ppm guideline, ranging from 0.3-3.0 ppm. In addition, we seized a shipment of the contaminated fish meal from East Coast Terminal that had not been recalled and contained in excess of 350 ppm PCBs.

Since November, 1969, the FDA has analyzed all raw agricultural commodities sampled under the pesticide surveillance program for PCBs as well as pesticide residues. Over 17,000 samples have been collected and analyzed. In the past 18 months, 684 samples of fish, cheese, milk, shell eggs and fish by-products, out of 3,505 samples of these commodities, have been found positive for the PCBs.

PCBs were encountered most frequently in fish in 363 of the 670 samples ranging from trace levels to 35.29 ppm. PCBs were detected in fresh water fish including catfish, chubs, and smelt, and also salt water species including porgies, sea trout, bluefish, bonita and sardines. PCBs have only been detectable in shellfish at trace levels. The results of the pesticide program for PCBs for that 18-month period are as follows:

PCB in selected food commodities—7/1/70 to 9/30/71.

	Number of samples examined	Number of samples positive	Percent positive	PCB levels (ppm) Low	PCB levels (ppm) high	Average
Fish	670	363	54	T	35.29	1.87
Cheese	1344	91	6	T	1.0	.25
Milk	941	60	7	T	27.8	2.27
Shell eggs	550	161	29	T	3.74	.55
Fish by-product	—	13	—	T	5.0	1.17
Total (excluding fish by-product)	3505	684	19			1.14

Above results reflect both surveillance and compliance* samples.

Average residue levels do not include values indicated as "trace." T indicates "trace", which in normal analyses would be less than approximately 0.1 ppm fish; 1–1.5 ppm milk or cheese fat; 0.2 ppm eggs.

* Compliance samples reflect followup of violative samples hence biased towards high levels.

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The FDA total diet studies for 60 market baskets representing a total of 720 composite samples during FY 70 and FY 71 show 22 composite samples to contain PCB residues ranging from a trace to 0.36 ppm. These positive findings were found in the meat, fish, poultry, dairy, and the grain and cereal composites. The 0.36 ppm PCB value reported in the total diet study was found to be caused by migration of PCBs from the grayboard container and dividers to packaged shredded wheat.

Subsequent information gathered on the occurrence seemed to point to the use of reclaimed, or recycled paper in the manufacture of the cardboard packaging material used for these food packages.

Consequently FDA initiated on September 8, 1971, a Survey of PCBs in Food and Food Packaging Material. This survey was designed to cover selected food categories nationwide to determine the extent of food contamination by PCB-containing cardboard packaging materials.

As of November 12, 1971, we had complete information from 12 of the 17 districts showing results on a total of 584 samples.

The packaging material analyzed in this survey included the following types of components:

Types of components comprising packaging material analyzed during survey

Inner wrappers		
	Waxed paper	Cellophane
	Foil	Paper
	Plastic	
Dividers		
	Paper cups (as used for cookies and candy)	
	Plastic trays	
	Paper trays	
Outer Wrappers		
	Foil	Plastic
	Cellophane	Paper
Other		
	Cellophane windows	Fiber cartons
	Plastic bags	Paper envelopes
	Metal can ends	Plastic can ends
	Plastic tear strips	Tea bags

Some of the findings from this survey as received as of that date are shown in the following Figs. 1, 2, 3, 4, 5, 6 and 7.

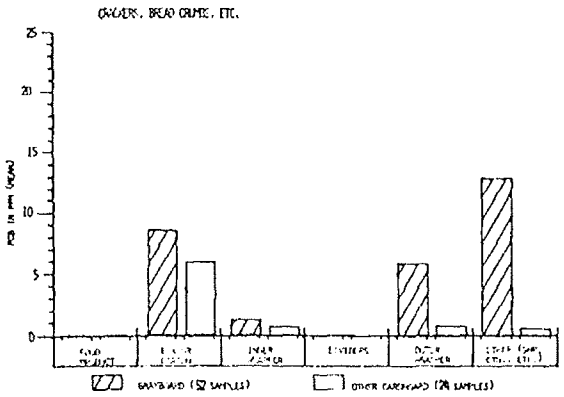

FIGURE 1. Survey--PCB in Food and Food Packaging Material Cumulative Analytical Findings—Sept. 8-Nov. 5, 1971.

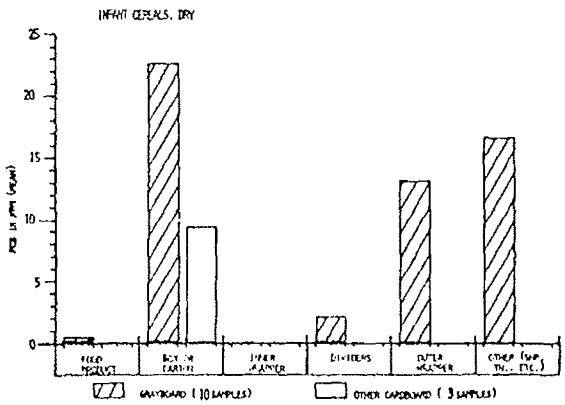

FIGURE 2. Survey—PCB in Food and Food Packaging Material Cumulative Analytical Findings—Sept. 8-Nov. 5, 1971.

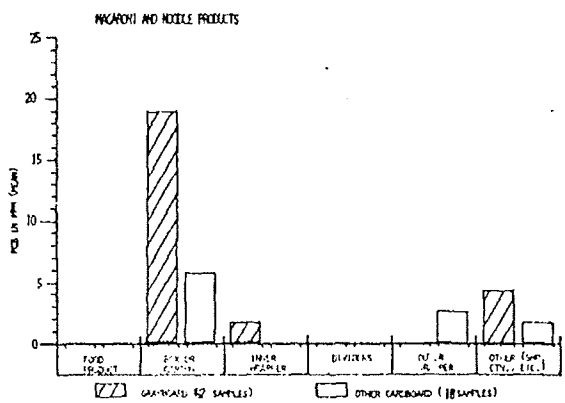

FIGURE 3. Survey--PCB in Food and Food Packaging Material Cumulative Analytical Findings—Sept. 8-Nov. 5, 1971.

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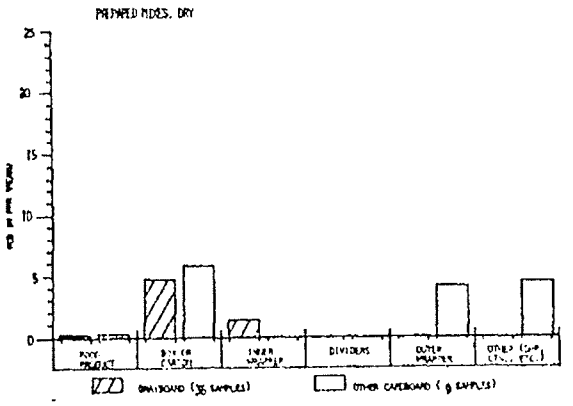

FIGURE 4. Survey—PCB in Food and Food Packaging Material Cumulative Analytical Findings—Sept. 8–Nov. 5, 1971.

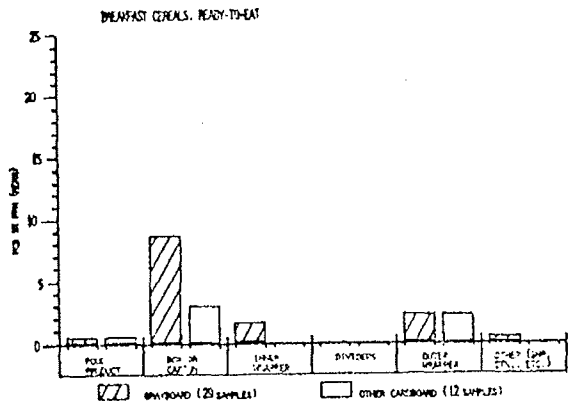

FIGURE 5. Survey—PCB in Food and Food Packaging Material Cumulative Analytical Findings—Sept. 8–Nov. 5, 1971.

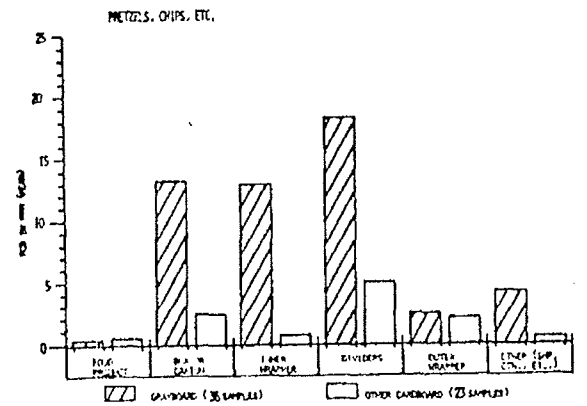

FIGURE 6. Survey—PCB in Food and Food Packaging Material Cumulative Analytical Findings—Sept. 8–Nov. 5, 1971.

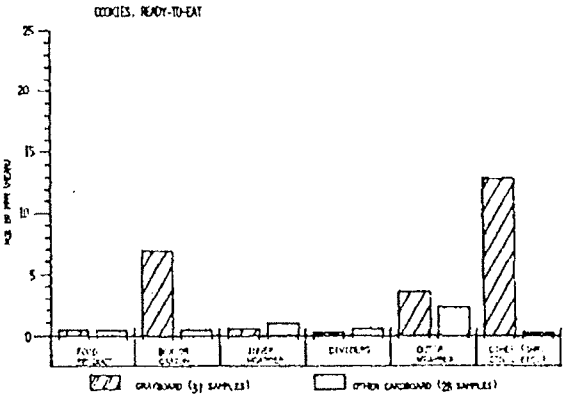

FIGURE 7. Survey—PCB in Food and Food Packaging Material Cumulative Analytical Findings—Sept. 8–Nov. 5, 1971.

It is significant (1) that no PCBs have thus far been reported in fresh fruits and vegetables, (2) that PCBs are found in fish quite frequently, (3) that in addition to poultry, PCBs are also found in milk and eggs, and (4) the PCBs have now been found in various food commodities apparently as the result of migration from their packaging components. At this point the significance of these findings has not been determined.

With the conclusion of the PCBs in Food and Food Packaging Material Survey, FDA has embarked on a program to determine the level of PCBs in animal feeds. Following the PCBs in feed program, FDA will further evaluate PCBs in milk by collecting milk for both bottling and manufacturing use on a nationwide basis. Specific attention will be then given to PCBs in shell eggs.

FDA's current working levels for PCB residue findings are based on specific occurrences of "accidental" contaminations. They are 5 ppm in the edible portion for fish; 5 ppm on a separate fat basis for poultry; 0.2 ppm in whole milk (5 ppm in the fat). Action has been taken against animal feeds and whole eggs associated with the East Coast Terminal incident at the 0.5 ppm level. These levels should not be construed as guidelines permitting consumption of foods containing these amounts of PCBs. If the FDA finds that the frequency of PCB contamination in the nation's food supply increases, the present working guidelines for PCB residues will be substantially lowered.

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Polychlorinated Biphenyls: Their Effects on Penned Pheasants*

by Robert B. Dahlgren and Raymond L. Linder† and C. W. Carlson‡

Introduction

Polychlorinated biphenyls (PCBs) have been useful industrial products since the beginning of their commercial production in 1929. The first report of PCBs occurrence in wildlife was made from the work of Sören Jensen, a Swedish Chemist, in the *New Scientist* in 1966 (1). Since then PCBs have been reported to be widespread in the world's ecosystem, building up in food chains as has been reported for organochlorine insecticides. The chemical structure of PCBs and their action on organisms, although less toxic, are similar to that of DDT. The contamination of human milk, poultry feed, and all classes of wild vertebrates, has created a concern for the need to determine possible harmful effects of PCBs.

* Funds for the study were supplied by the Bureau of Sport Fisheries and Wildlife through the South Dakota Cooperative Wildlife Research Unit, supported jointly by the South Dakota Department of Game, Fish, and Parks; the Bureau of Sport Fisheries and Wildlife; the South Dakota State University; and the Wildlife Management Institute. Tissue analyses were made by the Denver Wildlife Research Center and by Dr. Yvonne A. Griechus, Experiment Station Biochemistry Department. Dr. Robert J. Bury, Veterinary Science Department, conducted necropsies and histopathologic studies. Dr. W. L. Tucker, Experiment Station Statistician, gave statistical advice. Wild pheasants were collected by Fred E. Hartman and Edwin J. Kriz, Pennsylvania Game Commission; and Robert D. Feldt, Indiana Department of Natural Resources. This work is from a doctoral dissertation at South Dakota State University by the senior author.

† Assistant Leader and Leader, respectively, South Dakota Cooperative Wildlife Research Unit, South Dakota State University, Brookings, S.D. 57006

‡ Professor of Animal Science, and Leader, Poultry Research and Extension, South Dakota State University, Brookings, S.D. 57006

Because of the economic importance of the pheasant (*Phasianus colchicus*), it was chosen as the experimental animal using PCBs in this study. The objectives were (1) to determine the patterns of absorption, storage, and excretion for PCBs, and (2) the effects of PCBs on reproduction, behavior, and survival. Since many published reports indicated that the chromatograms of PCBs in wildlife most nearly resemble those of Aroclor 1254, this was the PCB product chosen for study.

The use of brand names in this paper does not indicate an endorsement of any product.

Materials and Methods

Aroclor 1254 (supplied by the Monsanto Co.) was mixed (w/w) in a 1:9 dilution with corn oil and weighed into No. 00 size gelatin capsules to within ±2.5 percent of the required weight. Capsules were administered into the esophagus using a glass tube. Capsules containing only corn oil were administered to control birds.

Analyses for residues were made by Dr. Yvonne A. Greichus, Experiment Station Biochemistry Department, South Dakota State University, and the Bureau of Sport Fisheries and Wildlife laboratory at the Denver Wildlife Research Center. Analytical procedures used at the South Dakota State University laboratory are described by Dahlgren et al. (2) and procedures of the Denver Laboratory by Dahlgren et al. (3).

Hens for breeding experiments were purchased in the winters of 1970 and 1971 (30 and 34 respectively) from the South Dakota Pheasant Co. of Canton, and cocks were raised from birds previously obtained from the Canton source. All birds used for breeding were about 1 year of age.

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In late January they were placed under 16 hours daily of artificial light to stimulate breeding. Both sexes were fed a pheasant breeder ration (Zip Feed Mills, Sioux Falls). One tablespoonful of oyster shells was added to the food cup of each hen weekly. Cocks were kept in breeder cages (30×36×18 inches high), and hens were kept in individual cages (12×18×12 inches high) designed to facilitate handling and identification of eggs. Temperatures were kept at near 65 F in colder months. Eggs were collected daily, individually numbered, set weekly for 15 weeks in a forced-draft incubator, and hatched in pedigree cages.

In 1970, cocks were first dosed with 25 mg PCBs on February 13, and weekly thereafter for a total of 17 times. Hens were first dosed 1 week later, February 20. Breeding was commenced February 16. Eggs were gathered for the first weekly egg group from March 7–13 and gathered last 15 weeks later, June 13–19. In 1971, the same sequence was followed, except that the first dosing began February 19 for cocks and February 25 for hens, and breeding began March 1. Eggs were gathered for 15 weekly egg groups beginning March 6 and ending June 18.

Within 36 hours after hatching, chicks were taken from the incubator, wingbanded, and one wing was removed to the alula. They were then placed on a visual cliff (4) and given 5 minutes to jump to either the visually deep or shallow side. Chicks from this study and those hatched from a concurrent dieldrin study were placed together in brooders and fed a turkey pre-starter diet (Zip Feed Mills) until they were 6 weeks of age. At 6 weeks, they were placed outside in 16×16 foot pens and fed a pheasant grower ration (Zip Feed Mills).

Several times in summer and fall, chicks were caught by hand by the same person, and wingband numbers were recorded. The number of birds caught in each category in the first and last half of all birds caught was compared with an expected 50:50 distribution using chi-square analysis. Each pen was a closed unit, and birds of several groups caught predominantly in the first half caused birds of other categories to be caught in the last half. Weights to the nearest gram were taken of all adult breeders weekly. Chicks were weighed to the nearest gram at 6 weeks of age.

To study effects of PCBs in combination with dieldrin, cocks and hens from 6 to 9 months old were stratified by sex and weight and randomized to five groups of 22 birds each. They were given capsules containing either (1) 4 mg technical grade dieldrin, (2) 8 mg dieldrin, (3) 50 mg PCBs, (4) 100 mg PCBs, or (5) 50 mg PCBs and 4 mg dieldrin together. Ten doses were given, one capsule each 3.5 days for 5 weeks. Three and one-half days after the last capsule was given, all surviving birds were sacrificed.

The Denver Wildlife Research Center analyzed for residues of PCBs in pooled livers from: three road-killed pheasants collected near Washington, Pennsylvania; six road-killed pheasants collected southeast of Lancaster, Pennsylvania; six pheasants shot east of Gary, Indiana; six pheasants shot in Benton County, Indiana; and six pheasants shot near Brookings, South Dakota. All of the above collections were made in the spring of 1971.

Results and Discussion

Effects on Reproduction

The averages of eggs laid per hen per day among hen treatment groups were significantly different in 1970 (5). With the use of an orthogonal test, the rate of egg production among hens given 12.5 mg PCBs was not found to be lower ($P>0.05$) than the rate of the control group; but the rate of the 50-mg group was lower ($P<0.01$) than the rates of the other groups. In 1971, the same pattern was evident (Table 1); there were significant differences ($P<0.05$) among hen groups. The production of hens given 12.5 mg was not lower ($P>0.05$) than that of controls, while production of 50-mg hens was lower ($P<0.01$). Egg production was lower in groups where cocks were given PCBs, but this is not to be expected, and we cannot explain it.

Differences among groups in egg fertility in 1970 were significant ($P<0.05$); however, variation in fertility did not follow a pattern related to PCB dosage, and, thus, the results may not have any biological meaning (5). In 1971, there were no significant differences in fertility among groups (Table 1).

Hatchability in both 1970 (5) and 1971 was highest in control groups and lowest in groups in

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Table 1. Reproductive statistics from control pheasants and pheasants given PCBs 1970–71.

Treatment group[a]	No. eggs per hen per day	No. eggs set in incubator	Fertile eggs		Fertile eggs hatched		Fertile eggs pipped but not hatched	
			No.	Percent	No.	Percent	No.	Percent
1970								
0-0[b]	0.621	277	101	36	74	73	6	6
0-12.5	0.459	220	128	58	87	68	29	23
0-50	0.292	136	50	37	32	64	8	16
25-0	0.335	168	91	54	73	80	9	10
25-12.5	0.362	179	71	40	50	70	18	25
25-50	0.198	98	63	64	41	65	10	16
1971								
0-0	0.337	168	82	49	65	79	6	7
0-12.5	0.436	199	128	64	64	50	20	16
0-50	0.328	148	96	65	51	53	4	4
25-0	0.465	233	170	73	116	68	8	5
25-12.5	0.385	288	153	53	109	71	15	10
25-50	0.228	95	42	44	10	24	5	12

[a] Each treatment group had five hens, except that the 25–12.5 mg group had nine hens in 1971.
[b] The first number is the weekly PCB level in mg given to cocks; the second, that for hens.

which hens had received 50 mg PCBs. In 1970, these differences tested by chi-square were not significant ($P>0.05$), while in 1971 hatchability was reduced ($P<0.01$) among hens given PCBs. Significant differences ($P<0.05$) were found in the number of eggs that were pipped but not hatched for hen groups in both 1970 and 1971. Apparently, the administration of PCBs to the hen adversely affected the viability of the embryo at hatching. McLaughlin et al. (6) injected both 10 and 25 mg of Aroclor 1242 into chicken eggs and found only 0–5 percent hatchability with growth retardation, edema, and beak deformities in embryos.

Eggshell thickness was measured only in 1970, and no significant differences ($P>0.05$) in eggshell thickness were found using analysis of variance (5). Eggshells from hatched eggs (without membranes) laid by control hens averaged 0.26±0.02 mm standard deviation, and those from all hen groups receiving PCBs averaged 0.23±0.02 mm. Eggshells from unhatched eggs averaged 0.32±0.02 mm for control hens, and 0.31±0.02 mm for hens receiving PCBs. Dahlgren and Linder (7) found the eggshell thickness of pheasants to be unaffected by weekly administration of capsules containing 6 mg dieldrin.

Heath et al. (8) fed 25 ppm of Aroclor 1254 to mallards (*Anas platyrhynchos*) through two breeding seasons. They also fed bobwhite quail (*Colinus virginianus*) 50 ppm PCBs and at a joint level of 25 ppm PCBs and 15 ppm DDE for one reproductive season. They found no effects on egg production, cracked eggs, eggshell thickness, embryonation, embryos alive at 3 weeks, normality of hatchlings, and normal hatchlings alive at 14 days.

Scott et al. (9), who fed 0, 0.5, 1.0, 10.0, and 20.0 ppm Aroclor 1248 for 8 weeks to chickens in full egg production, found no reduction in egg production on the lowest levels of PCBs after 8 weeks; however, they noted a 10 percent reduction was associated with 10 ppm, and a 13 percent reduction was associated with 20 ppm. They reported that 10 ppm Aroclor 1248 reduced hatchability of chicken eggs by 8 percent after 4 weeks and 44 percent after 8 weeks. The 20 ppm level nearly eliminated all hatchability. Most embryos died at 21 days of development; many after pipping. They also found that eggshell strength was not affected when as much as 20 ppm was fed.

Effects on Behavior

Seventy-one 11-week old hens given one 210-mg

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Table 2. Behavior on the visual cliff of chicks hatched from control pheasants and pheasants given PCB's 1970–71.

Treatment group	No. that jumped to visually deep side		No. that jumped to visually shallow side		No. not jumping within 5 minutes	
	1970	1971	1970	1971	1970	1971
0-0[a]	2	9	35	43	8	7
0-12.5	3	7	42	34	8	5
0-50	3	7	6	30	5	6
25-0	5	16	33	67	15	17
25-12.5	5	16	22	78	7	7
25-50	4	0	5	9	6	1

[a] The first number is the weekly PCB level in mg given to cocks; the second, that for hens.

capsule in the evening of the first day of testing appeared weak at the end of the following day. After receiving the second capsule, they sat with feathers fluffed, and some tremoring was noticed. During the 24 hours before death, they often showed tremors, particularly when disturbed. Shortly preceding death, birds were comatose and died without tremors.

Eleven 11-week old hens given 20 mg daily were hyperexcitable after 4 days of PCB treatment; after 10 days they sat with feathers fluffed. They exhibited weakness with occasional tremors about 30 days after dosage began. Eleven hens 11 weeks old given 10 or 20 mg daily appeared to have fewer body feathers after 30 days than controls, although they did not appear to peck one another more often. Flick et al. (10) reported feather loss in chicks of the domestic chicken given PCBs.

Visual Cliff Behavior—In 1970, when chicks were placed on a visual cliff for up to 5 minutes, significant differences ($P<0.01$) among groups were found in their behavior using chi-square analysis (5); offspring of hens given 50 mg jumped to the visually-deep side of the cliff more often than offspring in other groups. In 1971, no significant differences in behavior between groups were noted (Table 2). When data from both years were combined, no significant differences ($P>0.05$) were found. Baxter et al. (11) reported that pheasant chick behavior in a visual-cliff test was apparently affected by dieldrin given their parents.

Response to Hand Catching—Results of catching young pheasants by hand were analyzed by comparing the number of birds from each treatment group caught in the first half and last half of all birds caught to an expected number equaling 50 percent of the birds in that group. For example, 16 out of 20 young in the category where both parents had received PCBs were caught in the first half of all birds caught on July 22, 1970 (Table 3). The 16 caught in the first half and 4 caught in the second half were compared by chi-square analysis to an expected 10 (half the total category of 20), resulting in a highly significant difference ($P<0.01$). Since each pen was a closed unit and the PCB-treatment birds were penned with other young from a concurrent dieldrin study, it must be remembered that birds of several groups caught predominantly in the first half would cause birds of other groups to be caught in the last half. It is important to compare not only how the PCB birds were caught in comparison to an expected distribution, but also how categories compared with one another. The comparisons are shown in Table 3 as ratios. These indicated that the ability of penned pheasants to avoid hand capture was significantly less in 1970 offspring where both parents had been given PCBs. This is identical to the findings of Dahlgren et al. (12) for offspring of parents administered dieldrin. However, in 1971, the responses of offspring of treatment groups were similar to controls.

Data for 1970 and 1971 were combined and a contingency table chi-square technique was used to determine the effect of PCB treatment on the response to hand catching between groups. The response was similar whether PCBs were given to either the cock or hen but was significantly different ($P<0.05$) when both parents received PCBs from that where only one parent received PCBs.

During catching, pheasants were herded around and around the pen, and often the catcher was able to capture those birds which ran back directly toward the pursuer. However, there was no attempt made to determine or to quantify the elements of behavior that would explain why offspring of birds treated with PCBs were easier to catch.

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Table 3. Effects of PCBs on hand capture of penned pheasants, 1970–71. (Numbers represent birds caught in the first half of all birds caught; numbers in parentheses represent one-half the number of that category in the pen. Chi-square was used to compare numbers actually caught with half of the numbers in each category.)

Dates of capture	No. of hatches caught	No. of pens	Parents receiving PCB			
			Both	Hens only	Cocks only	Neither
1970						
July 22	1–8	14	16(10)**	8(8.5)	7(10.5)	11*(10.5)
July 29	1–9	15	19(11.5)**	7(10)	9(12)	6(13.5)**
Aug. 13	1–10	18	11(12.5)	12(12.5)	12(13.5)	8(13)*
Aug. 19	1–12	20	21(15.5)*	17(14)	18(17.5)	19(18)
Sept. 1	1–14	22	25(18)*	19(15.5)	22(20)	14(19.5)
Oct. 15	1–15	23	20(16)	14(16)	25(17.5)*	9(14)
Nov. 21	1–15	13	14(11.5)	14(12.5)	8(10.5)	12(11.5)
Dec. 5	1–15	13	11(11)	14(11.5)	12(10.5)	6(10)**
All 1970 catches combined			137(106)**	105(100.5)	113(112)	85(110)**
Ratio, 1970[b]			1.55	1.91	1.98	2.59
1971						
July 21	1–7	17	27(19.5)*	23(24.5)	25(21.5)	12(14.5)
Sept. 1–3	1–15	28	75(70.5)	41(37)	39(36.5)	21(22)
Sept. 21–23	1–15	28	71(70.5)	38(36)	37(36)	24(21.5)
Oct. 7–11	1–15	28	33(37)	33(35.5)	27(35)	19(20.5)
Oct. 19–22	1–15	28	34(35.5)	23(33.5)*	35(34.5)	17(21)
Nov. 2–5	1–15	28	30(30.5)	29(32.5)	23(28.5)	19(18)
All 1971 catches combined			270(264.5)	187(199)	187(192)	112(117.5)
Ratio, 1971			1.96	2.13	2.05	2.10
All 1970–71 catches			407(370.5)**	292(299.5)	300(304)	197(227.5)**
Ratio, 1970–71			1.82	2.05	2.03	2.31

[a] Totals of row numbers may exceed one-half total birds because odd numbers of birds in pens were rounded higher.
[b] Birds in pen/birds caught in first half.
* ($P<0.05$).
** ($P<0.01$).

Table 4. Weights and survival for the first 6 weeks of offspring from control pheasants and pheasants given PCBs, 1970–71.

Treatment group	Average weight at 6 weeks (g)		No. of chicks to brooder		No. of chicks alive after 6 weeks		Percent survival		
	1970	1971	1970	1971	1970	1971	1970	1971	Both years
0–0[a]	396	429	73	65	49	50	67	77	72
0–12.5	378	435	84	64	49	45	58	70	64
0–50	303	425	29	50	3	37	10	74	51
25–0	389	428	72	115	50	91	69	79	75
25–12.5	403	458	50	109	30	72	60	66	64
25–50	344	373	40	10	9	6	22	60	30

[a] The first number is the weekly PCB level in mg given to cocks; the second, that for hens.

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Table 5. Survival to the fall of offspring of control pheasants and pheasants given PCBs, 1970–71.

Group	No. of young alive in fall[a]		Percent survival from 6 weeks of age to fall[b]			Percent survival hatching to fall[c]		
	1970	1971	1970	1971	Both Years	1970	1971	Both Years
0–0[d]	20	36	41	72	57	27	55	41
0–12.5	23	36	47	80	63	27	56	40
0–50	0	29	0	78	72	0	58	37
25–0	21	57	42	63	55	29	50	42
25–12.5	18	59	60	82	75	36	54	48
25–50	4	4	44	67	53	10	40	16

[a] December 5, 1970; November 2–5, 1971.
[b] Using the number of chicks alive after 6 weeks, Table 4.
[c] Using the number of chicks to brooder, Table 4.
[d] The first number is the weekly PCB level in mg given to cocks; the second, that for hens.

Effects on Survival and Weights of Offspring

Survival—Chick survival was determined for treatment groups in 1970 (5) and 1971 during the first 6 weeks of age while chicks were kept in brooders (Table 4). The survival of chicks was not related to whether cock parents received PCBs, but chi-square analysis showed that significantly more ($P<0.01$) deaths occurred among chicks hatched from hens receiving 50 mg PCBs in 1970. These differences were not evident in 1971 data. When data from both years were combined, there was a significant difference ($P<0.01$) between offspring where hens received 0 and 12.5 mg PCBs and between offspring where hens received 12.5 mg and 50 mg PCBs ($P<0.01$).

Survival of young pheasants in outdoor pens was measured in December, 1970, and November, 1971 (Table 5). Survival of offspring from 6 weeks of age to the fall in both years appeared to be unaffected by level of treatment in either year. When overall survival from hatching to the fall in both years was considered, the overall survival of offspring from hens given 50 mg was significantly less ($P<0.05$) than that of the other groups. The lower rates of survival for the 50-mg groups were due to the effect on early survival, not that from mortality of birds older than 6 weeks of age. No meaningful departures from the expected 50:50 sex ratios in the treatment groups were determined (Table 6). Apparently PCBs did not affect the survival of one sex more than another.

Weight—Weights of chicks from hens on 50 mg PCBs weekly were lower ($P<0.01$) at 6 weeks of age (5) than those of other groups in 1970 (Table 4). In 1971, weights of offspring of 50-mg hens were lower ($P<0.01$) than that of controls, while weights of offspring of 12.5-mg hens were higher ($P<0.01$). When data from both years were combined, there was no relationship between weight and treatment level.

Although we did not adequately determine that

Table 6. Numbers of cocks and hens alive in November that were offspring of control pheasants and pheasants given PCB's, 1970–71. (Chi-square was used to compare numbers of each sex alive with an expected 50:50 distribution.)

Group	1970 November 21		1971 November 2–5		1970–71 Combined	
	Cocks	Hens	Cocks	Hens	Cocks	Hens
0–0[a]	11	11	17	17	28	28
0–12.5	14	11	15	20	29	31
0–50	0	1	13	17	13	18
25–0	5	16*	33	22	38	38
25–12.5	13	6	36	20*	49	26**
25–50	1	1	3	1	4	2
All PCB groups	33	35	100	80	122	115

[a] The first number is the weekly level in mg of PCBs given to cocks; the second, that for hens.
* ($P<0.05$).
** ($P<0.01$).

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PCBs via the egg depressed the weight of offspring, McLaughlin et al. (6) mentioned growth retardation of embryos as an effect of PCBs injected into the yolk sac of chicken eggs. We fed no pheasant chicks PCBs, but Flick et al. (10) found that 1-day-old chicks fed Aroclor 1242 at 200 and 400 ppm had depressed growth by the second week of feeding and that the growth depression was related to the level fed. Vos and Koeman (13) fed PCBs to 1-day-old cockerels and found bodyweight depression from 400 ppm Aroclor 1260. Platonow and Funnell (14) found that 1-day-old cockerels fed 250 ppm Aroclor 1254 had depressed body weights between the sixth and ninth week of their feeding trial; this depression was associated with reduced feed consumption. Rehfeld (15) also found depressed weight gains in 1-day-old chicks fed sublethal levels (10–50 ppm) of Aroclor 1254; chicks fed 30 and 50 ppm for 2.5 weeks and then fed a clean diet recovered from the growth depression, while chicks fed 40 and 50 ppm for 5 weeks and then fed a clean diet for 8 weeks did not show a recovery from the growth depression.

Body weights and Mortality in Birds Given PCBs

Adult hens in both the 1970 and 1971 breeding experiments were unaffected in body weight by administration of as much as 50 mg PCBs in single capsules weekly. It was characteristic, however, of birds that died on PCB treatment to stop eating and die within several days.

Hens 11-weeks old given a 210-mg capsule of PCBs in the evening ate very little the following day and appeared weak by the end of that day (3). After receiving the second capsule 24 hours subsequent to the first, birds sat with feathers fluffed; some tremoring was noticed; and no food was consumed. Birds of both sexes 6–9 months of age given 100 mg or 50 mg PCBs every 3.5 days for 5 weeks continued to eat, as did 11-week-old hens repeatedly given either 10 or 20 mg daily. Scott et al. (9) found no effect on feed consumption when laying chickens were fed up to 20 ppm Aroclor 1248, and no mortality that could be attributed to treatment. Prestt et al. (16) found no effect on weight of Bengalese finches (*Lonchura striata*) when they were fed up to 400 ppm Aroclor 1254 for 56 days.

Birds 11-weeks old given daily capsules containing 210 mg PCBs died within 1.3 and 5.9 days; the 16 designated to be analyzed upon death lived longer than the other birds in the experiment, from 2.2–5.9 days, averaging 3.8 ± 1.0 day standard deviation (3). They lost from 15–37 percent of their initial weight before death. All control birds lived. Correlations of the initial weight, days to death, and percentage weight loss were obtained for 53 birds in this experiment. Initial weight was correlated with days to death ($r = 0.699$, $P < 0.01$); heaviest birds lived longest. The birds which were heaviest initially lost the greatest percentage of their weight before death ($r = 0.589$, $P < 0.01$). Birds that lived the longest lost the greatest percentage of their weight before death ($r = 0.744$, $P < 0.01$). Time of death of the 11 birds that were not given PCBs, but were starved, ranged from 2.3 days to 8.6 days, averaging 3.9 ± 1.8 days. They lost from 27–51 percent of their weight by the time of death (3).

Of the birds given 50 or 100 mg every 3.5 days for 5 weeks, 4 of 22 died in the 50-mg group and 7 of 22 died in the 100-mg group. In addition, two birds in the 100-mg group were so weak they were near death at the conclusion of the experiment.

Mortality of birds given 10 mg began 30.6 days after the first capsule was given; the ninth bird died after 179.3 days. The other seven birds died between 50.3 and 60.6 days after initial treatment. The tenth bird of this group, still alive after 8 months of treatment, was sacrificed (3).

In the 20-mg group, the first bird died 39.6 days and the last bird 54.1 days after capsules were first given. The average number of days to death was 46.1 ± 5.3 days (3).

Mortality was light in breeding experiments in 1970 and 1971. In 1970, only 2 hens died from among the 30 hens and 10 cocks under study; both hens had received 50 mg PCBs weekly (5). In 1971, among 34 hens and 14 cocks in the study, three hens died, two that had received 12.5 mg and one that had received 50 mg weekly.

Tucker and Crabtree (17) reported that 2000 mg/kg of either Aroclor 1242, 1254, 1260, or 1268 given to mallards caused no mortality or symptoms. Prestt et al. (16) estimated that 254 mg/kg/day given to Bengalese finches would give 50 percent mortality at 56 days. Heath et al. (8) reported that Aroclor 1254 had an LC_{50} of 1090

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ppm when fed for 5 days as part of the diet to pheasants. Vos and Koeman (13) reported that, of 20 chicks fed 400 ppm for 60 days, only three died.

In the present study, 210 mg PCBs daily were given to 11-week-old hens. By the time the average bird died, it had received 840 mg PCBs, the first to die received 420 mg, and the last to die, 1260 mg. These same figures for 11-week-old hens on 20 mg daily were 940 mg, 800–1100 mg; and for birds given 10 mg daily (one was sacrificed after surviving 8 months) were >830 mg, 310–2410+ mg. Thus, for a group of pheasants given PCBs at 10 mg or more daily, the most susceptible individuals would probably die with 300–400 mg Aroclor 1254; the average bird would die with a cumulative dose totaling from 800 to 950 mg; and the least vulnerable would die after receiving 1200–2410+ mg PCBs.

Absorption, Storage, and Excretion

Dahlgren et al. (2) reported that retention of PCBs in the bodies of four hens, each given single 50-mg capsules and sacrificed 28 days later, averaged 40.5 mg in each hen. Levels of PCBs were highest in all tissues at 12 hours after capsule administration and declined most rapidly in liver at 24 hours. Throughout their experiment, levels were highest in liver, followed by brain and muscle. Brain tissue contained more PCBs than muscle per unit weight, but, having a higher lipid percentage than muscle tissue, brain tissue had a smaller concentration of PCBs per unit of lipid.

Analysis of whole bodies of four hens that received 12.5 mg PCBs weekly for 17 weeks revealed that they retained from 37 to 56 percent of the administered dose, while hens on the 50-mg level retained 60 to 82 percent of the administered dose. The hens on the higher dose averaged about six times as much PCBs in their bodies as the hens on the lower dose, although they had been given only four times as much PCBs (2).

A total of 48.7 mg of PCBs in the whole body plus that excreted in eggs and feces over 28 days was accounted for after administration of a 50-mg capsule (2). The PCB-corn oil mixture was readily absorbed in the gut of the pheasant; calculations showed that a maximum amount excreted unabsorbed was 6 percent of the 50 mg given, and as much as 98 percent may have been absorbed.

Five of the hens that had received 12.5 mg weekly for 16 weeks in the 1971 breeding experiment were sacrificed 1 week following the administration of the last capsule in the series. The ppm PCBs in the bodies of these five hens were 17.6, 18.6, 24.4, 28.3, and 30.5, averaging 23.8 ppm. Three months later, three other hens of the same group, which had been kept caged, were sacrificed. The analyses of their bodies showed 8.9, 12.0, and 20.0 ppm PCBs, averaging 13.6 ppm PCBs. After 6 months on a clean diet, three other birds similarly treated had whole-body ppm values of 18.3, 19.5 and 25.0, averaging 20.9 ppm. The apparent rise in PCB concentration at 6 months may be due to sample variation and/or the physiological state of the hens sampled. It is obvious that the rate of excretion of PCBs is relatively slow. These birds were analyzed at the Denver Wildlife Research Center.

Scott et al. (9) found that chicken hens given up to 20 ppm Aroclor 1248 in the diet for 8 weeks lost less than 50 percent of the stored PCBs after 4 weeks on either a standard diet or low-energy diet. He also found that a low-energy diet followed by a high-energy diet did not affect reduction of PCBs over time. Prestt et al. (16) fed 1500 mg Aroclor over 56 days to Bengalese finches and were able to recover only 9 percent of the amount fed, when they analyzed one bird from the experiment.

Excretion Via the Feces—Excretion of PCBs in feces was relatively low as reported by Dahlgren et al. (2). An average of 4.0 mg per single 50 mg dose was excreted in the feces of four hens over 28 days; the feces from these birds were analyzed as a pool. Two other hens excreted 2.2 and 2.9 mg per 50-mg dose over a 28-day period; the feces from these two hens were analyzed separately. PCBs in the pooled feces of the four hens were at a peak during the first week and declined to relatively lower levels thereafter. They also found that excretion in the feces was highest during the first 24 hours in the two other hens given single 50-mg capsules, and less than 1 mg PCBs appeared in the feces the first day. Variability in excretion between these two hens was low. An average of 2.6 mg per hen was passed in the feces of the two hens by the end of 28 days.

Excretion Via the Egg—In four hens given 50-mg capsules when egg laying was declining, levels of PCBs in the eggs were lowest in the first

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week, highest during the second week, and declined thereafter. The average excretion per hen via the egg was calculated to be 4.2 mg PCBs (2). Excretion of PCBs via the egg could be higher than excretion through the feces when hens are in full egg production. A single egg laid between 1 and 2 weeks after a hen was administered a single capsule containing 50 mg PCBs was shown to contain 1.5 mg of PCBs. This egg was one of two laid that week by one hen.

Heath et al. (8) reported that two mallard eggs from the second reproductive season, when birds had been on 25 ppm Aroclor 1254, had 56 ppm and 33 ppm PCBs, wet weight. Peakall (18) reported that ring dove (*Streptopelia risoria*) eggs taken from birds given 10 ppm Aroclor 1254 in the diet averaged 4.81±1.08 ppm standard error. The actual amounts in mg of PCBs in ring dove (18), mallard (8), and pheasant eggs in the present study would be far below the 10 mg injected by McLaughlin et al. (6) into chicken eggs. The 10 mg resulted in poor hatchability, edema, and beak deformation.

Scott et al. (9) found that PCBs deposited in chicken eggs were less than 0.5 ppm after 8 weeks with 0.5 and 1.0 ppm Aroclor 1248 in the diet. They found levels of over 3 ppm after 8 weeks with 10 ppm in the diet and levels of about 6–7 ppm in eggs of hens on 20 ppm. These values are much lower than those found in the present study from 3–4 weeks after administration of a single capsule of 50 mg Aroclor 1254. Their results, showing a drastic reduction in hatchability associated with 7 ppm or less residue in eggs, are not comparable to our findings with pheasants. Differences with the experimental animal or the Aroclor product used may have resulted in the gross differences in findings between our studies. Heath et al. (8) reported a nearly four-fold difference between bobwhite and Japanese quail in the LC_{50}; thus species differences may be important.

Residues in eggs of wild birds have been determined by several authors from diverse collection points. Anderson et al. (19) found PCBs in all egg pools of cormorants (*Phalacrocorax auritus*) and pelicans (*Pelecanus erythrorhynchos*); cormorants were shown to have an estimated 8 ppm in eggs and pelicans 0.6 ppm. Jensen et al. (20) reported 48 ppm in the egg of a heron (*Ardea cinerea*) collected in Sweden, and 8–21 ppm from 9 guillemot (*Uria aalge*) eggs from the Baltic Sea. Risebrough et al. (21) reported that an egg of a peregrine falcon (*Falco peregrinus*) contained 10 ppm; 8 black petrel (*Loomelania melania*) eggs had an average of 1 ppm; 5 eggs of a barn owl (*Tyto alba*) had <1 ppm; and a golden eagle egg (*Aquila chrysaetos*) had <1 ppm. Dustman et al. (22) reported median measurements of egg residues of bald eagles to be 1.65 ppm for Alaska and 9.7 for those from all other states. They further reported median egg levels of 15.9 ppm for the osprey (*Pandion haliaetus*) of Connecticut and 2.5 ppm for those in Maryland; 5–6 ppm in brown pelican (*P. occidentalis*) eggs from different areas; and 5 ppm in eggs of royal terns (*Thalasseus maximus*).

Prestt et al. (16) found residues ranging from 0-80 ppm in 363 eggs from 28 species of both land and water birds collected in Britain. Most of these had less than 10 ppm, but those which had higher levels included a single egg of the great crested grebe (*Podiceps cristatus*), 40 ppm; one egg among 101 of the heron that had 80 ppm, while the arithmetic mean was 5 ppm; one egg of a moorhen (*Gallinula chloropus*) with 15 ppm, among 13 samples that averaged 2.4 ppm; and a single egg of a great skua (*Sterocorarius skua*) that had 25 ppm.

Particularly in view of findings that about 5 ppm was associated with nearly complete negation of hatchability in the chicken (9), the above findings in some wild birds are alarming. Apparently, though, as earlier pointed out, there must be considerable differences among species in (1) the rate at which they deposit PCBs in the egg, and (2) what a particular ppm range may mean in associated deleterious effects.

Residue Levels in Tissues

We analyzed tissues from seven different groups of birds: (1) 16 birds given 210 mg PCBs daily that were designated for analysis upon death, (2) five additional birds on 210 mg daily that died, (3) nine birds that were killed at intervals for matching with the 16 designated to die, (4) pooled samples of birds killed 12 hours and 24 hours after receiving a single capsule containing 210 mg PCBs, (5) pooled samples of four birds dying and from four birds surviving capsules con-

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taining 50 and 100 mg PCBs, (6) a pooled sample of four birds dying on 20 mg and 10 mg PCBs, and (7) a pooled sample of two control birds (3).

Birds that died from daily doses of 210 mg PCBs had brain levels that ranged from 320 to 770 ppm wet weight and averaged 520 ppm±110 ppm standard deviation. Liver residue levels were much more variable than brain levels. They ranged from 390 to 9,300 ppm and averaged 2,500±2,000 ppm wet weight. Muscle residues were also relatively more variable than brain residues, as they ranged from 51 to 290 ppm, and averaged 140±53 ppm. Tissue levels from the birds killed for matching overlapped with ranges in the birds that died on 210 mg, but less so for brain than for liver or muscle ranges. Brain residue levels of nine birds sacrificed for matching ranged from 280 to 500 ppm, and averaged 370±65 ppm; livers ranged from 1,000 to 5,000 ppm, and averaged 1,900±1,300 ppm; and muscle ranged from 58 to 110 ppm, and averaged 83±17 ppm.

Brain showed less variability than other tissues. Further, brain was the only tissue independent of other parameters (initial weight, percent weight loss, days to death, and lipid content in brain, liver, and muscle) when testing correlations of all possible parameters using birds that died from capsules containing 210 mg PCBs. This lack of correlation with other parameters indicated the usefulness of the brain in assessing toxic levels of PCBs. The relationship of the liver with other parameters such as original bird weight and days to death tended to negate its usefulness. Further, the interrelationships of liver parameters with those of muscle, even though it is not known how many of these relationships were meaningful, tended to negate the usefulness of muscle as an indicator tissue.

Brain levels of PCBs were generally higher in birds that died than in birds that were sacrificed at the same time for matching of brain residue levels, although there was some overlap of the two groups. A brain residue level of 400 ppm separated the bulk of the birds (86 percent) that died from most (67 percent) of those that were sacrificed. There appeared to be a relationship between days to death and brain residue level; however, this was not significant ($P>0.05$). A pooled sample of four birds that died on treatment with 100 mg PCBs given each 3.5 days had 320 ppm in the brain tissue, while a pooled sample of four sacrificed birds in this group had 59 ppm. A pooled sample of three birds that died on treatment with 50 mg PCBs given every 3.5 days had 350 ppm in the brain, while a pooled sample of four sacrificed from this group had 34 ppm. Pooled brain tissues from four birds that died on daily doses of 10 and 20 mg had 360 and 380 ppm PCBs, respectively; no data were available for survivors of these groups. These latter data indicated that 300 ppm might be better than 400 ppm as a separation point that would indicate death from PCBs and that, when smaller amounts of PCBs were received by the birds over a period of time, the spread in brain levels between birds that died and survived might be greater.

Ratios of residue levels using wet-weight ppm values among brain, liver, and muscle in dead and sacrificed birds overlapped considerably, particularly in brain:liver and brain:muscle ratios. However, liver:muscle ratios appeared to be smaller in birds dying on 210-mg doses. If one establishes 19 as a liver:muscle ratio, 3 of 9 birds sacrificed on the 210-mg doses had smaller ratios and only 4 of 18 birds dying had higher ratios. However, it is necessary to gather more data to determine if this ratio is useful in diagnosing the cause of death as PCB toxicosis.

Vos and Koeman (13) found that, when 1-day-old cockerels were fed 400 ppm Phenochlor DP 6, Clophen A60, and Aroclor 1260 in the diet for 60 days, the chicks had brain residue levels ranging from 70 to 700 ppm among 11 birds that died on treatment and 40 ppm for one chick which survived. Liver levels in their experiment were more variable than brain levels and ranged from 120 to 2,900 ppm among 28 birds that died and from 210 to 340 ppm among four survivors. Seven of their 28 birds that died had liver residues of less than 250 ppm, while four of 11 birds had less than 300 ppm. It appears from their data that liver might be as useful as brain for diagnosing cause of death. Their liver:brain ratios were similar to those of the present study, but, since they had only one survivor from which brain and liver were analyzed, overlap could not be evaluated. Rehfeld (15) reported that 30–50 ppm Aroclor 1254 given to 1-day-old cockerels resulted in liver residues of 300–500 ppm. Prestt et al. (16) reported a range

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of 3–634 ppm in livers of survivors and 70–697 ppm in livers of Bengalese finches dying during treatment with Aroclor 1254, and that liver level was correlated with the amount received ($P<0.01$).

Prestt et al. (16) stated that the liver:brain residues should be compared and that brain ppm/liver ppm X 100/1 was three times higher in Bengalese finches that died during their experiment than in birds killed at the end. Data in the present study showed a complete overlap in brain ppm/liver ppm X 100/1; birds that died during testing had a range in ratios of 5.4 to 112.8, while matching birds sacrificed had 10.0 to 37.0.

Relatively little sampling of brain tissue for PCB residues has been done in wild birds. Dustman et al. (22) reported that a sick bald eagle had 230 ppm PCBs in its brain, and that PCBs may have contributed to its death. Jensen et al. (20) reported that three white-tailed eagles (*Haliaeetus albicilla*) had a brain residue range of 29–70 ppm PCBs, averaging 47 ppm; muscle residues ranged from 150–240 ppm, averaging 190 ppm. Risebrough et al. (21) found 0.04, 1.5, 21, and 34.6 ppm PCB in the brains of four peregrine falcons. Prestt et al. (16) reported liver residues ranging from 0 to around 900 ppm from a wide variety of British birds; arithmetic averages ranged from 0.5 ppm in the buzzard (*Buteo buteo*) to 98 ppm in the heron. The tissue levels reported above were below those at which mortality occurred in the present study, except that the muscle level for the white-tailed eagle was higher than those in this study associated with death in the pheasant from PCBs. However, these levels from wild birds do constitute substantial percentages of the brain and liver levels in the present study where death occurred from PCBs.

Histopathologic Effects

Dahlgren et al. (3) found that PCBs decreased weights of heart and spleen at all treatment levels ($P<0.01$). PCB treatment increased weights of kidney and liver in birds given 10- and 20-mg doses ($P<0.01$), but no effect was seen in the 210-mg group. Starved birds had smaller hearts ($P<0.05$) and livers ($P<0.01$) than controls.

Splenic atrophy, as described by Flick et al. (10) and Vos and Koeman (13) was found in all pheasants given 20 mg daily and in the 10-mg birds except for one that survived 8 months and was sacrificed (3). Splenic atrophy was characterized by almost complete absence of lymphatic nodules and an increase in the relative abundance of red pulp. In one of the birds given 20 mg, loci of necrosis were found in lymphatic nodules.

Prestt et al. (16), using the Bengalese finch, reported that kidneys were larger in birds that died from PCBs than in controls. Flick et al. (10), using chickens, mentioned both enlarged adrenals and kidneys from PCB treatment. In our study, kidneys were larger in pheasants given 10 or 20 mg daily, but neither kidney nor adrenal enlargement was visually detected during necropsy. McCune et al. (23), using Aroclor 1242 with chickens, mentioned both enlarged livers and kidneys in birds given PCBs. Platonow and Funnell (14) and Rehfeld (15), using 1-day-old chicks, reported enlarged livers with dietary intake of Aroclor 1254. Grant et al. (24) reported enlarged livers and decreased spleen size over a period of time in the rat. Flick et al. (10) and Vos and Koeman (13) reported small spleens in their studies. Although hydropericardium was found in varying degrees by many of the authors cited, it was found only rarely in the present study. Vos and Koeman (13) found that Phenoclor DP 6 and Clophen A60 caused much more liver necrosis and hydropericardium than Aroclor 1260; this was probably due to contaminants (25).

PCBs in Combination with Dieldrin

Mortality among pheasants of both sexes 6–9 months old given dieldrin, PCBs, or a combination of the two, varied with the level of chemical administered (Table 7). Among the 22 pheasants on each level, 3 died with 4 mg dieldrin per capsule, and 6 died with 8 mg dieldrin. The same proportions held true for PCBs, since 4 died with 50 mg PCBs per capsule, and 9 died with 100 mg PCBs. When 50 mg PCBs and 4 mg dieldrin were administered together, a total of 9 birds died. None of 11 control birds died during this period of time. These data suggest that effects of PCBs and dieldrin together are additive, not synergistic. Heath et al. (8) found that the joint toxicity of Aroclor 1254 and DDE given to Japanese quail was additive, and found no evidence of synergism in their joint effect.

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Table 7. Mortality occurring among 22 pheasants of both sexes that were 6–9 months of age when PCBs and dieldrin were administered separately and in combination. No mortality occurred among 11 control birds.

Group treatment	No. of deaths in week: 1	2	3	4	5	Total deaths
50 mg PCBs	1	0	0	1	2	4
100 mg PCBs	0	3	0	4	2[a]	9
4 mg Dieldrin	3	0	0	0	0	3
8 mg Dieldrin	3	0	0	1	2	6
50 mg PCBs and 4 mg Dieldrin	1	2	2	0	4	9

[a] These two birds were near death at the end of the experiment.

Residues in Wild Birds

A pooled sample of six pheasant livers taken from wild South Dakota pheasants had <0.1 ppm PCBs. A pooled sample of three livers collected near Washington, Pennsylvania, had <0.1 ppm PCBs, and a pooled sample of six livers collected southeast of Lancaster, Pennsylvania, had 2.0 ppm PCBs. A pooled sample of six livers collected east of Gary, Indiana, had 0.5 ppm PCBs, and a pooled sample of six livers taken from pheasants in Benton County, Indiana, had 1.5 ppm PCBs.

These relatively low levels in livers of wild pheasants taken both from industrial areas and rural areas indicate these pheasants were probably exposed to small amounts of PCBs. Prestt et al. (16) found that residue levels in birds in Britain were related to their food habits. They found the most PCBs in livers of fresh-water fish-eating birds (up to about 900 ppm); bird-feeding raptors had up to 70 ppm; birds which eat mammals had up to 50 ppm; birds with a mixed diet of mammals, birds, and carrion had up to 15 ppm; and those which eat insects had 0–1 ppm.

Summary and Conclusions

PCBs given to laying pheasant hens adversely affected egg production, hatchability, and viability of the embryo about the time of hatching but did not affect fertility or eggshell thickness. The chief effects on reproduction occurred through the PCBs taken in by the hen, not by the cock.

A subtle effect on behavior was indicated by studies on the visual cliff and of the ability of offspring to avoid hand capture. These behavioral effects have been reported for birds with organochlorine insecticides and could be deleterious to a wild species, in that instinctive patterns necessary for survival are involved. Behavioral differences may be affected through PCB administration to the cock as well as through the hen, as shown by the study of hand catching; this implies that the effect is, though unexplained as to mechanism, more than through the physical presence of the PCBs in the egg-yolk lipids. The presence of PCBs in egg lipids is probably responsible for the observed effect of increased mortality in young during the first 6 weeks of life, and in a possible depression of weight at 6 weeks of age. Only early survival of chicks was affected, as no effect was noted in survival from 6 weeks of age through the fall. Sex ratios of young pheasants surviving to the fall showed an expected 50:50 distribution, thus PCBs did not affect the survival of one sex more than another.

Large doses of PCBs, 210 mg daily, effected a loss of appetite, but lesser doses tested did not affect feed consumption until just prior to death. The death of birds given PCBs was not attributable to the starvation that occurred in the few days prior to death, because birds dying from PCBs did not lose as much weight as birds which were not given PCBs but were starved. Further, starved birds did not show the histopathologic effects observed in PCB-treated birds such as degeneration of liver cord cells and depletion of lymphatic nodules in the spleen.

PCBs were (1) rapidly and readily absorbed into the pheasant's body, (2) stored in the lipid fraction, and (3) excreted slowly in feces and eggs. Excretion via the egg may be an important means of ridding the body of PCBs for the hen, but the PCBs in the egg yolk lipid may be dangerous for the offspring because of altered behavior and lowered survival both of the embryo and hatched young.

Brain tissue may be a valuable indicator of PCB toxicosis; levels of 300–400 ppm or more were associated with death due to PCBs. Marked splenic atrophy was the most consistent characteristic noted among several organ parameters checked in birds that died from PCBs. Enlarged

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kidneys and livers were also useful characters in attempting to diagnose PCB toxicosis.

PCBs and dieldrin were not, when combined, synergistic in their joint toxicity to pheasants.

Livers from wild pheasants collected in Pennsylvania, Indiana, and South Dakota, did not exceed 2 ppm PCBs, indicating relatively low-level contamination.

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Embryonic Mortality and Chromosomal Alterations Caused by Aroclor 1254 in Ring Doves

by David B. Peakall, Ph.D., Jeffery L. Lincer, M.S.* and Stephen E. Bloom, Ph.D.†

The breeding success of Ring Doves (*Streptopelia risoria*) fed Aroclor 1254 at 10 ppm was determined over two generations. Six pairs of birds were fed PCBs for three months, then allowed to incubate their clutch of two eggs. Eggs were then collected again for six months, at which time the birds were allowed to incubate another clutch. Results are given in Table 1. The low-hatching success of the second generation was due to heavy embryonic mortality. The age of embryonic death varied considerably but was mainly in the range of 3–8 days. This finding is at variance with that of Scott et al. (1), who found a significant decrease in hatchability of chicken eggs in the first generation with 10 ppm Aroclor 1248 for eight weeks and almost complete failure at both four and eight weeks at 20 ppm Aroclor 1248. In this case mortality occurred immediately before hatching.

Eggshell thinning was not observed in either the first or second generation (2, 3). This is in agreement with the findings of Dahlgren and Linder (4) for the pheasant (*Phasianus colchicus*) and Heath et al. (5) for the Mallard (*Anas platyrhynchos*).

Cytogenetic studies were performed on 24 dove embryos at 3–6 days of incubation. Six embryos were from dove pairs not fed PCBs (control), 17 embryos were from PCB-fed (10 ppm in diet) pairs and one embryo was irradiated with 155 X-rays (positive control). Relative frequencies of chromosome aberrations (aneuploidy, polyploidy, breakage, rearrangements) were recorded for the largest 8 chromosome pairs occurring in metaphase cells of allantoic sac and limb bud origin. An average of 365 metaphases were examined per embryo. In control and PCB-treated groups primarily chromatid, some isochromatid and 1 rearrangement was observed. Mean aberration rates were as follows: control = 0.8% (range = 0–2.0%); PCB-treated = 1.8% (range = 0–9.4%); positive control = 18.0%. The chromosome rearrangement occurred in a PCB-treated embryo. 13 of 17 PCB embryos had aberration rates exceeding the mean control rate, and 4 PCB embryos exceeded the highest control rate with frequencies of 2.4%, 2.6%, 3.1% and 9.4%. These

Table 1. Breeding success of Ring Doves fed 10 ppm PCBs over two generations.

	No. of pairs	No. of eggs laid	No. of eggs hatched	No. of young fledged
Pre-experiment	6	24	22	22
1st generation PCBs	6	24	24	24
2nd generation PCBs	6	20*	4	2**
2nd generation Control	6	24	22	22

* One pair failed to produce any eggs during the period of the experiment.

** Fledging losses consisted of one young found outside the nest at one day of age and one deformed bird killed at the age of three weeks.

* Section of Ecology and Systematics, Cornell University.

† Department of Poultry Science, Cornell University, Ithaca, New York 14850.

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Table 2. Organ levels, ppm, wet weight basis.

	Muscle	Brain	Liver	Fat
Pre-stress (n = 4)	8.1±2.3 (4.1–9.9)	5.5±1.6 (4.8–8.0)	15.3±12.6 (3.6–27.5)	736.1±211.6 (481.2–1069.4)
Post-stress (n = 5)	172.9±38.3 (120.0–227.0)	293.0±27.6 (254.0–340.2)	1118±267 (937.3–1688.0)	***

Figures are means, standard deviations, and range.
*** No fat present.

results are indicative of a possible clastogenic (chromosome breaking) action of PCBs in dove embryos. Further studies are warranted to define more precisely the conditions under which PCBs might be mutagenic, clastogenic and possibly teratogenic to avian and mammalian embryos.

Residue analysis on eggs laid at various times from the start of the experiment showed that the levels increased for three months when an approximately steady level of 50 ppm (dry weight) was reached (6). The second generation eggs had similar levels; thus increased embryonic mortality was not caused by increased levels of PCBs.

The most striking finding of the seabird "wreck" in the Irish Sea in 1969 was the elevated concentration of PCBs in the liver (7). In birds shot as controls the concentration was only 0.4 ppm (wet weight), whereas birds found dead had 40 ppm in the liver. In an attempt to evaluate these findings, we subjected to stress five first generation birds after they had been exposed to Aroclor 1254 in their diet for six months. The stress was applied by reducing the food intake severely so that the birds lost approximately 10% body weight per week. Control birds were also stressed in a similar manner. No significant difference was found in either the time to death nor in the weight loss to death between controls and experimental groups. The organ levels of the first generation birds sacrificed at the end of the breeding experiment can be compared to organ levels after starvation (Table 2). A massive increase of levels as a result of mobilization of fat is clearly seen in all tissues studied. The fact that liver levels ranged from 937–1688 ppm at the time of death strongly suggests that the 40 ppm recorded in the livers of seabirds in the Irish Sea was not responsible for the death of the seabirds.

Increased occurrence of abnormal young in two species of tern has been reported from Long Island (8). In most cases, the levels of PCBs found in the muscle tissue of these abnormal birds were higher than those found in adult Ring Doves during our experiments. It is possible that the chromosomal aberrations noted in our study could be the cause of these abormalities.

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Toxicology of PCBs for Mammals and for Birds

by J. G. Vos*

Introduction

In the early days of its use, little work was done on the toxicology of the PCBs, and this was only in relation to the risks of occupational exposure. As will be shown many more studies were made as soon as it appeared that the extremely stable PCB's became a threat to the environment and its wildlife, and accidents occurred of acute poisoning in man and animals. These studies have been made with material with different contents of chlorine, from different manufacture, and—as we can now say in retrospect—with different and unknown contents of toxic impurities. For this reason the toxicological information of PCB's is difficult to summarize, but since the character of some important impurities has recently been elucidated, it is well to discuss these first in order to be able to consider their contribution to the overall toxicity of the different preparations studied.

These studies were started because of the analogy between the effects of the PCBs (1–6) and some toxic effects associated with toxic factors in crude chlorophenols and in "toxic fat". Effects of the latter are liver damage (7), chloracne (7,8) and edema formation (9).

Chemical, Toxicological, and Pathological Identification and Evaluation of Toxic Impurities in Technical PCB Preparations

The first indication of the presence of toxic impurities was obtained in a comparison of the toxicity of three commercial PCB samples (containing 60% chlorine on the average): Phenoclor DP6 (sample I), Clophen A60 (sample II) and Aroclor 1260 (sample III). These three mixtures showed a marked resemblance in their gas chromatograms and mass spectra (10). In this comparative feeding test in one-day-old chicks (11), it was found that 100% mortality, subcutaneous and abdominal edema, and centrolobular liver necrosis occured in only two groups (fed the samples I and II). Hydropericardium (Fig. 1), a common effect of these two mixtures, was only occasionally seen in the chicks fed the sample III. The

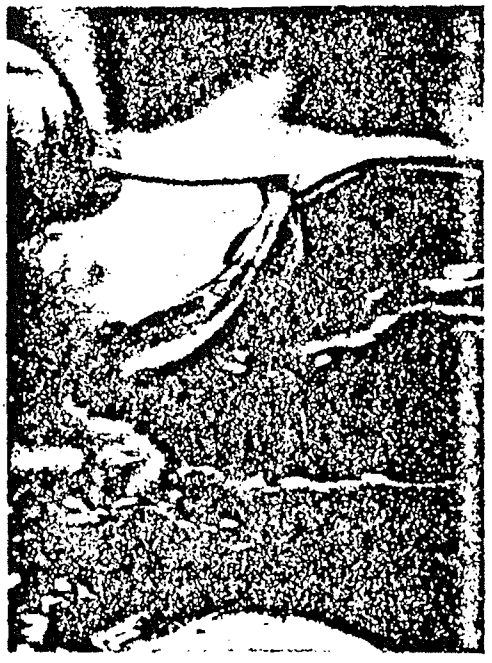

FIGURE 1. Hydropericardium in a chick fed 400 ppm of sample I.

* Institute of Veterinary Pathology and Institute of Veterinary Pharmacology and Toxicology, State University of Utrecht, The Netherlands.

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Table 1. Mortality, and Pathologic Observations of Chicks Fed 400 ppm PCB for 60 Days.

PCB sample	N	Number of deaths	Number of birds with edema			Number of birds with liver necrosis
			Hydro-pericardium	Abdominal	Subcutaneous	
I	24	24	18	8	5	9
II	22	22	20	9	7	9
III	20	3	3	0	0	0
Control	20	0	0	0	0	0

mortality in this group was only 15% (Table 1). The excretion of coproporphyrin and protoporphyrin in the feces was increased. Examination of tissues under Wood's light showed the presence of red fluorescence indicating porphyrins in the liver and other tissues of especially the birds that died. This hepatic porphyria was found in all three experimental groups.

In the subsequent study (12) by means of column and gas-chromatography the presence of relatively more polar compounds was demonstrated in the 25% diethylether fraction of samples I and II. In a chick embryo assay (Table 2), the difference in toxicity between the three samples was confirmed; the high toxicity of the 25% diethylether fraction of sample II is demonstrated by the similarity between the mortality levels in the group injected with 3.5 sample II/egg and the group injected with the 25% diethylether fraction from 3.5 mg sample II/egg.

Mass spectrometric analysis revealed that identical chlorinated compounds were present in the 25% diethylether fraction from samples I and II but not in that from sample III. They included compounds with mass number 304 and 338. The proposed identity of these compounds, tetrachlorodibenzofuran and pentachlorodibenzofuran, is indicated by the following chemical-analytical, pathological, and toxicological data.

Table 2. Chick-Embryo Assay of Three PCB samples and the 25% Diethylether Fraction from Sample II

PCB sample	Dose (mg/egg)	Number of eggs treated	Percentage hatch of fertile eggs
I	3.5	15	0
II	3.5	20	5
III	3.5	15	80
Fraction from sample II	35	15	0
	3.5	15	7
	0.35	15	92
	0.035	15	100
Ethanol control	0	20	94
Untreated control	—	20	90

From exact mass measuremen's it was found that the formulae of these peaks were $C_{12}H_4O^{35}Cl_4$ and $C_{12}H_3O^{35}Cl_5$. The formulae of the fragment ions 241 and 275 were $C_{11}H_4{}^{35}Cl_3$ and $C_{11}H_3{}^{35}Cl_4$. From this data it can be concluded that the parent ion has a preferential fragmentation for the loss of a single ClCO unit. Mass spectra were compared with the spectrum of a chick edema factor 1,2,3,7,8,9-hexachlorodibenzo-p-dioxin. The mass spectrum of this compound has a similar fragmentation pattern, i.e. the loss of two successive mass units of 63 suggests a loss of two ClCO units. With microcoulometric analysis a certain maximum level could be indicated. This maximum level was found to be five ppm of the compound with mass number 338 in sample II and 20 ppm in sample I.

Polychlorinated dibenzofurans are strong hepatotoxic and acnegenic compounds. Tri- and tetrachlorodibenzofurans in a single oral dose of 0.5 – 1.0 mg/kg caused severe and often lethal liver necrosis in rabbits (7,13), and application to the ear resulted in chloracne. Tetrachlorodibenzodioxin was about 10 times more toxic (7). Injection of the 25% diethylether fraction obtained from 35 mg sample II into the aircell resulted in 100% mortality (Table 2). It can be calculated that the maximum dose/egg is 0.2 μg pentachlorodibenzofuran (taking five ppm as the maxi-

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mum value). This confirms the order of toxicity found by Higginbotham et al. (9) in the case of chloro-dibenzodioxins. Tetra and pentachloridibenzofurans were considered responsible for the higher toxicity of samples I and II.

Confirmation is also obtained from the subsequent comparative toxicity study in rabbits (14). Again samples I and II were more toxic; the liver and skin lesions were more severe. Porphyria, especially of the liver, was present in all three groups (Fig. 2). A remarkable finding was the intense red fluorescence of small foci inside hepatic cells. They probably represent nuclei. This was confirmed in an additional cell culture experiment with the Aroclor sample and with 2,4,5,-2′,4′,5′-hexachlorobiphenyl. (This experiment was carried out by my colleague Dr. J. G. Wit of the Biochemical Section).

Application of the 25% diethylether fraction on the skin of rabbits resulted also in differences in toxicity (Fig. 3). So the presence of the hepatotoxic and acnegenic polychlorinated dibenzofurans as impurities in samples I and II was found to be established.

In another experiment (15), the toxicity of the Aroclor (60% Cl) sample was compared with the toxicity of 2,4,5,2′,4′,5′-hexachlorobiphenyl. From the increased fecal excretion of coproporphyrin in both experimental groups (Table 3), it is very likely that PCBs themselves are responsible for the porphyrogenic action of crude preparations. From the presence of slight skin lesions induced by 2,4,5,2′,4′,5′-hexachlorobiphenyl when compared with the Aroclor sample, it can be concluded that the major acnegenic action of crude mixtures comes from a possible contamination with chlorinated dibenzofurans. It can also be concluded that PCBs themselves have a slight acnegenic action. Liver damage was essentially the same after treatment with both 2,4,5,2′,4′,5′-hexachlorobiphenyl and the Aroclor mixture. The conclusion that the liver injury, caused by crude preparations, is predominantly due to the contaminants, is based on the differences in liver toxicity between the three PCB preparations (11,14).

The probable contribution of polychlorinated dibenzofuran (PCF) and pure polychlorinated biphenyl (PCBs) in the toxicity of crude preparations is summarized in Table 4. A proper evaluation of toxicity data and residue data can be hindered by the possibility that PCB samples may



FIGURE 2. Fluorescence of porphyrins under ultraviolet light in livers from rabbits treated with 60% chlorinated PCB's, 1, Aroclor; 2, Clophen; 3, Phenoclor; and 4, Control liver (14).

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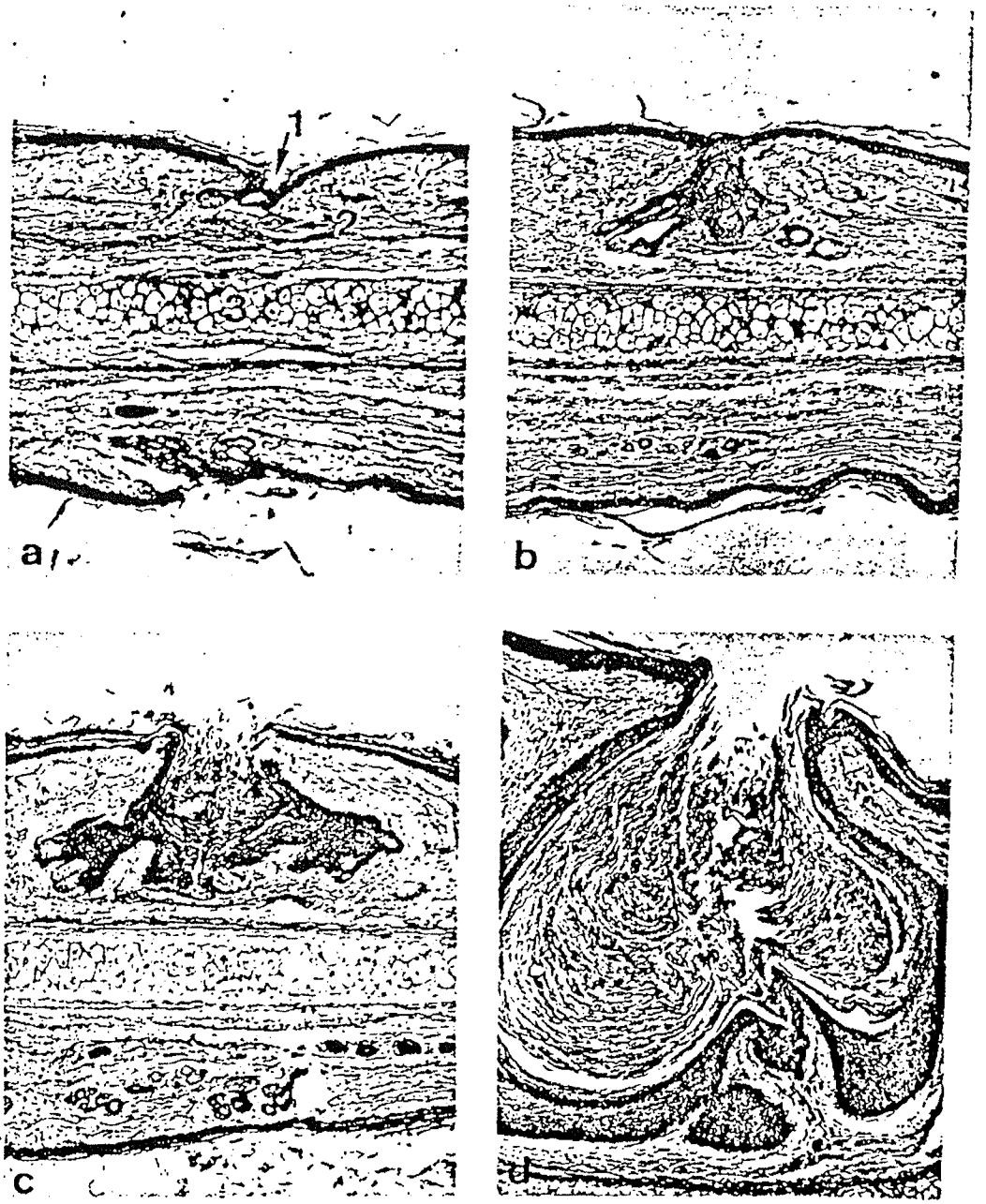

FIGURE 3. Response of the inside of the rabbit's ear after topical application of the 25% diethylether fractions from technical PCB's. Haemotoxylin and eosin. ×60. (a) Skin of control animal treated with ethanol. Note the hair follicle at 1, sebaceous gland tissue at 2, and cartilage at 3. (b). Ear skin of the animal treated with the fraction from sample III. Some hyperplasia and hyperkeratosis of the follicular epithelium can be seen. (c). Ear skin of the rabbit treated with the fraction from sample II. Considerable hyperplasia and hyperkeratosis of the follicular epithelium. (d). Ear skin of the rabbit treated with the fraction from sample I. Part of a section that shows the most severe lesion. The gravity of the response was in general the same as seen in (c). Note the cystic dilated hair follicle with prominent hyperplasia and hyperkeratosis of the follicular and epidermal epithelium (14).

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differ in an important respect: the presence of toxic impurities. The possibility of distinguishing between the effects of PCBs and their impurities can be further improved by using pure isomers with known positions of the chlorine atoms.

Mortality, Liver Effects, Edema Formation and Other Effects

These data, as presented by different authors, are summarized in Tables 5, 6, and 7. As can be seen in Table 5, the acute and subacute toxicity data of PCB's are poor. The established values are high. Semichronic oral toxicity studies are summarized in Table 6. Dermal and inhalation studies are given in Table 7.

Table 3. Coproporphyrin Contents (μg/g Dry Weight) of Feces of Rabbits Treated with PCB for 4 weeks, and of Controls.[a]

2,4,5,2',4',5'-Hexachlorobiphenyl	Aroclor (60% Cl)	Control
45.0	24.1	4.5
6.2	5.0	3.7
20.8	29.4	3.6
39.6	16.0	3.5
Mean 27.9[b]	18.6[b]	3.8

[a] Figures are the contents of feces, collected from the cecum of the individual animals.
[b] Significantly different from controls, $P \leq 0.025$.

It is very probable that the results of these studies may have been influenced by the presence of polychlorodibenzofurans or other toxic impurities. For example, in the study of Rehfeld et al. (23) general edema was already found in chicks fed 30 ppm Aroclor 48% Cl, while Kohanawa and co-workers (22) noted edema formation at the 100 ppm level of another 48% chlorinated mixture. Mortality in the latter study was also lower (Table 6).

Liver Effects

The most important liver effects, summarized in Tables 5, 6, and 7, are weight increase, fatty degeneration, hyalin degeneration and necrosis. Increased liver weights, as noted in several studies,

Table 4. Probable Contribution of Polychlorinated Dibenzofuran (PCF) and Pure Polychlorinated Biphenyl (PCB) in the Toxicity of Crude PCB Mixtures.

	Chloracne	Edema formation	Liver damage	Hepatic porphyria
Polychlorinated Dibenzofuran	++	++	++	–
Polychlorinated Biphenyl	+	–	+	++

Table 5. Acute and Subacute Oral Toxicity Studies of PCB Preparations.

Preparation	Animal	Treatment	Mortality	Liver effects	References
Unknown	Mouse	single dose of approx. 2000 mg/kg	LD50		(16)
Aroclor 54% Cl	Rat	single dose of 500 mg/kg	0%	Increase of weight and lipid; potentiation of CCl_4 toxicity	(17)
Aroclor 42, 54, 60, and 68% Cl	Mallard	single dose of 2000 mg/kg	0%		(18)
42% Cl	Rat	20 daily doses of 138 mg	0% in 3 months	Hyalin bodies in liver cells	(19)
42% Cl	Guinea pig	2 doses of 69 mg 1 week apart	100% between 11 and 29 days	Fatty metamorphosis; central atrophy	(19)
65% Cl	Rat	6 daily doses of 300 mg	70% in 14 days	Increase of weight; cell swelling: hyalin granules	(20)

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Table 6. Semichronic Oral Toxicity Studies of PCB Preparations.

Preparation	Animal	Treatment	Mortality	Liver effects	Other effects	References
65% Cl	Rat	Doses of 50 mg every second day	60% in 5 weeks	33% weight increase; cell swelling; hyalin globules		(20)
48% Cl	Cynomolgus monkey	From 641 mg in 40 days to 348 mg in 239 days	not given	Enlargement; SER proliferation	Main cause of death: pneumonia or diarrhea	(21)
48% Cl	Squirrel monkey	From 320 mg in 46 days to 67 mg in 48 days	not given	Enlargement; SER proliferation SER proliferation	Main cause of death: pneumonia or diarrhea; palpebral edema in 1 animal	(21)
48% Cl	Mouse	Daily doses of 0.001 ml for 13 to 26 weeks	0%	Enlargement; SER proliferation RER reduction; myelin figures; increase of microbodies, lysosomes and lipid	Skin: loss of hair, erosion and ulceration after 3 months	(21)
Aroclor 42% Cl	Chicken	100, 200, 400, and and 1000 ppm in diet for 4 weeks	0, 0, 50, 90, and 90% respectively	Enlargement; damage at the higher levels	Edema formation from 200 ppm; at high levels internal haemorrhage and tubular dilatation in kidneys	(5)
Aroclor 42% Cl	Chicken	200 and 400 ppm in diet for 3 weeks	0 and 12% respectively		Pronounced edema at 400 ppm; enlarged kidneys; small spleen; defeathering and dermatitis	(6)
48% Cl	Chicken	1, 5, 10, 25, 50, 100, 300, 600, 1200, 2400, and 4800 ppm in diet for 20 days	0% from 1 to 100 ppm; 100% from the 100 ppm level		Edema formation 100 ppm level	(22)
Aroclor 48% Cl	Chicken	10, 20, 30, 50, 100, and 150 ppm in diet for 4.5–5 weeks	After 3 weeks: 0, 0, 30, 30 and 20%; at the end 0, 0, 80, 60, and 80% respectively	Enlargement	General edema and depression of the secondary sexual characteristics from the 30 ppm level	(23)
Aroclor 54% Cl	Chicken	250 and 150 ppm in diet for 6 to 13 weeks	250 ppm 100% between 3 and 10 weeks; 500 ppm some mortality at the end		500 ppm at end: comb weights 20-fold and testes weights 2-fold lower than controls	(24)
Aroclor 54% Cl	Bengalese finch	Estimated dose rate at 56 days of 254 mg/kg/day	50%		Hydropericardium in some birds	(25)
Phenoclor 60% Cl	Japanese quail	2000 ppm in diet	100% between 6 and 55 days		Hydropericardium	(10)

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Table 7. Dermal Toxicity and Inhalation Studies of PCB Preparations.

Preparation	Animal	Treatment	Mortality	Liver effects	Skin effects	References
42% Cl	Guinea pig	11 daily skin applications of 34.5 mg	100% between 11 and 21 days	Fat; central atrophy; perinuclear basophilic granulation; focal necrosis in a few animals	Occasional thickening of the epidermis	(19)
42% Cl	Rabbit	Skin application at alternate days, total dose from 946 to 1980 mg	100% between 17 and 98 days	Fatty degeneration; central atrophy	Thinning of prickle cell layer and thickening of outer cornified layers	(19)
Aroclor	Rabbit	Daily skin applications of 0.3, 0.6, and 0.9 g	High dose died before liver necrosis developed	Moderate doses: mottled liver, subacute yellow atrophy, fatty degeneration, and marked necrosis	Reddening; formation of small papules and blisters; finally desquamation of external epidermal layers	(26)
Aroclor 65% Cl	Rat	Inhalation of 0.57 mg/cubic meter for 16 hours for 37 to 134 days	0%	Pale and yellow; cell swelling; hyalin degeneration; potentiation of CCl_4 and C_3H_4OH toxicity		(20)

are well explained by the proliferation of smooth surfaced membranes of the endoplasmic reticulum (SER) as was found by Nishizumi (21) in mice and monkeys and by Norback and Allen (27) in rats. The latter workers found a proliferation of the SER in rats fed PCB for 1 to 5 weeks. Concomitant with the structural changes, the activities of measured drug metabolizing enzymes (nitroreductase and aromatic hydroxylase) were increased. The induced level of drug metabolizing activity persisted as the proliferation of the SER decreased and concentric arrays pervaded the cytoplasmic reticulum (27). These concentric membrane arrays, probably representing the hyalin bodies described by Bennet et al. (20) and Miller (19), could have an enzymatic function similar to that associated with the SER (27).

Similar formations, the so-called myelin figures, were demonstrated in mouse liver by electron microscopy; in monkey liver they were not found (21). In both mouse and monkey liver a proliferation of the SER was found. In our comparative dermal toxicity study (15) in rabbits with 2,4,5,2′,4′,5′-hexachlorobiphenyl and Aroclor (60% Cl), the light microscopic findings included necrosis, hydropic degeneration (Figs. 4 and 5) as well as a peripheral and perinuclear shift of cell organelles (Fig. 5) and focal cytoplasmic hyalinization. In electron microscopy, the shift was found to be due to a proliferation of the SER resulting in a displacement of rough surfaced membranes (RER) and mitochondria. The focal cytoplasmic hyalin degeneration, often seen in hydropic cells, was recognized as tightly packed tubules of proliferated SER (Fig. 6). This very probably represents hypertrophic, hypoactive SER.

Sublethal effects caused by induction of hepatic enzymes have been noted by several authors. Increased steroid metabolism in pigeon liver homogenates has been demonstrated by Risebrough et al. (28). Lincer and Peakall (29) confirmed the effect of PCB on the hormone metabolism in birds at very low dose levels. They fed kestrels for 5 months with Aroclor 54% and 62% Cl at levels of 0.5 and 5.0 ppm. The higher

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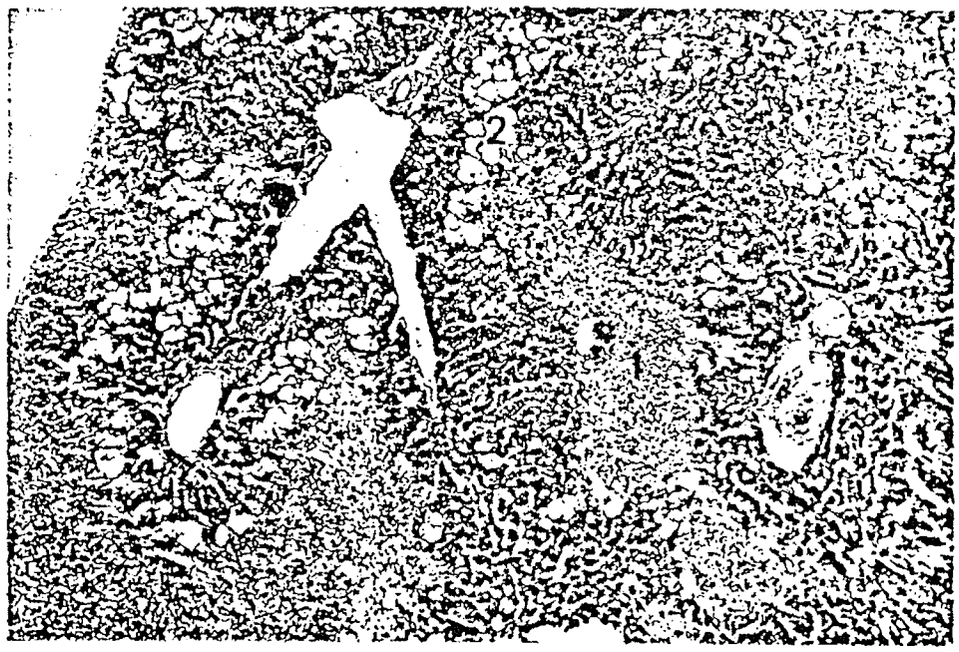

FIGURE 4. Liver damage in a killed rabbit treated with 120 mg Aroclor (60% Cl), 5 times per week, for 28 days. Note the centrolobular necrosis (1) and the hydropic cells (2) at the margin of the necrotic and vital tissue. Haemotoxylin and eosin. ×150.

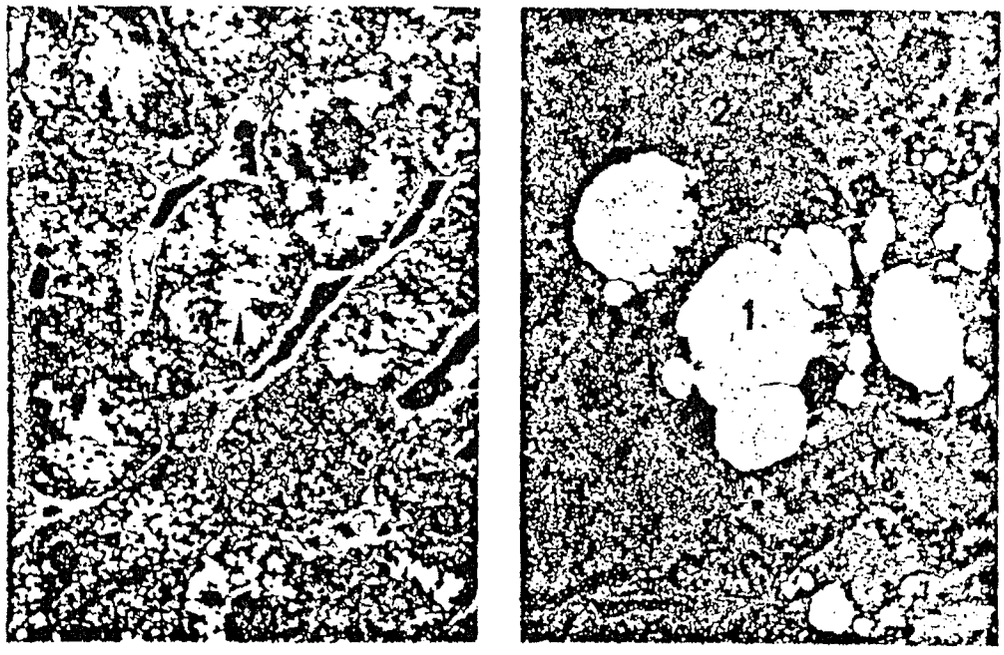

FIGURE 5(a). Liver cells of a control rabbit. Clear areas may represent negative images of glycogen (arrow). Toluidine blue. ×940. (b). Liver cells of an Aroclor treated killed rabbit. Note the hydropic cells (1) and the perinuclear and peripheral displacement of cell organelles with sometimes hyalin foci (2) inside hepatic cells. Toluidine blue. ×940.

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FIGURE 6. Hydropic liver cells from the same animal as seen in Figure 5b showing a large number of vacuoles (V) and a perivacuolar localization of mitochondria (arrow). Note the strong proliferation of the SER, consisting of tightly packed tubules. Uranyl acetate and lead citrate. ×5500.

dose being roughly equivalent to 2 mg/kg PCBs to each kestrel. A dose dependent in vitro breakdown of estradiol to a more polar metabolite occurred in the livers from kestrels fed either Aroclor 1254 or Aroclor 1262. No such conversion took place in the livers of the control birds. The increase in hepatic enzyme activity correlated with an increase in cytoplasmic RNA, as was measured cytophotometrically. A shortened sleeping time after treatment with hexobarbital, and enhanced in vitro rates of aniline hydroxylation and p-nitroanisole demethylations were demonstrated by Street and coworkers (30). These authors also found an increase of these effects with increasing chlorine content of the different PCB preparations (Aroclor 21 to 68% Cl).

Using enzyme induction as parameter, no-effect levels of some PCB preparations were established in the rabbit, rat, and Japanese quail. Oral administration of Aroclor 21% Cl and 54% Cl (1.0 and 10 mg/kg) for 28 days to pregnant rabbits resulted in liver enlargement and increased activities of the drug metabolizing enzymes aniline hydroxylase and aminopyrine n-demethylase at the 10 mg/kg level of the 54% chlorinated Aroclor. The no-effect level for enzyme induction in the pregnant rabbit appeared to lie between 1.0 and 10 mg/kg in the case of Aroclor 54% Cl, and higher than 10 mg/kg for Aroclor 21% Cl (31).

Another parameter for enzyme induction was used by Komatsu and Tanaka (32). They found that the hexobarbital induced sleeping times in rats were reduced by pretreatment with PCBs.

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The minimal effective dose was 5 mg/kg for 3 days with Kanechlor 400 (48% Cl) and 2 mg/kg for 3 days with the higher chlorinated Kanechlor 500. The porphyrogenic action of PCBs was further evaluated in a study with Japanese quail (33). The results (Table 8) indicate that the hepatic porphyria is closely associated with an increase of mitochondrial ALA synthase activity. A significantly increased activity of this enzyme was already noted after administration of daily doses of 1 mg/kg Aroclor 60% Cl for 1 week. Mean PCB content of the liver at that dose was 1.41 ppm. A less sensitive parameter is tissue fluorescence due to excess quantities of porphyrins. Liver fluorescence was only seen at the 100 mg/kg level. It develops, probably, only in animals showing clinical symptoms, such as loss of weight. A similar finding was done in the prior experiment with chickens (11).

Edema Formation

The most striking finding in birds is the accumulation of fluid. The pathogenesis of the edema formation is discussed by Flick and coworkers (6). The primary site of the edema causing factor could be the heart by increasing the permeability of the vascular bed, leading to cardiac congestion. Pulmonary edema could be the result of the cardiac congestion. The pulmonary edema might be followed by a flow of fluid into abdominal and subcutaneous air sacs. Decreased serum protein values (34) could also contribute to the edema formation. Liver damage can be responsible for reduced serum albumin levels.

As mentioned in Table 4, the edema formation is probably due to the presence of polychlorodibenzofurans. In our study the edema formation by the 60% chlorinated Aroclor sample was minimal at the 400 ppm level. As can be seen in Table 6, chick edema-like lesions were noted at low feeding levels and were caused by lower chlorinated Aroclors. Therefore the presence of toxic impurities in these Aroclor samples has to be considered.

Other Effects

An interaction of PCBs with duck hepatitis virus was found by Friend and Trainer (35). Ten-day-old ducklings were fed a 54% chlorinated Aroclor mixture at levels of 25, 50 and 100 ppm. The birds suffered no apparent clinical intoxications. Five days later they were challenged with duck hepatitis virus, and they suffered significantly higher mortality than birds which were not exposed to PCBs.

Effects of PCBs on the lymphoid system were noted in some studies. Feeding of PCBs to chickens resulted in small spleens (6,11). Lymphopenia, atrophy of the cortex of the thymus, and a reduction in the number of germinal centers in spleen and lymph nodes was found in rabbits (14). Therefore, an immunosuppressive action could be present. In an experiment with guinea pigs, this was established (36). Feeding of 10 ppm Aroclor 60% Cl, for 8 weeks resulted in a

Table 8. Formation of δ-Aminoevulinic Acid by Liver Mitochondria, Liver Residues, and Tissue Fluorescence in Female Japanese Quail Orally Dosed with PCB for Seven Days.

Aroclor (60% Cl) (mg/kg body weight)	ALA formed (mμ moles ALA/g liver/hr)	PCB content liver (ppm)	Tissue fluorescence incidence	
			Macroscopic	Microscopic (liver)
0	6.46±1.64	0.15[c]	0/5	0/5
0.1	8.76±4.06	0.45±0.27	0/5	0/5
1	10.50±1.21[b]	1.41±0.67	0/5	0/5
10	17.3±6.4[b]	27.0±9.4	0/5	0/5
100	119.9[c]	478±294	3/5	2/5

[a] Mean values±SD, 5 birds per group.
[b] Significantly different from controls, $P \leq 0.01$.
[c] Pooled samples

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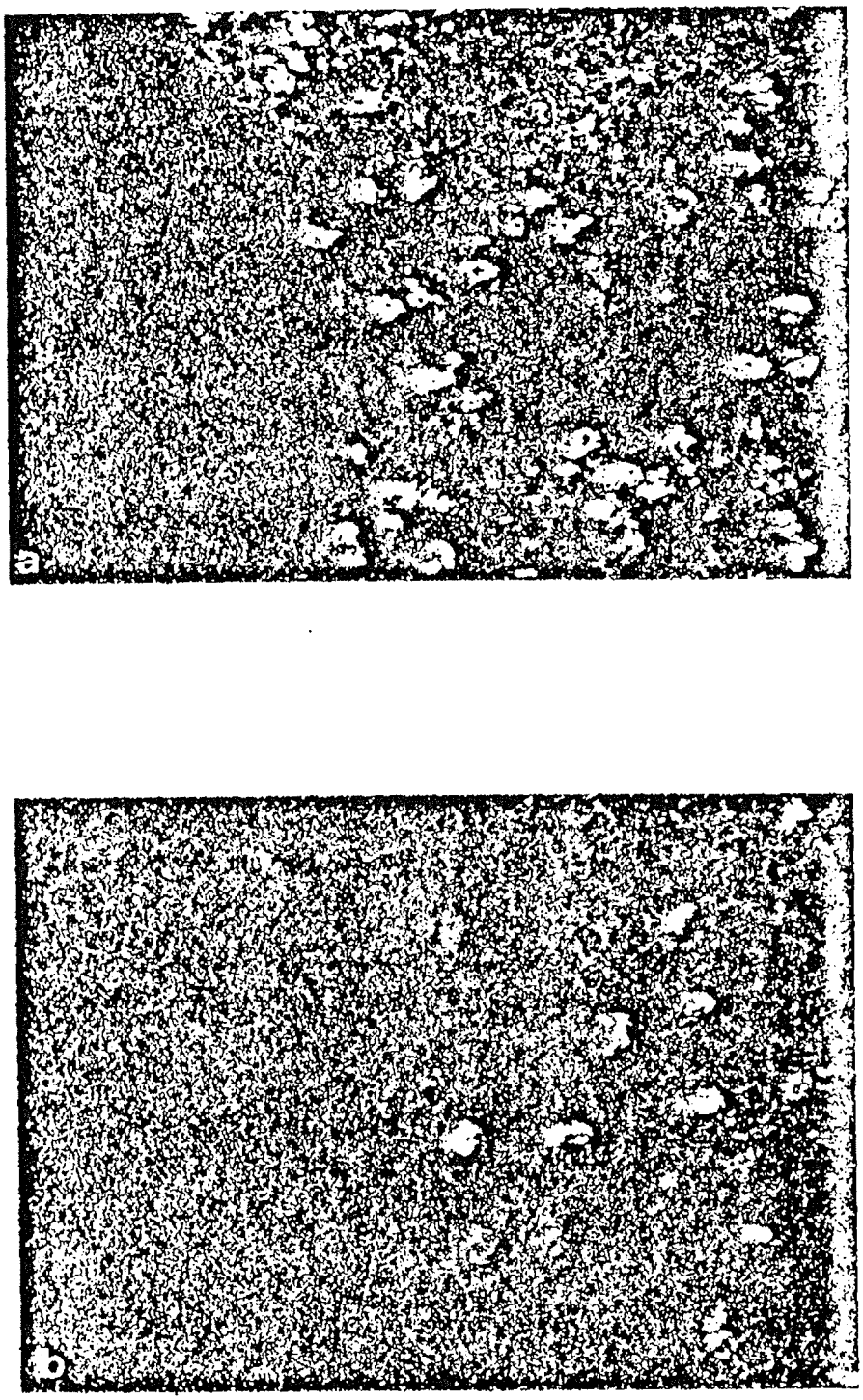

FIGURE 7. Representative areas of tetanus toxoid stimulated popliteal lymph nodes of guinea pigs. (a) Large number of antibody forming cells in a control animal. (b). Reduced number of antibody forming cells in an animal fed 10 ppm Aroclor (00% Cl) for 8 weeks. Direct fluorescent antibody technique. Cryostat sections. × 375. (36).

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decreased number of antibody-forming cells in the popliteal lymph node, after stimulation of the humoral lymphoid system with tetanus toxoid (Fig. 7). This suppression may explain the higher sensitivity of PCB-fed ducklings for duck hepatitis virus (35). In a comparative toxicity study in guinea pigs, an indication for an effect of PCB (Clophen and Aroclor 60% Cl) on the cell-mediated immunity was obtained. Feeding of these mixtures at 50 ppm levels for 6 weeks resulted in a decreased number of circulating lymphocytes (unpublished data).

An estrogenic activity of PCBs (Aroclor 21–48% Cl) was demonstrated by Bitman and Cecil (37). The estrogenic activity was evaluated using the 18-hr glycogen response of the immature rat uterus after a single subcutaneous injection. The minimum effective dose was 8 mg. The higher chlorinated PCB mixtures were inactive at the 8 mg level. In the above mentioned subacute feeding study of 60% chlorinated mixtures in guinea pigs, we found significantly increased uterus weights in the PCB treated animals. Both increased steroid metabolism, as mentioned by Rehfeld and coworkers (23), and the estrogenic activity could be responsible for the depression of secondary sexual characteristics (decreased development of comb and wattles) noted in cockerels (24).

Administration of Aroclor (54% Cl) at levels of 12.5, 25, and 50 mg/kg body weight during the first 28 days of gestation had embryotoxic effects in the rabbit (31). Edema and beak deformities in chicken embryos have been described after yolk-sac injection of 10 and 25 mg 42% chlorinated Aroclor, resulting in respectively 95 and 100% embryonic mortality (38).

An effect of PCB on the nervous system was noted by Ogawa (39). Oral administration of PCB (0.3–0.5 ml/kg/day) to rats for 14 or 21 days, resulted in marked or moderately impaired motor function, decreased motor conduction velocity and loss of large nerve fibres. He concluded that PCB caused neuropathy in rats.

Conclusion

Because of the possible presence of polychlorinated dibenzofurans (PCF) or other toxic impurities in crude PCB preparations, it is difficult to interpret many of the toxicity studie Pure samples are required for comparative i vestigations. Also the fate of the toxic impuriti in the environment has to be determined. A presented here, PCBs have several sublethe effects, such as microsomal enzyme induction porphyrogenic action, estrogenic activity, an immunosuppression. Since porphyria seems t be an effect of PCBs themselves and not fron PCF, the induction of ALA synthase could b used as criterion in the approximation of a no-effect level (at least for the 60% chlorine type of PCBs). The no-effect level could be about 0.1 mg/kg (mean PCB content of the liver in Japanese quail about 0.2 ppm). This is in the same order of magnitude as found in the other studies. Additional research is needed to determine fully the significance of these sublethal effects. Moreover, chronic and reproduction studies are necessary. The present results also make clear that manufacture of commercial PCB mixtures that are free from impurities is urgently requested.

Acknowledgment

The author gratefully acknowledges the helpful suggestions and critical reading of Prof. H. van Genderen, Head of the Institute of Veterinary Pharmacology and Toxicology. Many thanks are also due to colleagues of the working party of the Institute of Veterinary Pathology and the Institute of Veterinary Pharmacology and Toxicology: Dr. J. H. Koeman, Mr. H. L. van der Maas, and Dr. J. G. Wit. The author also thanks the students who studied for their degree in biological toxicology and Mr. M. C. ten Noever de Brauw, Mr. R. H. de Vos, and Dr. R. J. C. Kleipool of the Central Institute for Food and Nutrition Research, T. N. O., Zeist.

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Epidemiologic Study on Yusho, a Poisoning Caused by Ingestion of Rice Oil Contaminated with a Commercial Brand of Polychlorinated Biphenyls*

by Masanori Kuratsune, Takesumi Yoshimura, Junichi Matsuzaka, and Atsuko Yamaguchi

In October, 1968, an epidemic of a peculiar skin disease similar to chloracne was reported in Fukuoka-Ken (Fukuoka prefecture), Japan. The epidemic was later proved to have spread not only over Fukuoka-Ken but also over 20 other prefectures in the western part of Japan (Fig. 1). It produced 1,057 patients according to the latest tabulation (August, 1971) by the Ministry of Welfare.

Soon after the epidemic was announced, a study group was organized by the staff of the faculties of medicine, pharmaceutical sciences, agriculture, and engineering of the Kyushu University and by the staff of the local health departments, to clarify the cause of the epidemic and to effectuate its control (1). We participated in the study group and conducted, as members of its epidemiologic study subgroup, an extensive epidemiologic investigation following the basic methodology of epidemiology (2). Fortunately, the cause of the epidemic was soon demonstrated to be the ingestion of a brand of rice oil contaminated with a commercial brand of polychlorinated biphenyls, and the disease was called "Yusho", namely oil disease.

Although our observations and experiences are confined to the Yusho patients seen in Fukuoka-Ken, we believe that reporting of them will help many people in the world who are deeply concerned about the chronic deleterious effects of PCBs. The outline of our findings will, therefore, be presented, referring in addition to some other important observations reported by the clinical and chemical study subgroups of the Yusho study group and to those collected from other sources.

Clinical Symptoms

The most common initial symptoms experi-

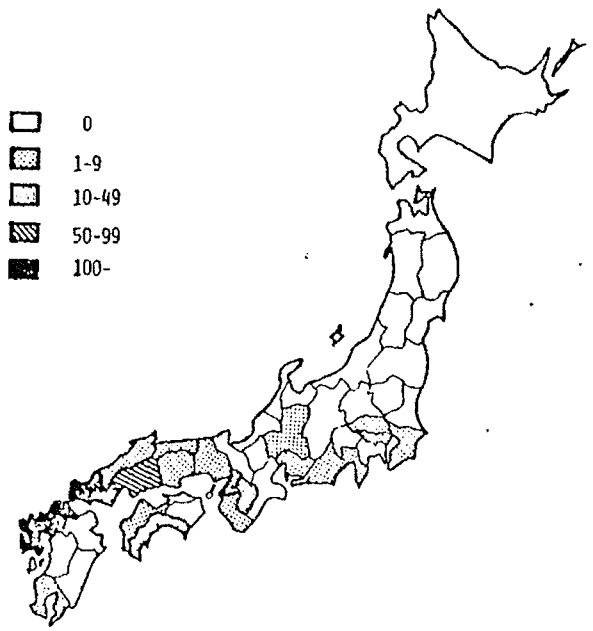

FIGURE 1. Number of patients with Yusho by prefecture.

* Prepared by Department of Public Health, Faculty of Medicine, Kyushu University, Fukuoka, Japan.

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Table 1. Initial symptoms Goto et al.

Initial symptoms	Patients No.	Patients %
Swelling of upper eyelids, increased eye discharge	52	38.3
Acne-form eruption, follicular accentuation	45	33.1
Edematous swelling of limbs	9	6.6
Languishment	4	2.9
Disturbances in digestive canal	4	2.9
Numbness and other neurological signs	9	6.6
Pigmentation of skin	13	9.6
Total	136	100.0

enced by 136 patients with Yusho were increased eye discharge and swelling of upper eyelids (38.3%), followed by acne-form eruption and follicular accentuation (33.1%), and pigmentation of the skin (9.6%) (3) (Table 1). With others which appeared later, the subjective symptoms of Yusho as stated by 189 patients diagnosed up to October 31, 1968, are summarized in Table 2. Dark brown pigmentation of nails and skin, follicular accentuation, acne-form eruption, increased eye discharge, increased sweating at palms, and feeling of weakness were the most notable symptoms (2).

Descriptive Epidemiologic Studies

First, 325 patients seen in Fukuoka-Ken from October, 1968, to January, 1969, were analyzed in order to know their distribution characteristics. One of the most important characteristics readily noted was a distinct familiar aggregation. The 325 patients belonged to 112 families. As shown in Fig. 2 and Table 3, 99 percent of these patients were affected during 1968, while the remaining stated that they became ill in December, 1967. Fifty-five percent of the patients were concentrated in the 3 months from June to August, and no significant difference was noted between sexes in monthly distribution (Table 3). For geographical distribution of the patients, crude incidence rates of Yusho were calculated for 3 large cities and for the jurisdictional areas of the 22 local health departments of Fukuoka-Ken. Excepting 10 health departments where no cases of Yusho were reported, the rates varied considerably from 1.0 to 58.9 per 100,000. Examination of such geographical distribution failed to indicate any common socioeconomic or environmental factor which might be associated with the disease. The 325 patients consisted of 158 males and 167 females, indicating that both sexes were equally affected. More than 90 percent of them were younger than 50 years (Table 4). Age- and sex-specific incidence rates again indicated no significant sex difference but lower risks for both males and females in the age group over 60 years (Table 5).

Analytical Epidemiologic Studies

When our study started, a commercial brand of rice oil produced by K company (abbreviated as K rice oil) in Kitakyushu-Shi (Kitakyushu city), Fukuoka-Ken, had vaguely been suspected as a possible cause of the disease, because most patients with Yusho seemed to have used it.

Table 2. Percent distribution of symptoms of Yusho reported by 189 patients examined before October 31, 1968.

Symptoms	Males (N = 89)	Females (N = 100)
Dark brown pigmentation of nails	83.1	75.0
Distinctive hair follicles	64.0	56.0
Increased sweating at palms	50.6	55.0
Acnelike skin eruptions	87.6	82.0
Red plaques on limbs	20.2	16.0
Itching	42.7	52.0
Pigmentation of skin	75.3	72.0
Swelling of limbs	20.2	41.0
Stiffened soles in feet and palms of hands	24.7	29.0
Pigmented mucous membrane	56.2	47.0
Increased eye discharge	88.8	83.0
Hyperemia of conjunctiva	70.8	71.0
Transient visual disturbance	56.2	55.0
Jaundice	11.2	11.0
Swelling of upper eyelids	71.9	74.0
Feeling of weakness	58.4	52.0
Numbness in limbs	32.6	39.0
Fever	16.9	19.0
Hearing difficulties	18.0	19.0
Spasm of limbs	7.9	8.0
Headache	30.3	39.0
Vomiting	23.6	28.0
Diarrhea	19.1	17.0

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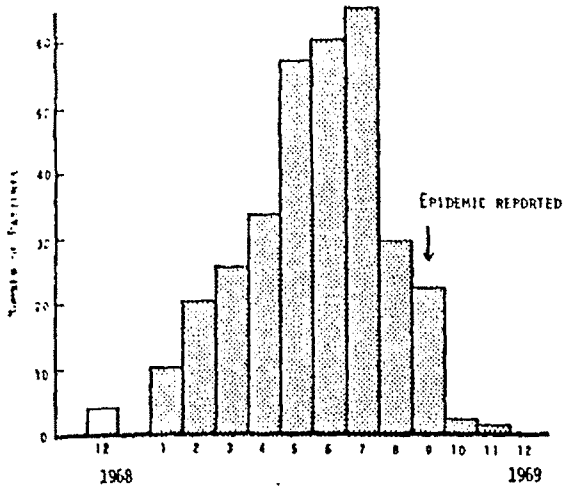

FIGURE 2. Distribution of patients by sex and month when symptoms appeared.

Therefore, a thorough investigation was undertaken to determine whether the patients had actually taken such special brand of oil before being affected. This was achieved by examining lot numbers appearing on the remaining containers of the oil used by some of the patients, shipping records of K company, and purchase and sale records at wholesale oil dealers' offices and at retail stores. It was soon disclosed that all the patients had used K rice oil, either canned (16.5 kg) or bottled (1.65 kg). Furthermore, an astonishing fact became evident: No matter where they lived, 166 of 170 patients who used only the canned K rice oil had used a very specific oil, produced or shipped by the company on February 5 and 6, 1968 (Table 6). For those who used bottled K rice oil, date of production or shipment could not be confirmed, because they had no old bottles at home. Examination of all available records concerning shipping, sale, and purchase at K company, wholesale dealers and retail stores, however, clearly indicated a possibility that 143 of 155 patients who used bottled K rice oil only had used a very specific rice oil produced or shipped from February 5 to 15, 1968. This was because such oil had been shipped to and had reached the retail stores from which they had purchased their oil (Table 6). Thus, nearly all of the patients had had a very peculiar common experience of use or possible use of a rice oil produced or shipped by one company in a specific period of time. An attack rate as high as 63.9 percent was noted for those who consumed the specific canned oil.

In an additional survey, we examined whether those who regularly used K rice oil, but did not use the specific K rice oil produced or shipped

Table 3. Distribution of patients with yusho diagnosed by January 20, 1969, by sex and month when symptoms appeared.

Month	Males		Females		Total	
	Number	Percent	Number	Percent	Number	Percent
Before 1968	2	1.3	2	1.2	4	1.2
1968						
January	0	0.0	0	0.0	0	0.0
February	4	2.5	6	3.6	10	3.1
March	8	5.1	12	7.2	20	6.2
April	15	9.5	10	6.0	25	7.7
May	18	11.4	15	9.0	33	10.2
June	23	14.6	33	19.8	56	17.2
July	27	17.1	32	19.2	59	18.1
August	34	21.5	30	18.0	64	19.7
September	17	10.8	12	7.2	29	8.9
October	8	5.1	14	8.4	22	6.8
November	1	0.6	1	0.6	2	0.6
December	1	0.6	0	0.0	1	0.3
Total	158	100.0	167	100.0	325	100.0

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Table 4. Distribution of 325 patients with yusho diagnosed by January 20, 1969, by sex and age group.

Age group (years)	Males		Females		Total	
	Number	Percent	Number	Percent	Number	Percent
0–9	37	23.4	27	16.2	64	19.7
10–19	38	24.1	28	16.8	66	20.3
20–29	28	17.7	36	21.6	64	19.7
30–39	30	19.0	39	23.4	69	21.2
40–49	11	7.0	23	13.8	34	10.5
50–59	9	5.7	11	6.6	20	6.2
60–69	4	2.5	3	1.8	7	2.2
70–79	1	0.5	0	0.0	1	0.3
Total	158	100.0	167	100.0	325	100.0

in the period in question, were free of the disease or not. A group of 113 persons of 29 households living in an apartment house had purchased canned K rice oil as a unit from one dealer and divided it among themselves fairly regularly from December, 1967, to September, 1968, except for the period from January to April, 1968, when no bulk purchases were made. Their disease experience in 1968 was carefully explored through investigation of their medical records at hospitals and doctors' offices. No case of Yusho was found among them.

All these results suggested that Yusho was caused by use of the K rice oil that was produced or shipped from K company on February 5 and 6, 1968, or soon thereafter. Nevertheless, some other factors or agents might have been the primary or secondary cause of the epidemic. Therefore two case-control studies were done. In one, the personal backgrounds of the patients and matche controls were compared. In the other, the use c oil and fats in the households of the patients an the controls were compared.

121 patients and their 121 healthy control (53 males and 68 females) matched by age, se and place of residence to each of the patient were selected and asked 60 questions concernin their occupations, medical background, genera health status, habits, customs, diet, pets, an other characteristics of their lives. As shown i Table 7, only one of the 60 personal factor examined, namely habit of "eating fried food or tempura nearly every day", was significantl more commonly seen among the patients tha among the controls.

In the latter case-control study, 69 household with Yusho patients were matched by place o residence with 207 control households withou

Table 5. Age-specific incidence rates for Yusho, by sex of patients.

Age group (years)	Incidence rates per 100,000	
	Males	Females
0–9	11.4	8.6
10–19	8.9	6.6
20–29	9.1	10.3
30–39	0.6	11.9
40–49	5.4	9.3
50–59	5.4	7.2
60–69	3.5	2.4
70 and over	1.7	0.0
Total	8.3	8.1

Table 6. Rice oil used by Yusho patients.

No. patients	Specifications of oil used	
	Type	Date of production or shipmen
166 (51.1)	Canned	February 5 and 6, 1968
4 (1.2)	Canned	Unknown
143 (44.0)	Bottled	Unknown, but use of oil shipped from February 5 to 15, 1968 is very probable.
12 (3.7)	Bottled	Unknown
325 (100.0)		

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Table 7. Results of case-control study.

Items investigated	Cases %	Controls %
Allergic to fish	5.0	7.5
Allergic to aspirin	0.0	4.2
Allergic to other drugs	7.5	6.6
Having bath facilities at home	84.7	85.5
Taking bath everyday	73.0	70.6
Having pets at home	18.3*	36.5*
Living in house smaller than 66 m² floor space	66.9	66.1
Handling agricultural chemicals	2.5	6.6
Taking cod liver oil	10.8	8.3
Taking vitamin pills	23.2	18.3
Taking other restorative drugs	9.1	7.5
Water supply available at home	81.3	74.7
Dining out occasionally	28.1	30.6
Dining the same meals with families	88.8	89.6
Eat green vegetables daily	63.1	58.9
Drink milk nearly everyday	49.0	39.0
Take butter nearly everyday	22.4	24.9
Eat eggs nearly everyday	64.7	59.8
Eat fried foods or tempura nearly everyday	22.4*	11.6*
Eat foods prepared with oil nearly everyday	21.6	29.1
Eat fish nearly everyday	21.6	29.1
Take mayonnaise nearly everyday	10.8	10.8
Eat instant "rahmen" or chinese noodle nearly everyday	10.8	10.0

* $p<0.05$.

such patients. A distinct difference was noted between the two groups only for regular use of rice-bran oil, namely 96 percent of the patients' households affirmed it while only 31 percent of the control households did (Table 8). Thus, the case-control studies clearly indicated that none of the factors tested except use of K rice oil could account for the disease.

Dose-Response Relationship

To prove a causal relationship, a definite dose-response relationship is needed. A rough estimate of the quantity of the specific K rice oil consumed by each patient and his family members was made regarding their age, sex, amount of food intake, and possible loss of oil during and after cooking [4]. Eighty of the 146 users of the specific canned K rice oil in question were believed to have consumed, individually, less than 720 ml. For these 80 light users, the attack rate of Yusho was 88 percent, while for those who were estimated to have used more than 720 ml, it was 100 percent (Table 9). It was also demonstrated that the proportion of severe cases of Yusho clearly increased with the amount of oil consumed. While the clinical severity of the disease was not found to differ significantly between the sexes, it did differ considerably according to age, as shown in Table 10 and Fig. 3. The proportion of severe cases among those aged 13 to 29 was significantly larger than that of other age-classes. Therefore, each of the three groups of users, with different levels of oil intake, was standardized for age using the age composition of the whole 146 users as standard. The figures, however, hardly changed from those shown in Table 9. Thus, a clear dose-response relationship could be demonstrated, even though the estimates of the dose were inaccurate (4).

Toxic Agent

In view of all these epidemiologic observations, we concluded that the K rice oil of specific production or shipments had caused Yusho. Now, why was the oil toxic? The chemical study subgroup (chief: Prof. H. Tsukamoto, Faculty of Pharmaceutical Sciences, Kyushu University) demonstrated that the canned K rice oil produced or shipped on February 5, 1968, and used by some of the patients contained about 2,000 to

Table 8. Results of case-control study on oils used.

Fat or oil	Cases		Controls	
	No. of households	%	No. of households	%
Butter	35	50.7	105	50.7
Margarine	44	63.8	127	61.4
Sesame oil	21	30.5	85	41.1
Rape-seed oil	10	14.5	77	37.2
Rice-bran oil	66	95.7	64	30.9
Lard	12	17.4	38	18.4
Other oils	13	18.8	117	56.5

Cases: 69 patient households.
Controls: 207 non-patient households.

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Table 9. Relation between the amount of the K rice oil used by patients and their clinical severity.

Amount of oil	Non-affected		Light cases		Severe cases		Total	
	No.	%	No.	%	No.	%	No.	%
Less than 720 ml	10	(12.0)	39	(49.0)	31	(39.0)	80	(100.0)
720–1,440 ml	0	(0.0)	14	(31.0)	31	(69.0)	45	(100.0)
More than 1,440 ml	0	(0.0)	3	(14.0)	18	(86.0)	21	(100.0)

3,000 ppm of Kanechlor 400, (Kanegafuchi Chemical Industrial Co. Ltd.) a brand of polychlorinated biphenyls (chlorine content: 48%) (5). In this discovery, Prof. K. Inagami of the Department of Food Technology, Faculty of Agriculture, Kyushu University, and his associates made most admirable contributions as members of the chemical study subgroup. The study subgroup also demonstrated that the oil was not contaminated with toxic agents such as Cu, Ni, Zn, Co, As, Hg and Pentachlorophenol. Furthermore, it found that most of the components of Kanechlor 400, particularly those corresponding to the peaks of higher retention times in the gas chromatograms, were retained in the sebum, subcutaneous fat, mesenterium, mesenteriolum, extraperitoneal adipose tissue, appendix vermiformis, heart, sternal marrow, small intestine, trachea, and other organs of patients, and can be transported into the fetus through the placenta (5,6,7). To examine whether only the K rice oil produced or shipped in the period in question was contaminated with Kanechlor, the chemical study subgroup analyzed 109 samples of the bottled rice oil which had been shipped between October, 1967, and October, 1968. Gas chromatographic analysis revealed that a significant contamination of the bottled oil was limited only to those produced or shipped between February 7 to 10, 1968 (5). No analysis could be made of the bottled K rice oil produced or shipped on February 5 and 6 because of the lack of samples. Similarly, 479 random samples of bottled K rice oil were analyzed for chlorine content by the X-ray fluorescence method with a count meter. Again, only the samples of February 7 to 10 contained a large amount of chlorine (maximum 462 ppm). None of the oils shipped in other months were contaminated with more than a trace amount of chlorine. Thus, the results of chemical studies completely coincided with those achieved by epidemiologic approaches. By the way, the Kanechlor had been used at the K Company in the equipment for heating the processed oil at a reduced pressure in order to remove the odorous matters of the rice oil (Fig. 4). It is

Table 10. Clinical severity by age.

Amount of oil used (ml)	Age	Number of patients by clinical grade			
		Non-affected	Light*	Severe*	Total
<720	0–12	3 (16.7)	13 (72.2)	2 (11.1)	18 (100.0)
	13–29	2 (8.3)	7 (29.2)	15 (62.5)	24 (100.0)
	30–	5 (13.1)	19 (50.0)	14 (36.9)	38 (100.0)
	Total	10 (12.5)	39 (48.8)	31 (38.7)	80 (100.0)
720–1440	0–12	0 (0)	5 (50.0)	5 (50.0)	10 (100.0)
	13–29	0 (0)	1 (6.7)	14 (93.3)	15 (100.0)
	30–	0 (0)	8 (40.0)	12 (60.0)	20 (100.0)
	Total	0 (0)	14 (31.2)	31 (68.8)	45 (100.0)

* "Light" corresponds to grades I and II while "severe" to grades III and IV used by Goto et al.

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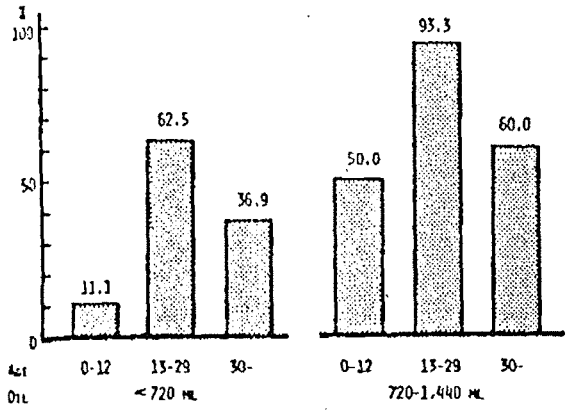

FIGURE 3. Percentage of severe cases of Yusho by age.

believed that it must have leaked from the heating pipe and contaminated the oil, because small openings were discovered in the old pipe.

Amount of Kanechlor 400 Ingested by Patients

One hundred forty-six patients who were proved to have used the contaminated canned K rice oil of February 5 and 6 by our investigation were used for the estimation of the amount of Kanechlor 400 ingested. As stated, the approximate amount of the oil consumed could be calculated for each of the patients. It was on average about 800 ml. Since the concentration of Kanechlor 400 in the oil was 2,000 to 3,000 ppm, the average amount of Kanechlor 400 ingested by a patient was estimated to be about 2 g (4). Similarly, the minimum dose of Kanechlor 400 consumed by a patient was estimated to be about 0.5 g.

Babies Born to Patients and Non-affected Wives of Patients

Thirteen women consisting of 11 with Yusho and 2 unaffected wives of patients were shown to have delivered 10 live born and two stillborn babies from February 15 to December 31, 1968 (8,10,11). As shown in Table 11, nine of them had unusually grayish, dark-brown stained skin and similar pigmentation of the gingiva and nails was noted in five of them. Increased eye discharge was also notable in most of them. Histological examination of a stillborn fetus showed a marked hyperkeratosis and atrophy of epidermis and cystic dilatation of hair follicle, especially at the head. A marked increase of melanin pigments in the basal cells of epidermis was also noted (9). Since no such symptoms at birth or stillbirth has been experienced in Japan during the period in which their incidence among the patients was extremely high, and also since none of the mothers had had any unusual experiences, for instance, in use of drugs or in health status, these unusual phenomena could be considered to have been caused by ingestion of the contaminated oil. However, a clear dose-response relationship between the oil consumption and such phenomena could not be shown because of the limited number of cases. The amount of the contaminated oil consumed during pregnancy ranged from 0.3 to

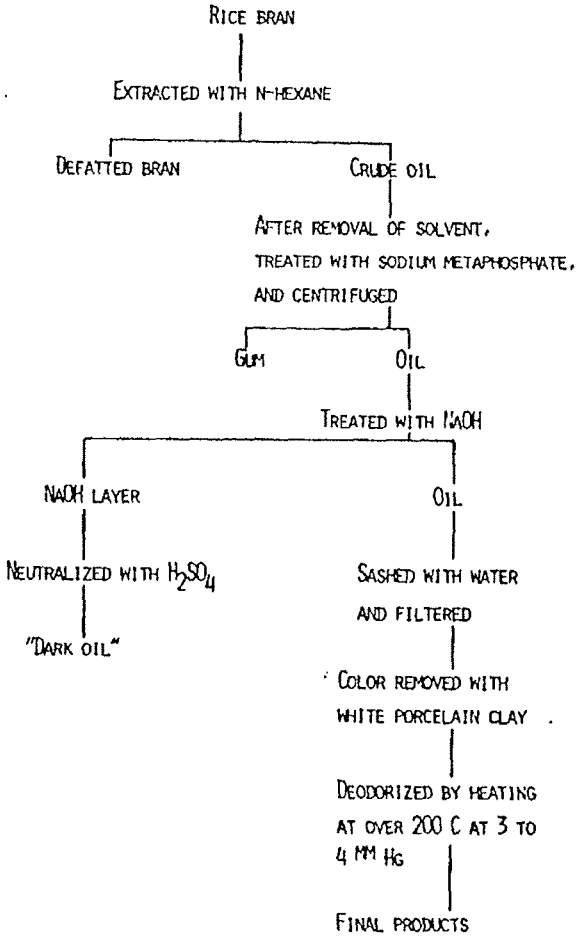

FIGURE 4. Flow sheet of rice oil production.

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Table 11. Babies born to patients and unaffected wives of patients and their use of K rice oil.

Mother No.	Age	Amount of oil used during pregnancy (L)	Pregnancy period when oil used	Grade of clinical severity of mother	Delivery	Sex of baby	Small-for-date	Stained skin	Stained gingiva	Stained nail	Increased eye discharge	Neonatal jaundice
1	28	0.3	3rd trimester	2	Normal	M	No	?	−	−	−	+
2	24	?	?	3	Normal	F	No	+	−	−	+	+
3	22	1.4	Later half of 2nd trimester	Normal	Forcep	M	No	?	−	−	+	++
4	27	0.7	2nd trimester	1	Normal	M	Yes	+	+	−	+	?
5	26	0.7	1st trimester	3	Normal	F	No	+	+	+	+	+
6	29	1.4	Later half of 2nd trimester	3	Normal	M	No	+	−	+	+	+
7	30	1.1	Whole	1	Normal	M	Yes	+	−	−	+	?
8	23	?	Whole	3	Normal	M	30	+	−	−	+	+
9	33	?	?	?	Caesarean section	M	Yes	+	+	+	+	?
10	29	0.3	Early half of 2nd trimester	Normal	Normal	F	No	?	−	−	−	+
11	26	?	?	2	Normal	M	No	+	+	+	+	+
12	33	2.6	Early half of 2nd trimester	4	Stillbirth	?	?	+	?	?		
13	25	?	?	3	Stillbirth	F	Yes	+	?	?		

Table 12. Prognosis of patients with Yusho.*

Grade of clinical severity**	Clinical conditions			
	Improved	Stational	Worsened	Total
0	16 (66.7)	6 (25.0)	2 (8.3)	24 (100.0)
1	30 (51.7)	16 (27.6)	12 (20.7)	58 (100.0)
2	23 (52.3)	16 (36.4)	5 (11.4)	44 (100.0)
3	12 (52.2)	10 (43.5)	1 (4.3)	23 (100.0)
4	0 (0)	10 (100.0)	0 (0)	10 (100.0)
Total	81 (50.9)	58 (36.5)	20 (12.6)	159 (100.0)

* Calculated from the figures reported by Toshitani and Kitamura.

** 0: Physical complaints without skin-lesions.
1: Pigmentation of the skin and the mucous membrane.
2: Comedo formation.
3: Acneform eruptions.
4: Extensive distribution of acneform eruptions.

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2.6 liters (Table 11) (8). Twelve of 13 fetuses were smaller than the national standards, and 4 of them were small-for-dates babies (8,10). As they grew older, the stain in their skin gradually faded (11). No evidence has so far been obtained with regard to any physical and mental retardation of the babies, but we suggest that a particularly careful and prolonged follow-up observation should be maintained for their future (8).

Growth of Affected Children

To examine whether Yusho disturbs children's growth, the affected school children, 23 boys and 19 girls, were compared in 1967, 1968, and 1969 with their 719 healthy classmates matched by sex. The gains of the affected boys in both height and weight decreased significantly after the poisoning, while the affected girls showed no definite change in this respect (12).

Current State of Patients

More than 3 years have passed since the epidemic was reported. It is a heart-breaking fact that many patients with Yusho are still tortured by the sickness because no curative treatments have been discovered yet. In summer of 1970, a mass clinical examination of the Yusho patients was carried out in Fukuoka-Ken, and the clinical state of the patients examined were compared with their previous findings observed in summer of 1969. As shown in Table 12, about a half of the 159 patients, for whom the comparison was feasible, were shown to have clinically improved signs, while the remaining half showed no such favorable signs, and more than 10 percent of the patients were even worsening (13). It should be noted, furthermore, that even many of those who appeared to be clinically improving had several serious complaints, such as persistent headache, general fatigue and feeling of weakness, numbness in limbs, weight loss, and others (14). All these facts clearly indicate that recovery from Yusho is extremely difficult. To cure them as quickly as possible and to prevent another epidemic of Yusho, all possible world-wide cooperative efforts are highly desired.

From the middle of February to the end of March, 1968, an epizootic of a strange disease closely resembling chick edema disease occurred involving more than two million chickens in the western part of Japan (15,18). More than 400,000 chickens were reported to have died. The disease was characterized by such clinical signs as labored breathing, droopiness, ruffled feathers, high mortality and decreased egg production (15,18). Autopsy revealed a marked subcutaneous edema, hydropericardium, ascites, and pulmonary edema, muscular ecchymosis in the thorax or inside of the thigh, and yellowish mottled appearance of the liver (15,17,18). An epizootiological investigation demonstrated that the disease was caused by feeding chickens with specific lots of commercial brands of formula chicken feeds manufactured by two companies. In view of the fact that the K rice oil used by Yusho patients had been contaminated with Kanechlor 400, the toxic feeds were analyzed for chlorinated hydrocarbons and proved to contain Kanechlor 400 (16,19,20). The experimental reproduction of the disease by administering this chemical mixture was successful (16,20,21). The reason the chicken feeds were contaminated with Kanechlor 400 could be reasonably understood by the following facts: both of the chicken feed manufacturers had been using "Dark oil" produced by the K company (Fig. 3) as an ingredient of their formula feeds, and they had actually used "Dark oil" purchased from the company from February 6 to 27, 1968. The remaining sample of "Dark oil," used for production of the toxic formula feeds, was also shown to contain about 1,300 ppm of Kanechlor 400 (16,20). Thus, it became clear that both the epidemic of Yusho and the epizootic of the disease among chickens had been very closely associated, being caused by intake of the oils contaminated with Kanechlor 400. It is very unfortunate, however, that the epidemic of Yusho could not have been prevented even though a large scale epizootic of such an unusual chicken disease had preceded the incidence of Yusho by more than 6 months.

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STLCOPCB4009105

An Abstract of Results of Laboratory Examinations of Patients with Yusho and of Animal Experiments

by Masanori Kuratsune*

An extensive clinical study of Yusho and many animal experiments were carried out by the Yusho study group in Kyushu University in order to clarify the mechanism of toxic actions of Kanechlor. Most of the findings have been published in Japanese with brief English abstracts. I hope this abstract will help in the understanding of the important observations obtained by these studies.

* Department of Public Health, Faculty of Medicine, Kyushu University, Fukuoka, Japan.

Table 1. Laboratory findings of patients with Yusho.

Item	Laboratory findings	Reference
Blood (I)		
1. Red blood cells	Decreased in most severe cases.	3
2. Leucocytes	Increased in most severe cases.	1
3. Blood platelets	Normal	3
4. Coagulation time	Normal	3
5. Prothrombin time	Normal	3
6. Blood sedimentation rate	Normal except for a few cases	3
7. Hemoglobin	Decreased in most severe cases	1, 3
Blood (II) serum lipids		
1. Total lipid	Increased 725.0±169.5 (mg %) 9 cases of grade IV*	3
2. Triglycerides	Increased	1, 3, 4, 8
	180.6±88.2 (mg %) 9 cases of grade IV*	3
	69–617 (mg %) 24 cases	4
	More than 300 (mg %) 9 out of 24 cases	4
	189±64 (mg %) 32 adult patients	8
	Fifteen percent of the patients showed unusually high levels (more than 300 mg %) of triglycerides	20

age	No. cases	TG>300 No. case	%
0–9	52	18	34.6
10–19	89	17	19.1
20–29	68	5	7.4
30–39	85	10	11.8
40–49	58	6	10.3
50–59	28	2	7.2
60–	16	2	12.5
Total	396	60	15.1

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Table 1—Continued.

Item	Laboratory findings	Referen
3. Cholesterol	Normal 181±43 (mg %) 27 adult patients	1, 8
4. Phospholipids	Normal 8.1±1.8 (mg %) 27 adult patients	8
5. Serum lipoprotein	β/α-liproprotein ratio increased. Woman with Yusho, aged 24: 2.0 Woman with Yusho, aged 60: 4.4 Man with Yusho, aged 35: 3.5	8
6. Fatty acid composition of serum lipids	Analysis of cholesterol esters, triglycerides, free fatty acids, and phospholipids showed no abnormal components, except that increase of oleic acid and decrease of palmitic acid were seen in serum-free fatty acids.	4
7. Effect of glucose administration on serum free fatty acids	Levels of glucose and free fatty acids in blood were determined 30, 60, 90, 120, 150, and 180 minutes after oral administration of 50 g glucose. Two types of variation of levels were noted. Type A: Blood glucose increased and later decreased. Fatty acids hardly changed. Type B: Blood glucose increased and did not return to normal even after 180 minutes after glucose administration. Fatty acids decreased markedly after administration. Palmitic acid decreased by glucose administration more markedly in patients than in normal persons.	8
8. Post-heparin plasma lipoprotein lipase activity (PHLA)	PHLA values (FFA μEq/min/ml) 10 minutes after heparin injection in 10 normal women aged 18 to 20 in years were 0.201±0.058. Four of 5 female patients showed PHLA 10 minute values lower than 0.143, namely the mean—1 S.D. value for control, while 2 of 8 male patients had similar low values.	20
9. Lecithin-cholesterol acyltransferase (LCA)	Five patients with Yusho were examined. Mean value was 41.7% for patients which was significantly lower than control ($p = 0.02$)	20
Blood (III) protein and others		
1. Total serum protein	Normal in most cases 6.87±0.73 (g %) 9 cases of grade IV* 7.3±0.6 (g %) 35 adult patients 7.3±0.5 (g %) 8 Yusho children	 3 8 8
2. Serum albumin	Normal 56.4±5.47 (%) 9 cases of grade IV* 59.4±4.2 (%) 33 adult patients 58.1±6.6 (%) 8 Yusho children	 3 8 8
3. Serum globulin	Increase in α_2-globulin in severe cases. No significant change in other fractions. α_2-globulin 12.7±2.74 (%) 9 cases of grade IV* 10.3±2.1 (%) 33 adult patients 13.1±3.0 (%) 8 Yusho children Increase in α_1-globulin 6.2±1.1 (%) 33 adult patients 6.0±1.0 (%) 8 Yusho children	 8 3 8 8 8
4. Non-protein nitrogen compounds	Normal	8
Blood (IV) electrolytes and metals		
1. Na, K, Ca	Normal Na and Ca were in normal range but K is slightly decreased. 4.0±0.4 (mEq/l) 53 adult patients	3 8

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Table 1—Continued.

Item	Laboratory findings	Reference
	4.2±0.5 (mEq/1) 17 Yusho children	
2. Cl	Normal	3, 8
3. Fe	Tended to decrease.	3
	66.0±30.9 (μg %) 6 cases of grade IV*	
	Decreased	8
	90±34 (μg %) 50 adult patients	
	83±26 (μg %) 17 Yusho children	
4. Cu	Increased in severe cases	3
	183.9±61.0 (μg %) 9 cases of grade IV*	3
5. Zn	Normal	3
Blood (V) enzymes		
1. Alkaline phosphatase, serum	Increased slightly in severe cases.	1, 3, 8
	10.3±3.5 (King-Armstrong units) 65 adult patients	8
	17.4±4.9 (King-Armstrong units) 11 Yusho children	8
	14.9±9.26 (King-Armstrong units) 9 cases of grade IV*	3
2. Lactate dehydrogenase (LDH), serum	Normal in most cases.	3, 8
	278±65 (Karmen units) 24 adult patients	8
	284±24 (Karmen units) 5 Yusho children	8
3. Transaminases		
GOT	Normal	3, 8
	24±19 (Karman units) 70 adult patients	8
	36±23 (Karman units) 12 Yusho children	8
	23.0±7.7 9 cases of grade IV*	3
	28.0±10.2 cases of grade IV*	1
	24.7±8.8 cases of grade III	1
	20.0±7.3 cases of grade II	1
GPT	Normal in most cases	3, 8
	22.6±11.9 7 cases of grade IV*	3
	23±16 (Karman units) 48 adult patients	8
	21±11 (Karman units) 10 Yusho children	8
	Mean 30.1 cases of grade IV*	1
	Mean 24.1 cases of grade III	1
	Mean 16.0 cases of grade II	1
Blood (VI) others		
1. Bromsulfalein (BSP) test	Delayed in several adult cases.	3
	5.9±5.19 (%) 8 cases of grade IV*	
2. Icterus index	Normal	3
	3.3±0.66 8 cases of grade IV*	
3. I^{131} resin sponge uptake (Triosorb test)	Normal	3
Urine		
1. Protein	Negative	3
2. Glucose	Negative	3
3. Urobilinogen	Negative in most cases	3
4. Urinary neutral 17-ketosteroids	Elevation of ratio of etiocholanolone/androsterone was noted, indicating adrenocortical hyperfunction but urinary excretion of total 17-ketosteroids was in normal range.	8
	Etiochol./androst.	
	2 Normal women aged 28: 0.6, 1.0	
	2 Normal men aged 20 and 28: 0.4, 0.8	8
	2 Yusho women aged 35: 1.7, 2.0	
	2 Yusho men aged 37 and 39: 0.9, 2.6	

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Table 1—Continued.

Item	Laboratory findings	Reference
	Total 17-ketosteroids 23 adult Yusho women: 5.7±1.8 (mg/day) 13 adult Yusho men: 7.3±2.8 (mg/day)	8
	Urinary total 17-ketosteroids (17-KS) and 17-hydroxycorticosteroid (17-OHCS) were determined in 50 male and 45 female patients. Positive correlation was found between 17-KS and 17-OHCS. 42% of patients exceeded normal range in both steroids in males and females. Analysis of major components of urinary 17-KS was made in 9 male (19 to 57 years in age) and 7 female (16 to 51 years in age) patients. For androsterone, etiocholanolone, and dehydroepiandrosterone, female patients seemed to be lower than control, while male patients seemed to be higher.	19

Sex	17-Ks	Normal (mg/day)	Patients (mg/day)
M	Pregnanediol	0.70±0.17	0.84±0.46
	Androsterone	2.95±1.02	3.28±1.19
	Etiocholanolone	1.83±0.55	2.64±0.84
	Dehydroepiandrosterone	1.55±0.75	1.96±1.18
	Total	6.4±1.8	7.9±3.2
	Determined by Zimmermann reaction	10.6±2.3	11.6±3.2
F	Pregnanediol	0.95±0.55	1.34±0.91
	Androsterone	2.02±0.97	1.32±0.60
	Etiocholanolone	1.35±0.48	1.03±0.40
	Dehydroepiandrosterone	1.01±0.40	0.93±0.48
	Total	4.4±1.7	3.8±1.0
	Determined by Zimmermann reaction	6.1±2.4	7.2±1.9

5. Amino acids	Two patients were examined. A remarkable increase in excretion was noted for taurine (5,641; 8,103 μM/day), β-aminoisobutyric acid (247; 1,042 μM/day), glycine (1,865; 2,997 μM/day), and histidine (743; 1,170 μM/day). Increase was also noted for threonine, serine, and Tyrosine.	8

Fatty acid analysis of acne and skin fat

1. Cheese-like material collected from acne eruptions and fat in the sweat of patients — 1

	cheese-like material %	Fat in sweat	
		Face %	Body %
myristic	2.7	11.7	9.1
palmitic	21.5	41.2	41.0
palmitoleic	10.4	23.5	22.7
stearic	16.3	11.8	9.1
oleic	30.4	11.8	18.1
linoleic	18.3		
linolenic	0.4		

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Table 1—Continued.

Item	Laboratory findings	Reference
2. Yusho acne and non-Yusho acne	Yusho acne contained more stearic and oleic acids than did non-Yusho acne. Linoleic acid was present only in the former. The former contained 10 times more cholesterol than the latter.	8

	Cheese like materials		
	from ordinary acnes of many persons %	from acnes of members of a Yusho family	
		1 %	2 %
myristic	11.6	3.0	8.3
palmitic	41.2	22.1	23.4
palmitoleic	17.0	10.7	6.1
stearic	13.7	16.6	31.2
oleic	16.5	31.5	23.5
linoleic	none	16.1	7.5

Biopsy		
1. Liver	Reduction of rough endoplasmic reticulum and hypertrophy of smooth endoplasmic reticulum. Mitochondria showed morphological heterogeneity. Many filamentous inclusions were present in the matrix. Giant mitochondria were frequently encountered.	5, 22
2. Skin	Twenty-four biopsy specimens from 18 patients were examined. Marked hyperkeratosis and cystic dilation of hair follicles; marked increase of melanine in basal cells of epidermis.	9
Neurological findings	Slowing of sensory nerve conduction velocities of radial and sural nerves was noted in 9 of 23 patients examined. Motor nerve conduction velocities of ulnar and tibial nerves were shown to be unaffected except for 2 patients in whom they decreased.	6
Otorhinolaryngological findings	No audiometric abnormality and no disturbed vestibular function.	7
Ocular signs	Hypersecretion of meibomian gland; abnormal pigmentation of conjunctiva; no particular lesion in intraocular tissues. Increase of melanine granules was noted electronmicroscopically in cytoplasm of epithelial cells, especially in basal cells of conjunctiva. Innumerable electron dense particles, 300–400 Å in diameter, were in the cytoplasm of basal cells.	

* Grade IV means clinically most severe cases.

Table 2. Findings of animal experiments

Experimental	Results	Reference
1. 2 ml of olive oil containing 1% Kanechlor 400 orally given to rabbits daily for 10 days	Increase of serum triglycerides and slight increase of total cholesterol	4 (Uzawa et al.)
2. Oral administration of Kanechlor 400 to female mice, . . cynomolgus monkeys, and	Enlargement of liver; slight fatty metamorphosis of liver; swelling of sebaceous gland. Increase of smooth endoplasmic reticulum and decrease of rough endoplasmic reticulum in	10 (Nishizumi et al.)

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Table 2—Continued.

Experimental	Results	Reference
squirrel-monkeys for a month or longer.	liver.	
3. Oral administration of 0.1 g of Kanechlor per Kg per day to rats for 4 weeks.	Loss of body weight, hepatomegaly, and marked increase in serum lipid components were noted. Serum triglycerides reached a level as high as 10 times the control level. Serum cholinesterase activity was not affected.	11 (Tanaka et al.)
4. Oral administration of the rice oil used by a Yusho patient to hairless mice.	Hyperkeratosis, cystic dilatation of hair follicle and sweat-gland, marked hepatomegaly, fatty degeneration of liver.	12 (Inagami et al.)
5. Distribution and elimination of components of Kanechlor 400 orally administered in a single dose of 2.0 mg/body to female mice.	Each component of Kanechlor 400 was equally absorbed from gastro-intestinal tract. Concentration of Kanechlor 400 in skin one day after administration was twice that in liver and kidney. Kanechlor 400 was retained in skin more than in other tissues. Tetrachlorobiphenyls were almost completely eliminated from tissues in three to four weeks, but penta- and hexa-chlorobiphenyls were retained 9 to 10 weeks after administration.	13 (Yoshimura et al.)
6. Distribution and excretion of ^{3}H-Kanechlor orally administered in a single dose of 500 μc or 25 mg/body to Wistar King male rats weighing about 150 g.	Radioactivity in skin, adipose tissue, liver, adrenal gland and gastrointestinal tract was higher than in plasma 3 days after administration. Radioactivity was noted in some tissues after 8 weeks. Urinary excretion of Kanechlor was limited, while 70% of the dose given was in feces. A small portion of radioactivity excreted during the first day was in the phenolic fraction. More phenolic metabolites and less unchanged components were excreted in the feces on the second and third day.	14 (Yoshimura et al.)
7. Effect of oral administration of Kanechlor 400, 500, 600 and their components, 0.2 ml of a solution of 6.8 mg/0.2 ml/100 g of body weight, daily for 3 days, on liver microsomes of male rats.	Kanechlor 400, 500, 600: Hepatomegaly. Microsomal protein per gram of liver increased in proportion to chlorine content of Kanechlor. Amount of cytochrome P-450 and specific activities of O-dealkylase (p-nitrophenetol), N-demethylase (p-chloro-N-methylaniline), and aniline hydroxylase, increased, particularly with Kanechlor 500. 4,4′-dichlorobiphenyl: Slight increases seen. 3,4,2′,4′-tetrachlorobiphenyl } : Increases noted, but less than 3,4,3′,4′-tetrachlorobiphenyl } with Kanechlor 400. 3,4,3′,4′-tetrachlorobiphenyl was a more potent inducer than the others. 2,4,5,3′,4′-pentachlorobiphenyl: Most marked increases were noted in cyt. P450 and specific activities of 3 enzymes. 3,4,5,3′,4′,5′-hexachlorobiphenyl: The increase was larger than that induced with the tetra chlorobiphenyls but less than that with the pentachlorobiphenyl. Phenobarbital: Resembling Kanechlor 400 in enzyme induction capability. The above microsomal enzyme activities and the microsomal components started to increase 12 hours after administration of 3,4,3′,4′-tetrachlobiphenyl, reaching the maximum after about 1 week. Thereafter, activity levels tended to decrease but did not return to normal even after 6 weeks. Microsomes, particularly their membranes, contained the largest amount of H^{3}-Kanechlor-400 administered orally, as compared with mitochondria or lysosomes.	15 (Fujita et al.)
8. Oral administration of Kanechlor 400 or 500 to female rats	Hexobarbital sleeping time was markedly shortened by pretreatment with Kanechlor 400 or 500 in rats. Minimum effec-	16 (Komatsu et al.)

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Table 2—Continued.

Experimental	Results	Reference
(Wistar King), about 100 g in weight, in order to see effects of the administration on *hexobarbital sleeping time*	tive dose was daily 5 mg and 2 mg/Kg for 3 days for Kanechlor 400 and 500, respectively. When 40 mg/Kg of Kanechlor 400 or 500 was given daily for 3 days, the effect lasted for 3 weeks by the former and for 6 weeks by the latter. Ethionine inhibited the shortening of hexobarbital sleeping time by Kanechlor. Anabolic steroids, chloroquine, glutathione, α-mercaptopropionylglycine, essential phospholipids, diphenyl, and sulfadiazine did not modify the sleeping time reduction by Kanechlor.	
9. Oral administration of daily dose of 0.5, 2.5, 5, 50 mg of Kanechlor to rats for 13 to 21 days.	Growth was inhibited by the treatments. No weight increase at daily dose of 5 mg, and a weight decrease at 50 mg. Plasma triglycerides did not increase at daily dose up to 5 mg, and slightly exceeded the control at dose of 50 mg, while plasma cholesterol and phospholipid increased definitely by increasing amount of Kanechlor.	17 (Nagai et al.)

Daily dose Kanechlor (mg)	Triglyceride μM %	Cholesterol mg %	Phospholipid mg %
Control	109±26	83±12	4.7±0.8
0.5	98+19	118+26	5.8+1.7
2.5	89+21	102+11	7.2+1.2
5.0	89+18	125+28	7.1+1.3
50.0	130+37	178+18	13.4+2.2

The administrations hardly affected concentration of triglyceride, cholesterol, phospholipid in liver except for daily administration of 50 mg Kanechlor, which caused slight increase of triglyceride and cholesterol. They slightly increased triglyceride in skin but hardly affected cholesterol and phospholipid. A definite reduction of triglyceride in perirenal adipose tissue was noted.

10. Oral administration of Fraction A and B of Kanechlor 400 to rats, each daily dose 1 mg on every fourth day for 40 days, 10 mg total. Fr. A: Boiling point 123–124°C/0.01 mmHg, 3.12 chlorine atoms per biphenyl average. Fr. B: Boiling point 140–142°C/0.01 mmHg, 3.84 chlorine atoms per biphenyl average.	Animals grew in weight with Fr. A but lost weight with Fr. B, which caused marked atrophy of abdominal adipose tissue. Lipid content of adipose tissue around kidney decreased when Fr. B was given but did not when Fr. A given. Plasma triglyceride decreased with Fr. B but did not with Fr. A.	17 (Nagai et al.)

Animal	Triglyceride μM %	Cholesterol mg %	Phospholipid mg %
Control	130±18	66±5	5.7±0.4
Fr. A	128±10	66±7	5.1±0.4
Fr. B	83±21	57±5	5.2±0.3

11. *Lipid in liver.* Oral administration of daily dose of 8 ml of olive oil containing 1% Kanechlor for 3 and 11 days to 2 female rabbits.	Total lipid and glycerides of liver increased, but concentration of serum glycerides was normal after administration for 3 consecutive days, but increased abnormally after administration for 11 consecutive days.	18 (Ito et al.)

Lipid	Control (n=1)	Poisoned for 3 days	Poisoned for 11 days

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Table 2—Continued.

Experimental	Results			Reference
		(n = 1)	(n = 1)	
	Total lipid %/dry wt.	17	29	30
	Glycerides	7.1%	24.3%	25.3%
	Cholesterol	6.3	6.1	10.8
	CGP Lecithin	22.5		9.7
	Lysolecithin	7.4	30.0	7.6
	Sphingomyelin	2.9		4.8
	EGP	16.9		14.4
	SGP	9.0		4.5
	PI	6.8	39.6	5.1
	Glycolipid	5.2		6.7
	Others	15.9		11.1
	Serum glycerides (mg %)	52	65	1,690
12. Oral administration of daily dose 0.5 or 0.3 mg/Kg of Kanechlor 400 each to 40 adult Wistar rats.	Marked or moderately impaired motor function, decreased motor conduction velocity, early stage of segmental demyelination and loss of large nerve fibers were noted.			21

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DSW 025158

STLCOPCB4009113

Chlorinated Aromatic Hydrocarbon Induced Modifications of the Hepatic Endoplasmic Reticulum: Concentric Membrane Arrays*

by D. H. Norback and J. R. Allen†

A number of commercial compounds having chlorinated aromatic hydrocarbon structures are used extensively for medical(3), industrial(4) and agricultural(5) purposes. Quantities of certain parent chlorinated aromatic hydrocarbons and metabolites have been repeatedly detected as residues in the food supply and tissues of birds, fish, and mammals(6-9) including man(10). Chlorinated diphenyl-p-dioxins have been identified as contaminants of many commercial products, such as certain edible fats, herbicides and disinfectants. It is suggested that the compound results from the conjugation of commercial chlorinated phenols(11). Polychlorinated polyphenyls are among the most abundant chlorinated hydrocarbon global pollutants. Their contamination of the environment is a result of the widespread commercial use of these compounds for their properties of insulation, adhesiveness, thermoplasticity, chemical inertness, and insolubility.

Following this investigation of sequential biochemical and ultrastructural alterations within the liver produced by three chlorinated aromatic hydrocarbons — chlorinated diphenyl-p-dioxin, polychlorinated biphenyls (PCBs), and a highly chlorinated triphenyl (PCT)—certain similarities of biochemical and ultrastructural effects of the compounds were observed. The livers of rats fed the chlorinated aromatic hydrocarbons consistently contained numerous multi-layered concentric membrane arrays, proliferated smooth endoplasmic reticulum (ER), and altered microsomal enzyme activity.

Materials and Methods

Separate groups of male Sprague-Dawley rats initially weighing 100 grams were fed diets containing the following chlorinated aromatic hydrocarbons: 1% PCTs (Aroclor 5460), 0.02% PCBs (Aroclor 1254), and 0.002% chlorinated diphenyl-p-dioxin, respectively. At regular intervals the rats were deprived of food for 24 hours and sacrificed by exsanguination. The livers were weighed and portions were removed for electron microscopy. This tissue was immediately placed in veronal acetate buffered osmium tetroxide and cut into small cubes. Following one and one half hours of fixation, the tissues were dehydrated in a graded series of ethanol, cleared in propylene oxide and embedded in an Epon-Araldite mixture. Sections were cut at a thickness of 0.5–0.8 microns for light microscopic evaluation, and thinner sections at a thickness of approximately 60 millimicrons were cut and stained with uranyl acetate for electron microscopic examination. The remaining portions of the livers were perfused through the portal vein with isotonic KCl, weighed, and homogenized at 0° with two volumes of the salt solution. Microsomes were isolated from the post mitochondrial supernatant obtained by centrifugation at 9,000 × G for 20 minutes, washed by homogenization with KCl and recentrifuged, resuspended in KCl equal to three volumes of the original liver material, and immediately assayed for the activities of aromatic

* Portions of this paper were previously reported (1, 2).

† Department of Pathology, University of Wisconsin Medical School, Madison, Wisconsin 53706.

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hydroxylase(12), nitroreductase(12), N-demethylase(12), nitrophenyl acetate hydrolysis (esterase) (13), and glucose-6-phosphatase(14). RNA(15), phospholipid(16), and cholesterol(16) concentrations were determined from samples that had been frozen at −80°. Protein determination(17) on alkaline-washed(18), microsomal material provided estimation of membrane protein in the microsomal suspensions, which is subsequently referred to as microsomal protein.

Results

The rats from the dioxin and PCT groups readily ate the experimental diets and attained at least 80% of the weight of the controls at three weeks. Hypertrophy of the liver, varying from a moderate enlargement, following chlorinated diphenyl-p-dioxin ingestion, to an increase in the relative liver weight of 8 grams per hundred grams body weight, following PCB and PCT ingestion, was observed. Neurological side effects, including tremors, convulsions, or sedation, were not observed. The rats from the dioxin and PCT group remained on the diet for four weeks without apparent extra-hepatic effects other than the small decrease in weight gained. The rats from the PCB group did not gain weight and were sacrificed at 8 days.

The ultrastructural alterations within the hepatocytes of rats from the three experimental groups were similar and will be considered collectively. The changes were primarily limited to the endoplasmic reticulum and consistently affected all experimental animals. The hepatocytes contained numerous multi-layered, concentric membrane arrays and proliferated smooth ER. Preceding the formation of the concentric arrays, the parallel cisternae of the rough ER, representative of the control hepatocytes (Fig. 1) were reorganized into sinuous patterns that encompassed lipid droplets, mitochondria and other organelles (Fig. 2). Annular profiles consisting of paired membranes with ribosomes attached to the

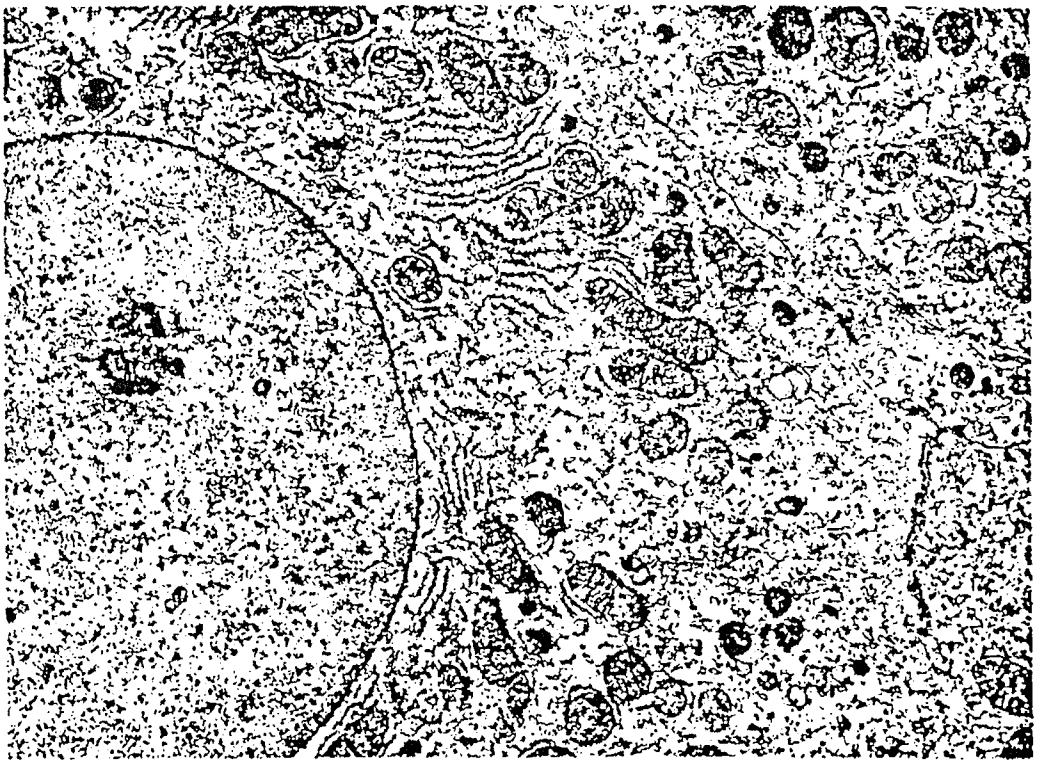

FIGURE 1. Elements of the rough endoplasmic reticulum are characteristically arranged in parallel lamellae in hepatocytes of rats fed a control diet (× 8,620).

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FIGURE 2. Following the ingestion of a chlorinated aromatic hydrocarbon, the parallel membranes are reorganized into sinuous cisternae that encompass lipid droplets, mitochondria, and other organelles (following ingestion of chlorinated diphenyl-p-dioxin; × 15,500).

central surface of the inner membrane and the peripheral surface of the outer membrane were also prevalent (Figs. 3 & 4). Shortly after the development of the annular formations, concentric annuli composed of variable numbers of degranulated paired membranes were observed (Fig. 5). These structures composed of multi-layers of membranes were also evident following light microscopic evaluation of the toluidine blue-stained sections. Within three weeks, each experimental group developed multi-layered membrane arrays composed of several units of concentric annuli (Fig. 6). Lamellar profiles of paired membranes resembling flattened cisternae partially circumscribed the

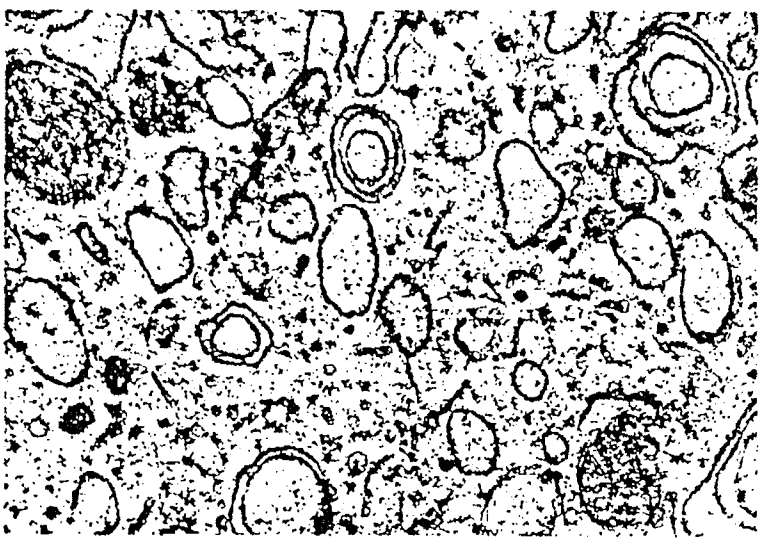

FIGURE 3. Membranes of the endoplasmic reticulum are also represented as annular profiles in hepatocytes of the experimental rats (following ingestion of chlorinated diphenyl-p-dioxin; × 24,300).

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FIGURE 4. The concentric annuli with attached ribosomes are apparently derived from the rough endoplasmic reticulum (following ingestion of chlorinated diphenyl-p-dioxin; ×35,600).

annular units. The outer membrane pairs of the lamellae were continuous with elements of the smooth ER and rough ER. Ribosomes were occasionally attached to the surfaces of the outer membrane pairs of the multi-layered arrays. The concentric membrane arrays increased in size and complexity in direct relationship to the time the rats remained on the experimental diets.

Associated with the modified membrane structures were alterations in enzyme activity and biochemical composition of the microsomal fraction of these cells (Table 1). Following the ingestion of PCTs for 21 days, there was an increase in microsomal protein per gram of liver. Compositional changes of the membrane material were indicated by the reduction in cholesterol to protein ratio and slight increase in phospholipid to protein ratio. The increase in esterase activity was parallel to that of protein, whereas, increases in N-demethylation and nitroreduction were greater than the increase in membrane material. Specific activities of glucose-6-phosphatase and aromat hydroxylase were reduced. The enzymatic chang following PCB ingestion were parallel to those the PCT group.

Discussion

Ingestion of the chlorinated aromatic hydr carbons by rats induces liver hypertrophy wit proliferation of the ER and formation of lar concentric membrane arrays within the cytoplas of the hepatic cells. These changes measured aft 21 days PCT ingestion result in increased hepat function. An increase in total activity of eac enzyme investigated is evident when the nearl three-fold liver hypertrophy and more tha doubled quantity of microsomal protein per gra liver is considered. Associated with the enzymati alterations are an increased ratio of phospholipi to protein and a decreased ratio of cholesterol t protein. Increased enzyme activity and proli erated smooth ER are commonly produced by

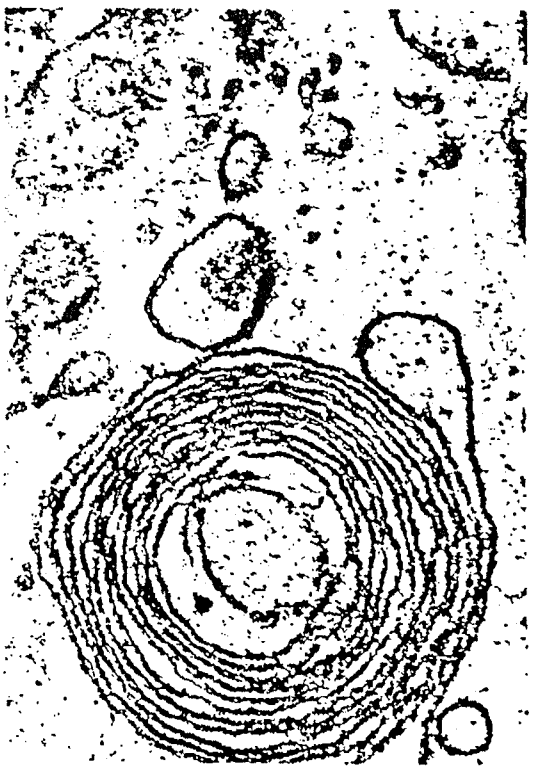

FIGURE 5. Larger concentric membrane arrays consist of numerous distinct rings of paired membranes (following ingestion of chlorinated diphenyl-p-dioxin; ×30,300).

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FIGURE 6. Following ingestion of polychlorinated triphenyl for three weeks extensive concentric membrane arrays develop within the hepatocytes. The membrane arrays encompass lipid droplets, mitochondria, and other cellular organelles (× 3,700).

wide variety of agents including phenobarbital; however, the phospholipid/protein are unaltered and cholesterol/protein increased(18). Thus the membranes low in cholesterol and high in phospholipid composition, which are characteristic of chlorinated triphenyl induced microsomes, likely represent the concentric membrane arrays.

Structures similar to concentric membrane arrays have been observed following the administration of numerous other compounds including phenobarbital(19), thiohydantoin(20), triparanol(21), aflatoxin(22), ethionine(23), carbon tetrachloride(24), and amino azo dyes(25). An evaluation of the sequential changes that occurred following consumption of the chlorinated aromatic hydrocarbons suggests that arrays of concentric paired membranes originate from cisternal elements of the ER. Modification of the rough ER included reorganization of parallel cisternae into sinuous cisternae and concentric annular profiles of paired membranes. The concentric annuli are similar to the compositional units of the multilayered membrane arrays. This similarity suggests that the reorganized cisternae of the rough ER are incorporated into the arrays of the concentric paired membranes. Continuity of these arrays with the rough and smooth ER further support a postulate of a rough ER derivation.

Enzymatic and compositional changes of the microsomes suggest the modification of the ER may be correlated to several probable functions. Consistent with the increased proportion of phospholipids within the membrane structure is the proposal that the proliferated membranes provide a site for storage, for isolation, or for contact with membrane enzymes of the hydrophobic chlorinated aromatic hydrocarbon. The increased proportion of phospholipid enhances the suitability of the membrane to sequester the foreign material. The proximity of the concentric

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Table 1. Hepatic alterations in rats fed chlorinated triphenyl for 21 days.

	Cont.	Exp.
Liver weight (g/100 g body weight)	3.00[a]±0.28	8.63±0.79
Microsomal protein (mg/g liver)	20.1[a]±0.5	54.5±4.1
RNA (μg)[c]	180[a]±33	63.7±4.3
Phospholipid (μg)[c]	455[b]±24	508±30
Cholesterol (mg)[c]	44.0[a]±8.9	17.9±1.5
Glucose-6-phosphatase (μmol PO4/15 min)[c]	3.98[a]±0.51	1.08±0.08
Esterase (μmol p-nitrophenol/min.)[c]	9.75±3.0	10.3±1.21
Aromatic hydroxylase (mμmol p-aminophenol/30 min.)[c]	30.2[a]±6.2	6.06±0.29
N-demethylase (mμmol formaldehyde/30 min.)[c]	103[a]±19	166±7
Nitroreductase (mμmol p-aminobenzoate/hr.)[c]	32.6[b]±6.8	45.4±6.2

[a] Difference between mean and following mean is significant ($p < .001$).

[b] Difference between mean and following mean is significant ($p < .01$).

[c] per mg protein.

membrane arrays to lipid droplets, also a probable location of the lipid soluble compound, provides accessibility of the lipid component of the membrane to the compound. Hydroxylation activity present within the microsomes converts a lipid soluble hydrophobic compound into a form which has the potential to bind with activated glucuronide (uridine diphosphate glucuronic acid) to form a glucosiduronate. The resulting water soluble conjugate could then be excreted through the biliary or urinary system. Although the specific activity of this enzyme is reduced, the overall activity within the liver is substantially increased over the control value. Cholesterol within the membrane structure has been related to the rigidity of the membrane by its ability to condense the unsaturated fatty acids of the phospholipids(26). Lack of cholesterol suggests a less stable structure or, alternatively, regions available for insertion of other compounds, such as the chlorinated aromatic compounds among t fatty acids and the phospholipids. Thus t enzymatic and compositional alterations of t newly formed membranes suggest a membra suited for localizing the chlorinated aromat hydrocarbon for possible sequestration of met bolites for excretion.

(Supported by NIH Grant ES00472)

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Biological Effects of Polychlorinated Biphenyls in Rats and Quail

by Joel Bitman, Helene C. Cecil and Susan J. Harris*

Polychlorinated biphenyls (PCBs) have been administered to rats and to quail to discover the nature of the biological effects which these environmental pollutants produce in mammals and birds. Biological effects evaluated were:

(1) estrogenic activity of the PCBs in rats,
(2) alterations of pentobarbital metabolism induced by the PCBs as judged by sleeping time of Japanese quail,
(3) alterations of liver vitamin A, liver lipids and liver weight induced by Aroclor 1242 in rats and quail, and
(4) egg production of Japanese quail.

Estrogenic Activity

Estrogenic activity was assessed by determining the stimulation of the uterine glycogen response of the immature rat uterus 18 hours after administration of the test compound(1). The potency of active compounds is reported in terms of the minimal subcutaneous dose which will increase glycogen to a level significantly different from control. In a series of 11 polychlorinated biphenyls and terphenyls, the compounds containing up to 48% chlorine were estrogenically active (Table 1). A polychlorinated terphenyl containing 42% chlorine was found to be 8 times more active. This is a low level of estrogenic activity and probably arises from hydroxylated metabolites during in vivo metabolism.

* United States Department of Agriculture, ARS, Beltsville, Maryland 20705.

Effects on Pentobarbital Metabolism in Japanese Quail

The polychlorinated biphenyls have been demonstrated to stimulate liver enzyme systems which metabolize barbiturates in rats(2). We utilized a sleeping-time test with pentobarbital in quail as a means of determining effects of polychlorinated biphenyls and terphenyls on liver microsomal enzyme activity. The liver is the major site for the metabolism of foreign chemicals, and a standard dose of pentobarbital will produce a standard sleeping time. Stimulation of the liver enzymes which metabolize the barbiturate will be reflected in a shorter sleeping time, and conversely, any inhibition of the liver enzymes will be reflected in a longer duration of pentobarbital hypnosis(3).

The PCBs and PCTs were tested for their acute or short-term effects (48 hours) and for longer-term effects at 3 and 7 days. At appropriate times after administration of the PCBs or PCTs, sodium pentobarbital was injected intramuscularly at a dosage rate of 50 mg/Kg body weight. Birds were considered asleep as long as they could not right themselves when placed on their backs.

A single dose of Aroclor 1268 or Aroclor 5460 administered orally at a dosage level of 100 mg/Kg to mature male or female Japanese quail initially increased sleeping times (Fig. 1). At 18 hours, however, sleeping times were less than untreated control values in the male quail, and by 48 hours, the PCBs and PCTs had stimulated the liver enzymes which metabolize pentobarbital, so that sleeping times were only about 50% of control sedation times. In the female quail, there was little effect of the single oral dose after 5 hours. In both male and female quail, Aroclor 1268 appeared

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Table 1. Estrogenic Activity of Polychlorinated Biphenyl (PCB) and Terphenyl (PCT) Compounds.

Name	Minimum effective dose mg
PCB Aroclor 1221 21% Chlorine	8
PCB Aroclor 1232 32% Cl	8
PCB Aroclor 1242 42% Cl	8
PCB Aroclor 1248 48% Cl	8
PCB Aroclor 1254 54% Cl	Inactive
PCB Aroclor 1260 60% Cl	Inactive
PCB Aroclor 1262 62% Cl	Inactive
PCB Aroclor 1268 68% Cl	Inactive
PCB Aroclor 4465 60% PCB, 40% polychlorinated triphenyl (PCT), 65% Cl	Inactive
PCT Aroclor 5442 42% Cl	1
PCT Aroclor 5460 60% Cl	Inactive

to exert greater effects than Aroclor 5460. The diphasic pattern elicited suggests that the PCBs and PCTs first inhibited the enzymes which metabolize pentobarbital, but then, at later times, stimulate the induction of greater enzymic activity.

Continuous feeding for 3 and 7 days at levels of 100, 300, and 625 ppm ad libitum stimulated the metabolism of the standard dose of pentobarbital

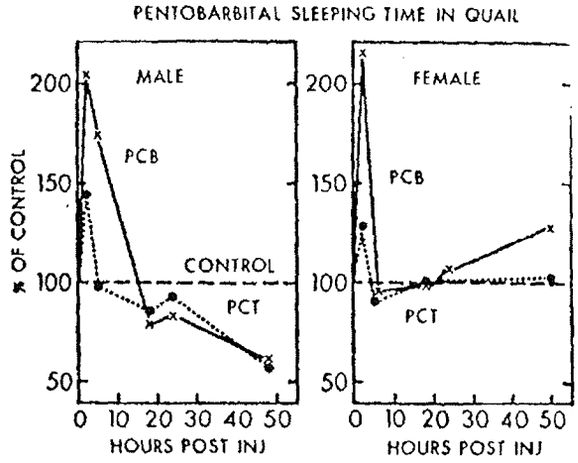

FIGURE 1. Sleeping times of quail fed PCB 1268 or PCB 5460. Each point represents six quail.

(Fig. 2). Sleeping times were about 50–70% of control levels, thus indicating greater metabolism of the barbiturate. Higher dose levels exerted greater effects and, again, Aroclor 1268 appeared to be more stimulatory than Aroclor 5460. Sleeping times were not decreased to as great an extent in the female quail as in the male.

The sleeping time pattern elicited by PCBs and PCTs is in marked contrast to that which the organochlorine pesticide analogs of DDT produce in quail(3). DDT and its analogs produce an

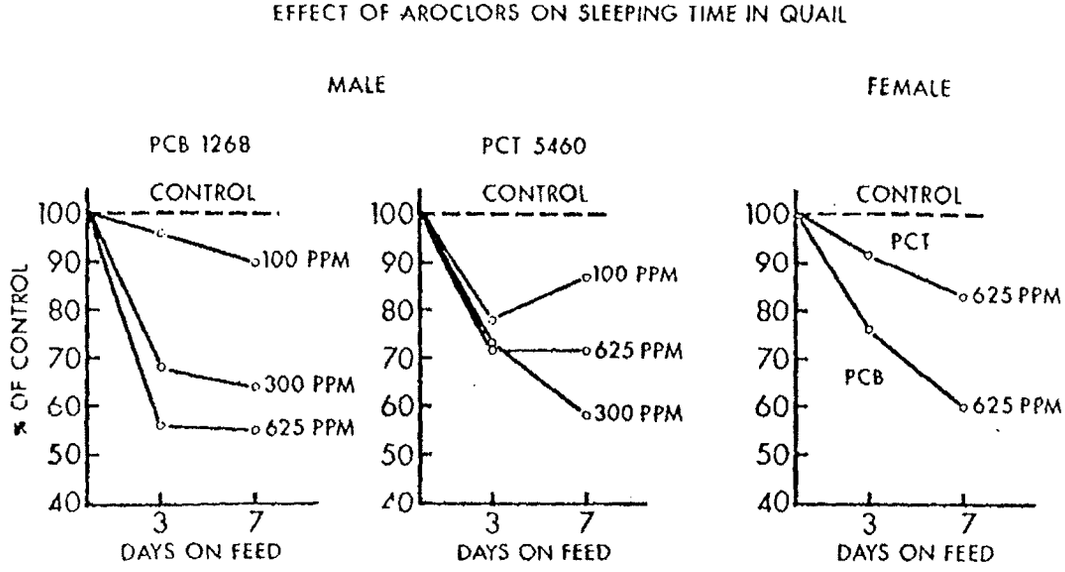

FIGURE 2. Sleeping times of quail fed PCB 1268 or PCB 5460. Each point represents six quail.

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Table 2. Liver Changes in Male and Female Rats after Feeding Ad Libitum for Two Months Diets Containing 100 ppm Aroclor 1242.

Treatment	n	Liver wt. g±SE	Liver lipid %±SE	Vitamin A μg/g liver	Vitamin A mg/liver
			Female rats		
Control	10	6.4±.2	3.15±.04	479±23	4.54±.29
Aroclor 1242	10	7.4±.3[a]	3.46±.08[b]	244±15[c]	2.25±.11[a]
			Male rats		
Control	10	12.2±.4	5.14±.12	564±20	6.82±.22
Aroclor 1242	8	13.0±.8	7.12±.18[c]	270±15[c]	3.46±.21[c]

[a] $p<0.01$.
[b] $p<0.005$.
[c] $p<0.001$.

initial inhibition of pentobarbital metabolism, and sleeping times are longer during the first week of feeding in males and during the first 3 weeks of feeding in female quail.

Effects on Liver Vitamin A in Rats and Quail

Repeated exposure of rats to DDT results in degenerative changes in liver tissue, an increase in liver size and an increase in liver lipids(4), and a decrease in liver storage of vitamin A (5). The possibility that PCBs would produce similar effects was investigated in rats by feeding Aroclor 1242 for 2 months at a dietary level of 100 ppm (Table 2). In both male and female rats there was an increase in liver weight and liver lipids. There was a very striking decrease of about 50% in the liver concentration and content of vitamin A.

Aroclor 1242, fed to male Japanese quail for 2 months at a dietary level of 100 ppm, produced similar effects, an increase in liver weight and

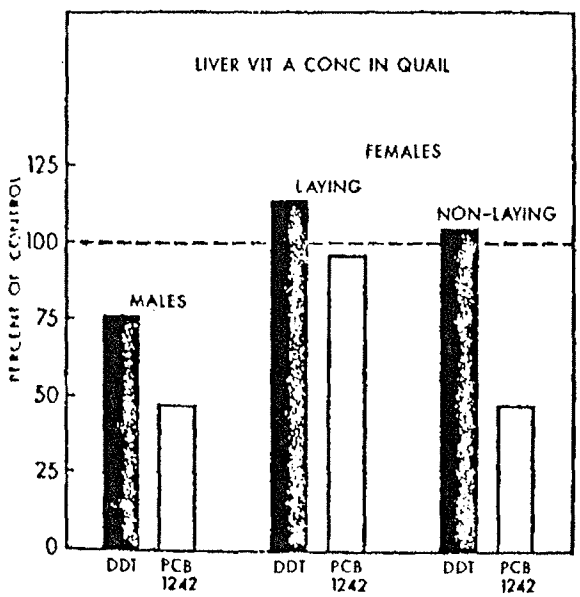

FIGURE 3. Liver vitamin A concentrations of quail fed 100 ppm DDT or PCB 1242 for two months. Groups contained 14 to 20 quail.

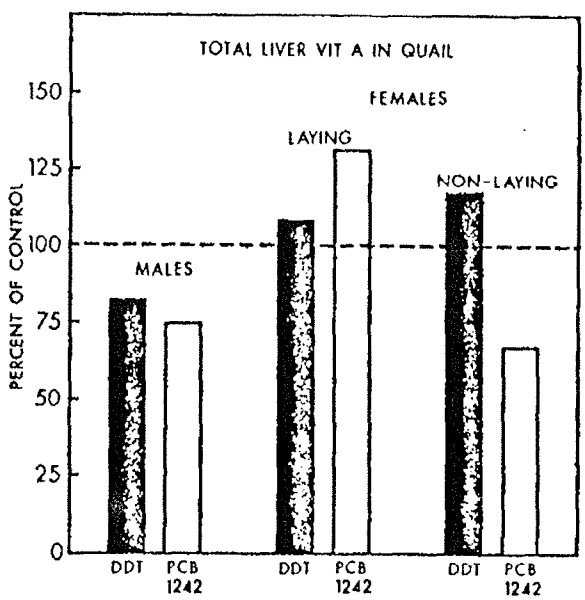

FIGURE 4. Total liver vitamin A of quail fed 100 ppm DDT or PCB 1242 for two months. Groups contained 14 to 20 quail.

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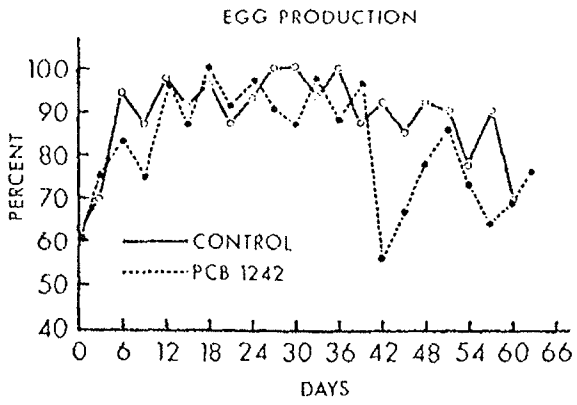

FIGURE 5. Egg production of Japanese quail fed PCB 1242. Average production for three day periods is plotted for groups of 16 quail.

lipids, and a large decrease in liver vitamin A (Fig. 3 and 4). Female quail that were laying did not show consistent changes. During the ovulatory cycle, large amounts of vitamin A stores are mobilized to provide the vitamin A for the yolk of the eggs. Because of these large, almost daily

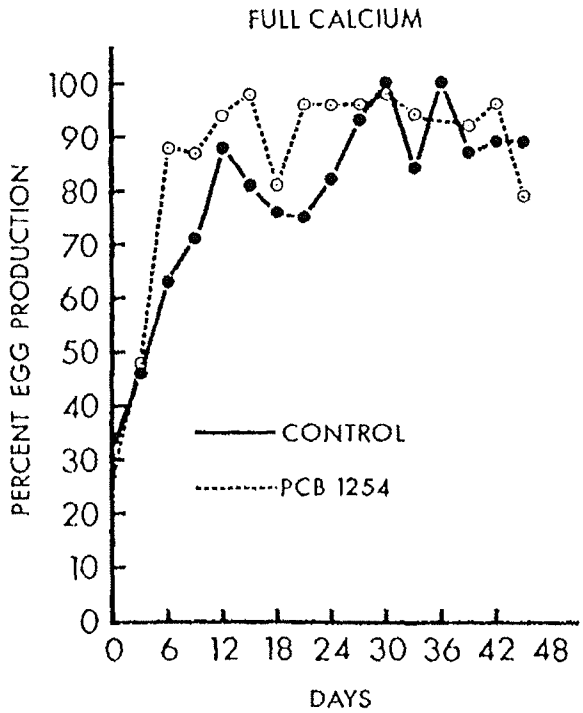

FIGURE 6. Egg production of Japanese quail fed PCB 1254 on a full calcium diet. Average production for three day periods is plotted for groups of 16 quail.

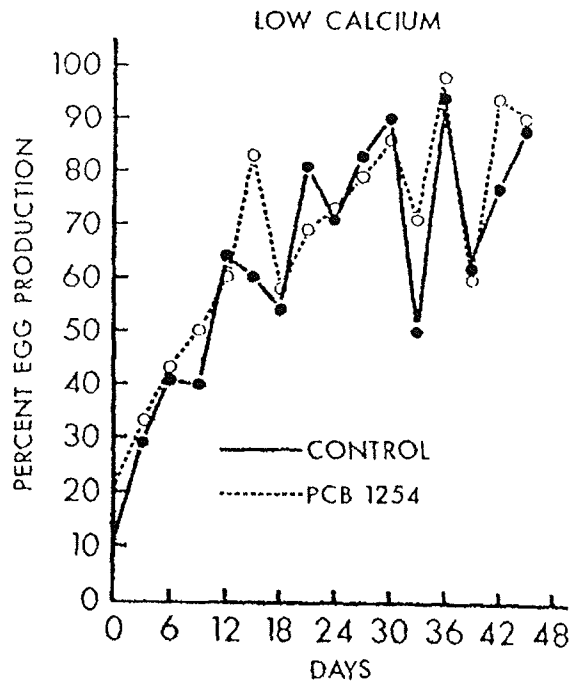

FIGURE 7. Egg production of Japanese quail fed PCI 1254 on a low calcium diet. Average production for three day periods is plotted for groups of 16 quail.

changes, variability in liver vitamin A levels is great. In order to determine whether PCBs exert similar effects in female quail, a group of young females were kept in the dark, from the time they matured (39 days of age), and fed Aroclor 1242 for 2 months. Under these conditions, egg-laying is inhibited, and the cyclic mobilization of vitamin A for deposition in the egg yolk does not occur. In the birds kept in the dark, Aroclor 1242 caused about a 50% reduction in liver vitamin A (Figs. 3 and 4).

Effects on Egg Production

The effects of Aroclor 1242 and Aroclor 1254 on egg production was studied in Japanese quail. Aroclor 1242 was incorporated into an adequate calcium laying mash at a level of 100 ppm for 60 days. Egg production (Fig. 5) was similar for controls and Aroclor 1242-fed birds during the first 40 days (>90%). Average egg production in the Aroclor 1242-fed quail declined to about 71% during the next 3 weeks. The number of membranous and broken eggs in the Aroclor 1242 group was over 2 times that of the control quail.

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Aroclor 1254 was fed at a level of 100 ppm in both a full calcium (2.55%) and low calcium (0.66%) diet. Egg production was not suppressed by the PCBs in either case (Figs 6 and 7), although there was a negative effect of the low calcium diet. There were no differences in the numbers of broken or membranous eggs attributable to Aroclor 1254.

A depression in egg production of White Leghorn hens fed Aroclor 1248 was recently reported by Scott et al.(6). Heath et al.(7), however, found little effect of Aroclor 1254 on eggshell thickness or reproduction in mallards or bobwhite quail. An effect upon egg production and reproduction of Aroclor 1242 and Aroclor 1254 has been previously reported in White Leghorn chickens(8). Recently, Peakall(9) found no effects upon eggshell weight in Ring doves fed 100 ppm Aroclor 1254. Thus, there is a notable lack of consistency in the published reports of the effects of PCBs on egg production and reproduction in birds.

Conclusion

A study was made of the effect of PCBs on estrogenic activity, liver barbiturate metabolism, liver vitamin A and egg production using a limited number of PCB materials. It appears that the biological effects of specific PCB compounds are different, as might be expected with this series of materials containing compounds which differ so greatly in chlorine content and in chemical and physical properties. More extensive research on individual PCB compounds must be undertaken to assess fully the biological effects of polychlorinated biphenyls on mammals and birds.

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PCBs and Interactions with Insecticides

by E. Paul Lichtenstein*

The effects of polychlorinated biphenyl plasticizers, like those of many other environmental chemicals ("synthetic chemicals to which man is exposed by either voluntary or involuntary means"), on biological systems are not too well understood. When research by our group at the University of Wisconsin indicated in 1969 (1) that several of the Aroclor compounds increased the toxicity of DDT and dieldrin to insects, their potential biological interaction with environmental chemicals was indicated. Since then further studies were conducted in our laboratory to investigate the effects and interactions of environmental chemicals on human cells in tissue culture by determining the toxicity of some of these chemicals on HeLa cells and skin fibroblasts (2). Aroclor 1254 (a polychlorinated biphenyl plasticizer), carbaryl, parathion, DDT, several insecticide metabolites, aspirin and caffeine were used to determine their effects on cell growth and synthesis of proteins and nucleic acids. The dosage causing a 50% inhibition in culture growth was determined, and the interaction or combined effects between these chemicals relative to their effects on protein and nucleic acid synthesis was studied. Both cell types responded nearly equally to the presence of an individual chemical. Aspirin and caffeine were 8 to 20 times less toxic than the other chemicals studied, while Arcolor 1254 was as toxic as DDT (Fig. 1). Parathion was more toxic than its metabolite paraoxon. Para-nitrophenol was as toxic as parathion. 1-Naphthol was less toxic than its parent compound carbaryl. The toxicities of DDT and DDE were dependent upon cell type. Little significant interaction on nucleic acid or protein synthesis was seen (2).

* Department of Entomology, University of Wisconsin, Madison, Wisconsin 53706.

Utilizing insects as the test objects, the biological interaction between plasticizers and some insecticides was also investigated (1). Because of the structural similarity to DDT-related compounds, 11 plasticizers were tested for their toxicological interaction with dieldrin and DDT. Many of these polychlorinated biphenyl compounds were toxic to *Drosophila melanogaster* Meigen and house flies, *Musca domestica* L., but to a lesser extent than dieldrin or DDT. Their toxicity increased with a decrease in their chlorine content. Dieldrin, DDT, or any one of the less chlorinated and more volatile plasticizers (Aroclor 1221, 1232, 1242, 1248, 1254) were toxic to both test insects (Table 1). While these polychlorinated biphenyls contain increasing amounts of chlorine, their toxicity towards the insects decreased. Dieldrin was the most toxic of the com-

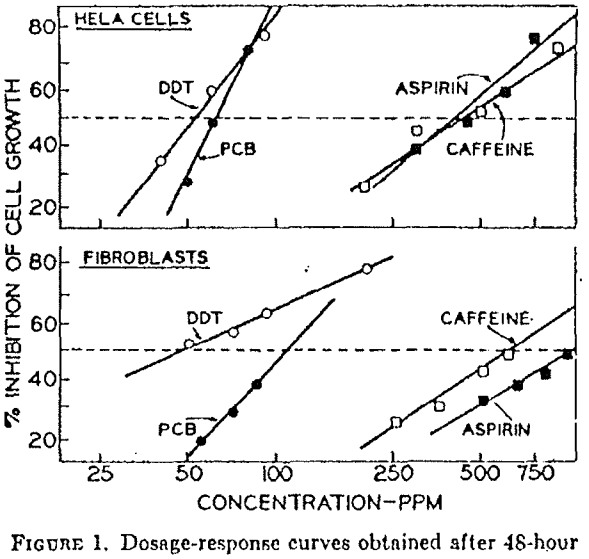

FIGURE 1. Dosage-response curves obtained after 48-hour exposure of human cell cultures to various concentrations of p,p'-DDT, Aroclor 1254 (PCB), aspirin and caffeine. (Ref. 2).

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Table 1. Effect of plasticizers or insecticides on insect mortalities.

Material	D. melanogaster			House flies	
	Applied[a]	% mortality		Applied[b]	% mortality
	(μg)	24 hr	48 hr	(μg/g fly)	24 hr
None[c]		0	0		0
Dieldrin	0.1	31	84	1.0	73
p,p′-DDT	3.0	24	58	25.0	57
Aroclor 1221	200	0	0	500	17
	800	57	92	1000	43
Aroclor 1232	200	0	0	500	10
	800	35	64	1000	30
Aroclor 1242	200	0	4	500	17
	800	40	73	1000	20
Aroclor 1248	200	0	0	500	13
	800	15	45	1000	13
Aroclor 1254	2000	0	0	1000	10
Aroclor 1260	2000	0	0	1000	0

[a] Applied in 8 ml hexane to 4-oz glass jars. *D. melanogaster* exposed to dry residue.
[b] Applied topically to house flies in 2 μliter of acetone. Weight of 1 fly = 20 mg.
[c] Controls were treated with solvents only.

pounds, as measured by the percent mortality of the insects within a 24- or 48-hr exposure period. Approximately 25 and 30 times more DDT than dieldrin, and 8000 and 1000 times more plasticizers than dieldrin had to be used to obtain similar mortalities of *D. melanogaster* or of house flies, respectively, within a given time of exposure to these chemicals. The more highly chlorinated and less volatile plasticizers were nontoxic even when used at dosages of 2000 μg with *D. melanogaster* and 20 μg/fly (1000 μg/g fly) with house flies.

Table 2 summarizes the results obtained after the insects had been exposed to sublethal dosages (200 μg for *D. melanogaster*, 10 μg/house fly) of either one of the plasticizers (Aroclor 1221, 1232, 1242, 1248 or 1254) and in combination with dieldrin or DDT. Though no appreciable insect mortalities were observed with plasticizers alone, they significantly increased the toxic effects of both dieldrin and DDT. This effect was most noticeable with DDT on house flies, and differences observed were highly significant. After a 24-hr exposure period, 38% of the house flies had died after only DDT had been applied. However, all the insects were killed when DDT was applied in combination with any one of these plasticizers. The polychlorinated biphenyls also increased the toxic effect of DDT with *D. melanogaster*, but to a lesser extent. The most striking effect was observed with Aroclor 1221. Aroclor 1260, 1262, 1268, 1265, 5442 and 5460 applied with dieldrin or DDT to glass surfaces to which *D. melanogaster* were exposed reduced the toxicity of the insecticide, resulting in mortalities of 30% to none within 48 hr (1).

In particular, though, a substantial increase in the toxicity of organophosphorus insecticides to houseflies was achieved with polychlorinated biphenyl plasticizers (3). Aroclor 1248—although nontoxic by itself to house flies at dosages of 10 μg/fly—significantly increased the toxicity of the oxygen analogs of five organophosphorus insecticides after simultaneous topical application to houseflies. Both p,p′-DDT and p,p′-DDE, applied at sub-lethal dosages, also increased the mortality of houseflies due to paraoxon from 2 to 60 and 73 per cent, respectively. The polychlorinated biphenyl plasticizer, applied simultaneously with parathion, however, decreased the toxicity of parathion to houseflies but increased mortalities when applied to flies one-half hour after the application of the insecticide. The plasticizer apparently inhibited the conversion of parathion to its toxic

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Table 2. Combined effects of plasticizers and insecticides on insects.

Aroclor	% mortalities of insects after exposure to: No insecticide	Dieldrin	p,p'-DDT
		D. melanogaster after 48 hr[a]	
None[b]	0	66±6	59±6
1221	0	87±4[c]	93±5[d]
1232	0	81±3[c]	84±6[c]
1242	5±2	77±9	83±6[c]
1248	0	72±4	77±3[c]
1254	0	10±3[c]	47±3[c]
		House flies[f] after 24 hr	
None	0	27±5	38±8
1221	4	49±3[c]	100[d]
1232	4	27±5	100[d]
1242	0	47±5[c]	98±3[d]
1248	6	58±11[c]	100[d]
1254	0	24±6	100[d]

[a] *D. melanogaster* were exposed in 4-oz glass jars to the dry durface deposit of the chemicals: plasticizers, 200μg; dieldrin, 0.1 μg; DDT, 3.0 μg.

[b] Bioassay jars without plasticizers contained 250 μg of corn oil each.

[c,d] Differences obtained between treatments with the insecticide only and those with insecticide plus plasticizer are significant at the c = 1%, d = 0.1%, or e = 5% level.

[f] Chemicals were applied topically in 2 μliter of acetone to houseflies: plasticizers, 500 μg/g fly; dieldrin, 0.75 μg/g fly; DDT, 15 μg/g fly. Control: only acetone was applied. Weight of 1 fly = 20 mg.

activation product, paraoxon, and the breakdown of paraoxon into nontoxic compounds. This was also indicated by the recovery of larger amounts of ^{14}C-paraoxon and smaller amounts of water soluble ^{14}C-products from flies treated simultaneously with Aroclor 1248 plus paraoxon, in comparison to those which had been treated with ^{14}C-paraoxon only. Utilizing the 10,000×G supernatant from housefly homogenates, the addition of Aroclor 1248 with ^{14}C-paraoxon in vitro also resulted—after 2 hours incubation at 30°C—in higher recoveries of ^{14}C-paraoxon and in a reduction of the appearance of water soluble ^{14}C-products. Identical results were obtained with subcellular fractions (10,000×G supernatant) from flies that had been treated with Aroclor 1248 eighteen hours before homogenate preparation. No difference was observed in the degradation of paraoxon or the appearance of water soluble products with Aroclor applied to the living flies or to the 10,000×G supernatant of homogenates from untreated flies (3).

Since plasticizers are in the environment, their potential effects on biological systems, especially in combination with other synthetic chemicals, should not be disregarded.

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Polychlorinated Biphenyl Interactions with Tissue Culture Cells†

by Roger Hoopingarner, Albert Samuel, and David Krause*

The successful use of tissue culture cells in biological work is now almost 60 years old. However, the applicability or the "fitness" of these cells to demonstrate biological principals is still debated. It is well known that many of the cell lines bear little resemblance to the parent tissue or organ cells. Yet isolated functional cells of serially-propagated clonal strains, that can perform specialized, organ-specific functions for prolonged periods, have been produced in nearly limitless numbers (1). The loss of specific function has been ascribed to (i) selective overgrowth by connective tissue cells, (ii) a phenotypic change in the cultured cells, or (iii) inadequate or harmful environmental conditions (2). In order to circumvent these problems, primary cell lines are used and tested before the line "adapts" to culture conditions through aneuploidy or other means.

The use of tissue culture cells for the examination of environmental chemicals has been relatively recent (3). The effects measured have been (i) inhibitory dose, (ii) toxic dose, (iii) cytotoxic effects, and (iv) chromosome aberrations. Samuel (4) has used both established and primary cell lines also to study the metabolism of certain pesticides. Litterst and Lichtenstein (3) using HeLa cells and a cell line of non-malignant origin found that the interaction of DDT and PCBs (Aroclor 1254) that had been found in flies was absent. They concluded that the various interactions of chemicals with the cell lines had no correlation to intact animals.

The use of human primary cell lines was enhanced greatly with the discovery of the mitotic stimulus of the mucoprotein phytohaemagglutinin (5). The leucocyte culture techniques developed by Moorehead et al. (6) proved to be most suitable for in vitro cell studies of human cells and chromosomes. The method also allows an in vivo check for chromosome effects (7) and, as such, may offer a comparison of in vivo with in vitro effects on essentially the same cell.

In the studies to be reported here, we used Chinese hamster cells (quasi-diploid epithelial cells, CH-461) and primary human lymphocyte cells. The Chinese hamster cell line has a generation time of approximately 24 hours. They were grown in Eagle's minimum essential medium supplemented with 10% fetal calf serum.

The lymphocyte cultures were obtained from blood of laboratory volunteers and were grown in chromosome medium 1A from Grand Island Biological Company. The cell cycle time varies with individual donors but is approximately 24 hours after a "lag" period of from 24 to 48 hours prior to activation.

The PCBs were obtained from the Monsanto Company and were used without alteration. The gas chromatography of the PCBs was accomplished using a 50 foot ×0.02 inch S.C.O.T. column of Apienzon L at 200°C. Detection was by hydrogen flame ionization detector.

The growth suppression of Chinese hamster cells with PCBs and DDT are shown in Fig. 1. These data are comparable to those found by Litterst and Lichtenstein (3) though they did not directly study an ID_{50} using both chemicals

† A contribution from the Michigan Agricultural Experiment Station (Article 5832). This research was supported in part by funds provided by the Food and Drug Administration DHEW under contract FDA 71-285.

* Department of Entomology, Michigan State University, East Lansing, Michigan 48823.

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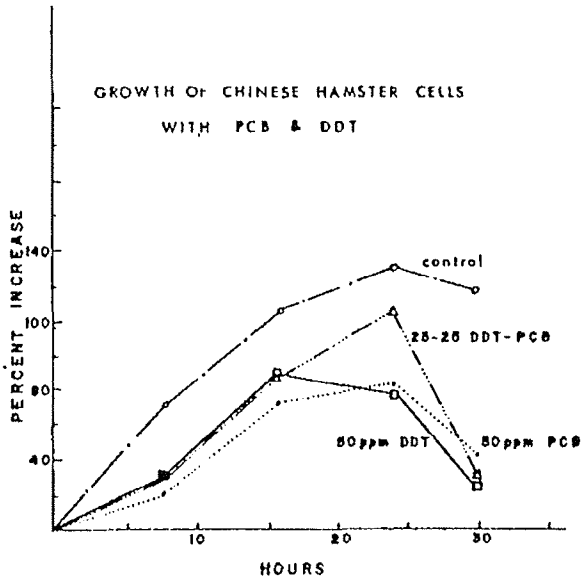

FIGURE 1. Growth of Chinees Hamster cells with PCB and DDT.

simultaneously. The toxic dose of the chemical was several times greater than is usually found biologically.

It is of some interest to note the disintegration of cells in the period from 24 to 30 hours, when the cells were being stressed from culture conditions. This same phenomenon can be seen from the differences between 24 and 30 hours in Table 1. This Table gives the relative growth information for the different PCBs. As you can see, there was a steady drop in cell numbers as one decreases the percentage of chlorine. The Aroclor 1016 is a distillation product of 1254, which essentially excludes the biphenyls of five chlorines

Table 1. Populations of Chinese Hamster Cells Cultured with 50 ppm of Various PCB's

PCB	24 hrs*	30 hrs
None	35±5.9	36±4.4
1260	27±5.6	7±1.7
1254	23±5.8	9±1.7
1248	18±6.1	5±1.4
1221	16±6.4	11±3.4
1016	4±1.1	1±0.4

* Average of ten replicates. Count of cells/unit ocular grid area.

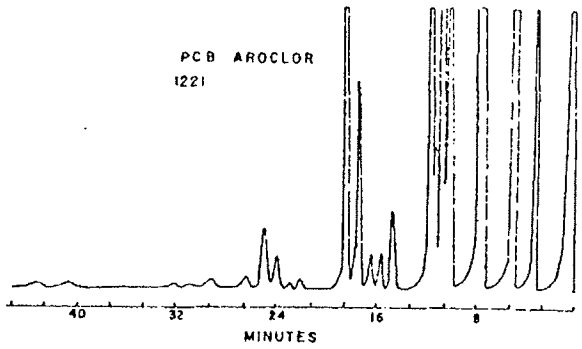

FIGURE 2. Gas Chromatogram of Aroclor 1221.

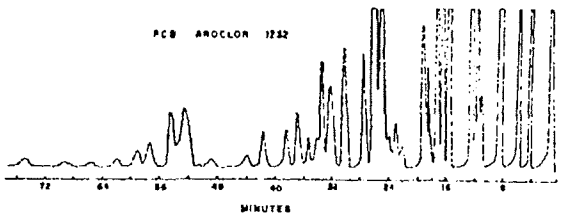

FIGURE 3. Gas Chromatogram of Aroclor 1232.

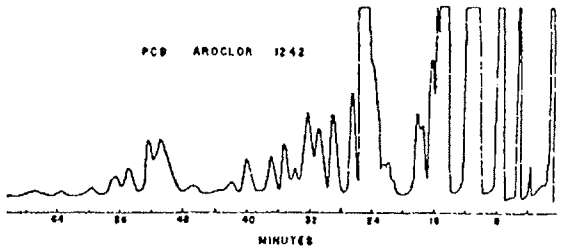

FIGURE 4. Gas Chromatogram of Aroclor 1242.

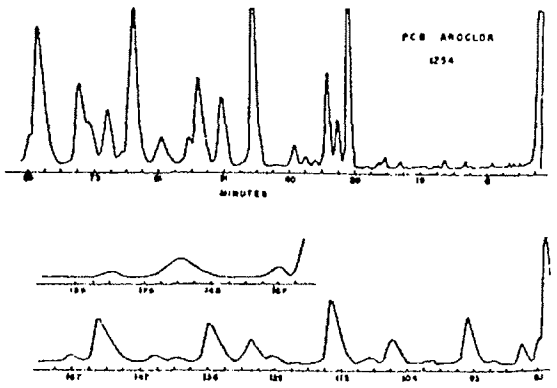

FIGURE 5. Gas Chromatogram of Aroclor 1254.

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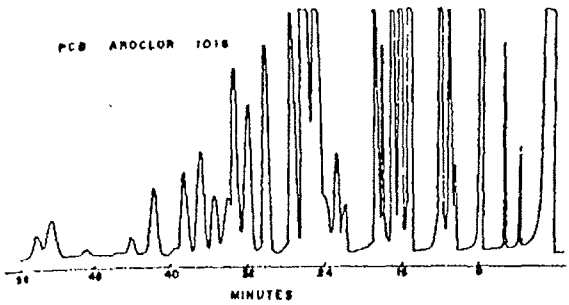

FIGURE 6. Gas Chromatogram of Aroclor 1016.

and above. The product is still a biphenyl, but since it is derived differently, the prefix is changed, from 12 to 10. Based on the percent chlorine, Aroclor 1016 falls out of the pattern of toxicity. If you look at the gas chromatographic patterns, this toxicity is somewhat of an anomaly. For example, the Figures 2 through 6 are the chromatograms for the various PCBs using the support-coated open tubular, or S.C.O.T., columns.

The pattern of 1016 was very much like that found for 1232 or 1242 yet 1016 was several fold more toxic to the tissue culture cells. It would appear that there may be certain components that are in higher proportion in the 1016 product, and these components (or component) are causing the increase in toxicity. We have fractionated most of the chromatographic peaks into their component parts and are currently assaying them to determine the relative toxicity of each.

The human lymphocyte cultures are of course a primary cell line, and in fact we examine the cells cytologically at the first in vitro division.

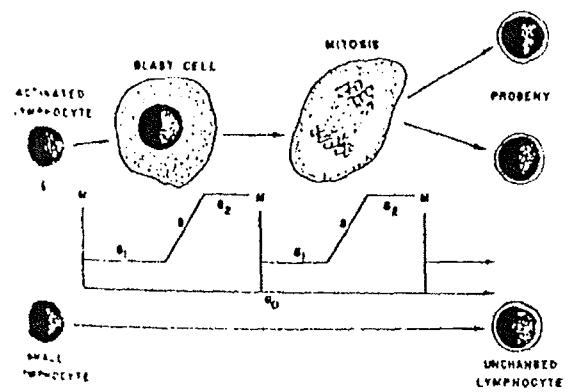

FIGURE 7. Diagram of Cultured human lymphocytes at first in vitro division.

Table 2. Human Lymphocytes Treated with 100 ppm Aroclpr 1254.*

Period of PCB Treatment	Mitotic Index	Satellite Association	Chromatid Gap*	Chromatid Breaks
Test 1				
0–24 hrs.	.032	9	5	1
44–52 hrs.	.009	15	3	1
49–52 hrs.	.017	3	4	0
Acetone check	.022	6	3	0
Control	.012	4	5	1
Test 2				
0–24 hrs.	.017	10	1	0
44–52 hrs.	.007	4	4	0
49–52 hrs.	.012	4	2	1
Acetone check	.009	4	3	0
Control	.008	8	2	1

* 50 cells analyzed/test/treatment

The cells are shown diagramatically in Fig. 7. Within the body, the lymphocyte is in a differentiated state G_0. These cells are then activated or transformed into a division cycle with phytohemagglutinin. Sasaki and Norman (8) have shown that the small lymphocyte finishes its transformation in 24 to 48 hours after stimulation and begins a period of cell proliferation with a generation time of 22 hours; G_1, S, G_2, and mitosis are 6, 11, 3, and 2 hours, respectively. We treated these cells with Aroclor 1254 at 100 ppm for the first 24 hours and again during the last eight (S plus G_2), and the last three hours (mitosis). During the first 24 hours, the cells would be in some stage between G_0 and G_1. The cells were then examined cytologically for chromosome aberrations for the various stage treatments. The results are shown in Table 2. PCBs seem to have no effect on chromosomes under these conditions.

The results of this study would indicate that established tissue culture lines are more sensitive than primary cultures to at least one PCB mixture. The various PCB mixtures are generally more toxic as one decreases the percentage of chlorine. The distillation product 1016, however, was more toxic to these cells than was indicated from the percent chlorine content or from its gas chromatographic pattern. At 100 ppm of

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1254 to human lymphocyte cultures, there was no apparent effect to the chromosome integrity as measured by cytological evidence.

Tissue culture systems may not always be indicative of whole body conditions. However, these systems do offer some uniform conditions for many definitive studies of environmental chemicals. With substantial data for comparative tests they may be good indicator systems. The human lymphocytes system also offers the comparative cytological test for both in vivo or in vitro exposure.

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Toxicities of PCBs to Fish and Environmental Residues

by David L. Stalling and Foster Lee Mayer, Jr.*

Polychlorinated biphenyls (PCBs) have been found in fish and wildlife from many parts of the world at levels which may adversely affect aquatic organisms and interfere with pesticide residue analyses (1, 2, 3, 4). The environmental occurrence, uses, and present toxicological aspects of PCBs were recently reviewed by Peakall and Lincer (5), Gustaffson (6), and Risebrough (7). PCBs have gas chromatographic characteristics similar to many organochlorine insecticides. Jensen (8) first identified unknown gas chromatographic peaks as PCBs in extracts of pike and an eagle which were analyzed for organochlorine insecticides.

The Monsanto Company is the sole manufacturer of PCBs in the United States (6) and markets eight formulations of chlorinated biphenyls under the trademarks Aroclor 1221, 1232, 1242, 1248, 1254, 1260, 1262, and 1268. The latter two digits designate the percent chlorine of each formulation. Aroclor 1248 and 1254 are the materials produced in greatest quantities and are used as a dielectric fluid in capacitors and in closed-system heat exchangers (9). Aroclor 1242 is used as a hydraulic fluid, and Aroclor 1260, as a plasticizer. Chlorinated terphenyls are marketed under the trademark Aroclor 5442 and 5460. A mixture of bi- and terphenyls is designated Aroclor 4465. The Monsanto Company has restricted the sale of PCBs for uses in which disposal of the end products cannot be controlled, such as the use of PCBs as plasticizers (6).

* Chemist and Fishery Biologist, respectively, Fish-Pesticide Research Laboratory, Bureau of Sport Fisheries and Wildlife, Columbia, Missouri 65201.

Chemical Composition and Analysis

PCBs occur in the aquatic environment as mixtures of chlorinated biphenyl isomers (10, 11). The biphenyl structure may be substituted with one to ten chlorine atoms, and over 200 compounds are possible (6). The isomer composition and chromatographic characteristics of each formulation have been described by Stalling and Huckins (12) and Bagley (13). The PCBs can cause serious interference in gas chromatographic (GC) determination of chlorinated insecticides (4). Analysis of PCBs is best accomplished by GC after separation of PCBs and pesticides during sample cleanup using silicic acid column cleanup (14). No standardized GLC method has been proposed for the analysis of mixtures of PCB formulations in environmental samples. The solubility of these formulations in water have not been precisely determined but is reported in the range of 100–1000 μg/1 (9). Considerable research remains to be done on the structure of the isomers of each technical material and how environmental residues of various Aroclors are to be reported.

Environmental PCB Residues

Analyses of 40 fish (cross-check sample) from the 1970 National Pesticide Monitoring Program were made using sample preparation techniques which separated PCBs from pesticides and other industrial residues (Table 1). These analyses employed GC and gas chromatography-mass spectrometry (GC-MS) for confirmation. The samples were prepared using gel permeation chromatography (23), and PCBs were separated from the pesticides with silicic acid (14). Only

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Table 1. Composition of PCB residues in selected fish samples from the 1970 National Pesticide Residue Monitoring Program.

River	Location	Species	PCB residue as Aroclor type (μg/g whole body)				
			1232	1248	1254	1260	total
Ohio	Cincinnati, O.	Carp	10.2	75	42	6.0	133
Ohio	Cincinnati, O.	White crappie	15.9	17	27	5.6	66
Ohio	Marietta, O.	Channel catfish	38	23	11	4.9	77
Ohio	Marietta, O.	Channel catfish	15.6	5.2	12.6	4.6	38
Yazoo	Redwood, Miss.	Smallmouth buffalo	72	—	1.4	—	73
Hudson	Poughkeepsie, N.Y.	Goldfish	8.6	173	32	—	213
Allegheny	Natrona, Pa.	Walleye	—	5.2	25	4.6	35
Delaware	Camden, N.J.	White perch	—	8.0	6.8	3.9	19
Cape Fear	Elizabeth Town, N.C.	Gizzard shad	18.9	—	2.6	1.1	23
Lake Ontario	Port Ontario, N.Y.	White perch	12.9	—	4.6	1.2	19
Mississippi	Memphis, Tenn.	Drum	11.2	—	4.5	3.4	19
Merrimac	Lowell, Mass.		13.8	75	6.1	3.2	98

one of the forty samples contained less than 1 μg/g PCB residue. The composition of PCB residues in a lake trout and coho salmon were examined by GC-MS and found to be a mixture of Aroclor 1248 and 1254 (Figure 1), and residues of PCBs were in excess of the "DDT" residue levels (Table 2).

Widespread pollution of the major waterways has occurred, and appreciable PCB residues exist in fish. In view of the PCB bioconcentration in fish, PCB levels as high as 5 μg/l may exist in the waters of the Hudson River. These levels are certain to have adverse effects on aquatic organisms.

Toxicity and Bioaccumulation

Fish

Chronic and acute toxicity studies on aquatic organisms are quite limited. Due to the low solubility of PCBs, tests to obtain 96-hr LC_{50} values do not adequately reflect their toxicities to fish (Table 3). For Aroclor 1221-1268, 96-hr LC_{50} values ranged from 1,170 to 50,000 μg/l for cutthroat trout (15). The acute oral toxicity of Aroclors 1242, 1248, 1254, and 1260 was greater than 1500 mg/kg in rainbow trout.

Intermittent-flow bioassays of Aroclor 1242, 1248, and 1254 to bluegills resulted in 15-day

Table 2. Pesticide and PCB Residues in a Coho Salmon and a Lake Trout from Lake Michigan.

	Residues in μg/g whole fish									
	Dieldrin	p,p′ isomers			o,p′ isomers		Total "DDT"	PCB (Arcolor)[1]		
		DDE	DDD	DDT	DDE	DDT		1248	1254	Total
Lake trout[2]	0.10	13.0	1.8	5.2	0.9	1.8	22.7	12.0	16.0	28.0
Coho salmon[3]	0.10	4.3	0.8	2.3	0.4	0.5	8.3	6.7	6.7	13.4

[1] Calculated from ratios of characteristic 1248 and 1254 peaks.

[2] Lake trout (33 L)—taken 10-14-69. 690 mm, 3590 gm, immature M. Taken in 2.5 fathoms in Little Bay de Noc, Michigan.

[3] Coho salmon (7Cs)—taken 9-3-69. 675 mm, 5718 gm F. Taken in 17-19 fathoms 4 miles NW of Manistee, Michigan.

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LC_{50} values of 54, 76 and 204 μg/1, respectively (Table 4). Exposures to channel catfish gave 15-day LC_{50} values of 107, 127, and 741 μg/1 for the same Aroclors. All LC_{50} values decreased significantly with longer exposures. The 20-day LC_{50} values for Aroclors 1248, 1254, and 1260 were 10, 135, and 245 μg/1 for bluegills and 300, and 296 μg/1 for Aroclors 1254 and 1260 with channel catfish at 20°C. The toxicity of Aroclor 1248 to bluegills and channel catfish increased two-fold at 27°C. The toxicity of the PCBs to fish is inversely related to percent chlorine (higher Aroclor number).

In chronic tests to determine the effects of Aroclor 1242 and 1254 on fathead minnow reproduction, all fish exposed to greater than 8.3 μg/1

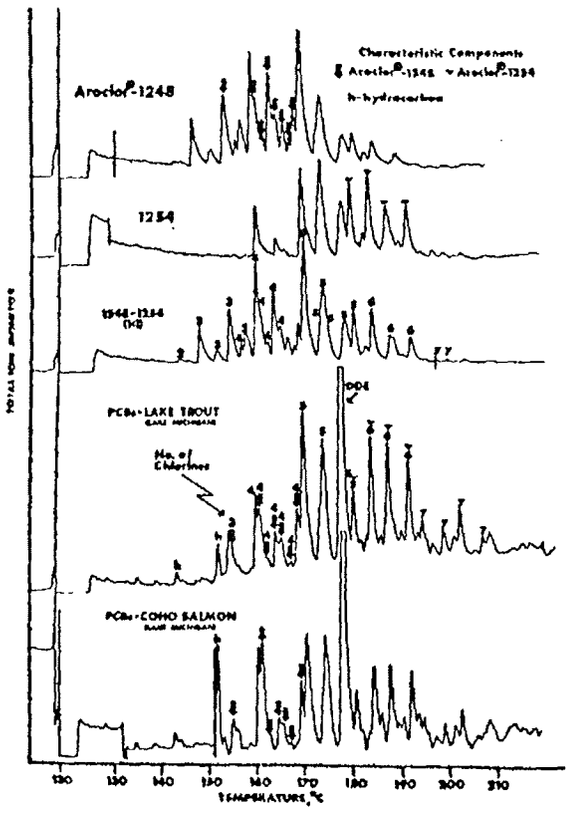

FIGURE 1. Composition of PCB residues in fish. Arrows and triangles designate the most characteristic peaks of the Aroclor. All numbered peaks in chromatograms of fish extracts are PCBs. These numbers designate the number of chlorine atoms in the compounds as determined by mass spectrometry. Temperature program rate, 4°C/min, He 9 psig, 50'×0.020" i.d. SE-30 SCOT column, PE-2708 GC-MS.

Table 3. Acute toxicity[1] of several Aroclors to selected fishes.

Aroclor	Species	Temperature (C)	96-hr LC_{50} (μg/1)
1221	Cutthroat trout	8.9[2]	1,170
1232	Cutthroat trout	8.9	2,500
1242	Cutthroat trout	8.9	5,430
1248	Cutthroat trout	8.9	5,750
1254	Cutthroat trout	8.9	42,500
1260	Cutthroat trout	8.9	60,900
1262	Cutthroat trout	8.9	50,000
1268	Cutthroat trout	8.9	50,000
1248	Channel catfish	18.3[3]	6,000
1254	Channel catfish	18.3	12,000
1248	Bluegills	18.3[3]	278
1254	Bluegills	18.3	2,740

[1] Static bioassay.
[2] Alkalinity, 159 ppm; pH 7.6.
[3] Alkalinity, 35 ppm; pH 7.1.

of each Aroclor died. Reproduction occurred at and below 8.3 μg/1 Aroclor 1242 and at and below 2.8 μg/1 Aroclor 1254 (17).

Accumulation of Aroclor 1248 and 1254 by bluegills chronically exposed to 2–10 μg/1 was from 26,300 to 71,400 times the exposure levels for both PCBs. Concentration factors were not strongly dependent upon water exposure levels, but a direct correlation of water concentrations and whole-body residues may exist. No major modification of the PCB isomer ratios was observed in the tissue residues, and no new components were identified (18).

Coho salmon fed Aroclor 1254 for 240 days at concentrations of 14.5 to 14,500 μg/kg body weight per day (dietary concentrations of 0.4 to 580 μg/g) accumulated whole-body residues which were 0.9 to 0 5 times the exposure levels (300 mg/kg highest residue value). Growth rates were not affected; however, all fish exposed to the highest treatment died after 240 days' exposure, and thyroid activity was stimulated in all but the lowest treated group (19).

Direct water exposures appear to represent a greater hazard to fish than dietary exposures, although both contribute to tissue residues. However, in the environment the kinetics of residue uptake from dietary sources could be more important, since PCBs have a high affinity for

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Table 4. Intermittent-flow bioassays of Aroclor against three fishes.[1]

Aroclor	Species	LC_{50} (μg/1)					
		5 days	10 days	15 days	20 days	25 days	30 days
1254	Rainbow trout	156	8	—	—	—	—
1260		—	240	94	21	—	—
DDT		2.26	0.87	0.26	—	—	—
1242	Bluegills	154	72	54	—	—	—
1248		307	160	76	10	—	—
1254		—	443	204	135	54	—
1260		—	—	—	245	212	151
1242	Channel catfish	—	174	107	—	—	—
1248		—	225	127	—	—	—
1254		—	—	741	300	113	—
1260		—	—	—	296	166	137
1248[2]	Bluegills	137	76	—	—	—	—
1248[2]	Channel catfish	—	94	57	—	—	—

[1] Temperature, 20°C; alkalinity, 260; pH, 7.4.
[2] Temperature, 27°C.

sediments and PCBs can readily enter food chains (20, 21).

Aquatic Invertebrates

Daphnia magna: Aroclor 1248 is the most toxic of the Aroclor series to *Daphnia*, and preliminary studies indicate that levels above 5 μg/1 are not safe for reproduction (17). After 48 hours' exposure to 300 μg/1, *Daphnia* concentrated Aroclor 1254 by 48,000 times (22).

Gammarus pseudolimnaeus: The level of Aroclor 1248 that did not affect reproduction was comparable to that for daphnids, ca 5 μg/1 (17). The 96-hr LC_{50} values for Aroclor 1248 and 1254 were 52 μg/1 and 2,400 μg/1, respectively (Table 5). Scud were more sensitive to Aroclor 1242 (96-hr LC_{50}=10 μg/1). After exposing another species of scud (*Gammarus fasciatus*) to 1.6 μg/1 Aroclor 1254 for 14 days, the PCBs were concentrated 27,000 times the exposure level. No further increase in PCB residue resulted after an additional 21 days of exposure (22).

Orconectes nais: Crayfish were more susceptible to Aroclor 1242 (7-day LC_{50}=30 μg/1) than they were to Aroclor 1254 (7 day LC_{50}=80 μg/1). PCB residues in crayfish did not reach equilibrium after a 28-day exposure to Aroclor 1254, and the uptake was linear during this period (22).

Palaemonetes kadiakensis: Glass shrimp were very sensitive to Aroclor 1254, having a 7-day LC_{50} value of 3 μg/1 compared to a 5-day LC_{50} of 1 μg/1 for DDT (22).

Marine Organisms

Immature pink shrimp were extremely sensitive to Aroclor 1254 exposures. Fifty-one percent of the shrimp died within 15 days when continuously exposed to 0.94 μg/1 Aroclor 1254 (25). Mortalities in two estuarine fishes (*Lagodon rhomboides* and *Leiostomus xanthurus*) exposed for 14 to 45 days to Aroclor 1254 were observed at 5 μg/1. Fish mortalities were not observed at 1 μg/1 during the same exposure time (24). PCB concentration factors in these fish were similar to uptake by freshwater fish ($1–5\times10^4$ times the exposure levels).

Correlation of Reproduction and PCB Residues

In preliminary investigations, Jensen et al. (25) reported a possible relationship between PCB residues in salmon eggs and egg mortality in Sweden. On a fat basis, PCB residues in eggs ranged from 7.7 to 34 μg/g and mortality ranged from 16 to 100 percent. A regression analysis of their data resulted in a coefficient of correlation of 0.85, and the correlation was significant at P .001.

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Table 5. Aroclor and DDT toxicity to invertebrates.

Compound	Organism[1]	Bioassay type[2]	Exposure (days)	LC_{50} (μg/l)
Aroclor 1242	Crayfish	static	7	30
Aroclor 1254	Crayfish	static	7	100
Aroclor 1254	Crayfish	continuous-flow	7	80
DDT	Crayfish	static	4	100
Aroclor 1242	Scud	continuous-flow	4	10
Aroclor 1242	Scud	continuous-flow	10	5.0
Aroclor 1248	Scud	static	4	52
Aroclor 1254	Scud	static	4	2,400
DDT	Scud	static	4	3.2
DDT	Scud	continuous-flow	5	0.6
Aroclor 1254	Glass shrimp	continuous-flow	7	3.0
DDT	Glass shrimp	static	5	1.0
DDT	Glass shrimp	continuous-flow	5	1.3
Aroclor 1242	Dragonfly	static	7	800
Aroclor 1254	Dragonfly	static	7	1,000
Aroclor 1242	Damselfly	continuous-flow	4	400
Aroclor 1254	Damselfly	continuous-flow	4	200
DDT	Damselfly	static	4	56

[1] Crayfish (*Orconectes nais*), Scud (*Gammarus fasciatus*), Glass shrimp (*Palaemonetes kadiakensis*), Dragonfly (*Macromia sp.*), Damselfly (*Ischnura verticalis*).

[2] Temperature, 15.6°C; alkalinity, 35 ppm; pH, 7.1.

Summary

The occurrence of high levels of PCB residues in aquatic organisms (fish and invertebrates) as complex mixtures of Aroclor formulations has been determined by GC analysis and confirmed by GC-MS. Suitable methods for sample cleanup include gel permeation and silicic acid column chromatography. Agreement on methods for reporting PCB residues is lacking.

The highest concentrations of PCB residues in freshwater fish occur in rivers which are associated with industrialized areas, i.e. Hudson River (ca 200 μg/g whole body residue) and the Ohio and Allegheny Rivers (ca 100 μg/g). Residue levels associated with these rivers approach PCB residues which were associated with fish mortalities in chronic continuous-flow exposures (500–600 μg/g).

Concentration of PCBs by fish is greater than 40,000 times the exposure levels. Similar concentration factors have been observed for invertebrates. Adverse effects on reproduction of aquatic organisms may occur at PCB concentrations of 5 μg/l or less. Further studies are required to determine more definitely the exposure levels which do not adversely effect aquatic organisms.

The problem of PCB residues in industralized areas can be greatly improved by close monitoring of effluents and by identification of those PCB uses which contribute to effluent contamination. Elimination of widespread PCB residues in fish at concentrations of 1–20 μg/g will require greater efforts, such as the control of PCBs from sewage treatment plants and other as yet unidentified sources of PCBs into the aquatic environment.

Related Materials

Chlorinated napthalenes are recommended for uses similar to those of the PCBs (27). These materials are recovered by the same analytical procedures as the PCBs (28) and may be recognized by their gas chromatographic elution patterns if PCBs are not present. The materials are designated Halowax 1000, 1031, 1099, 1013, 1014, and 1051 and are listed in an order of increasing chlorine content (27). Aquatic toxicity information is not presently available, nor has

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the persistence and fate of these materials been examined in the aquatic environment.

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The Sensitivity of Fish ATPases to Polychlorinated Biphenyls*

by L. K. Cutkomp, H. H. Yap, D. Desaiah, and R. B. Koch†

In spite of widespread concern about the possible hazards of polychlorinated biphenyls (PCBs), little is known about the action of these materials. We have therefore examined the in vitro (1, 2) and in vivo (3) effects of PCBs on the adenosine triphosphatase (ATPase) enzyme system. We have found some effects which are similar, yet distinctive, to chemically related compounds of the DDT type. A background of earlier research with DDT and closely related chemicals has shown the greatest inhibition on Mg^{2+}ATPase and less on Na^{+}-K^{+}ATPase (4–7). Subsequent research showed that mitochondrial Mg^{2+}ATPase of fish and insects was most sensitive to DDT (8). The results from testing several PCBs on the ATPase enzyme system are presented in this manuscript.

Materials and Methods

Tissues from bluegill fish, *Lepomis machrochirus*, were dissected, homogenized, and fractionated by centrifuging at 13,000×g for 20 minutes, and the sediments were resuspended in 0.32 M sucrose, 1 mM EDTA, and 10 mM imidazole. The fraction obtained (B) contained mitochondrial and nerve ending particles. Each preparation was appropriately diluted and the samples were quick frozen in liquid nitrogen and stored at −20°C. until the ATPase assay. ATPase activity was determined using the enzymatic or continuous procedure in which catalysts pyruvate kinase and lactic dehydrogenase were present, and NADH (reduced nicotinamide adenine dinucleotide phosphate) is oxidized to NAD (nicotinamide adenine dinucleotide phosphate) for spectrophotometric determination at 340 nm. The procedure is detailed in Pullman et al (9), and Fritz and Hamrick (10), and in Yap and Cutkomp (11). Mg^{2+}ATPase activity was measured when one mM ouabain was in the reaction mixture. Ouabain is a cardiac glycoside which specifically inhibits the Na^{+}-K^{+}ATPase. Mg^{2+}-ATPase was further separated into oligomycin-sensitive (mitochondrial) and oligomycin-insensitive portions by adding 0.03 μg oligomycin (oligomycin A 15%, B 85%) per ml reaction mixture. Oligomycin insensitive Mg^{2+}ATPase is generally more prominent in microsomes (endoplasmic reticulum and plasma membrane) and is present to a small extent in mitochondria.

Four PCB preparations (Aroclor 1221, Aroclor 1242, Aroclor 1254 and Aroclor 1268) and a polychlorinated terphenyl, Aroclor 5460 were investigated for effects on the ATPase systems. Aroclors 1221, 1242 and 1254 were dissolved in ethanol, and Aroclors 1268 and 5460, in acetone, and added to the reaction mixture by slowly releasing the solution from a Hamilton microsyringe under the liquid surface of the vortex of a rapidly-stirred reaction mixture. This prevented sedimentation and promoted the formation of colloidal mixtures. Reaction temperature was 37°C, except for muscle homogenates, where the temperature was maintained at 27°C. The amount of solvent added (never exceeding 5 μl) with the PCBs had no effect on the ATPase

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* Prepared by the Department of Entomology, Fisheries and Wildlife, University of Minnesota, St. Paul, Minnesota 55101. A review and condensation of publications (1), (2), and (3).

† Staff Scientist, Honeywell Corporate Research Center, Hopkins, Minnesota 55343.

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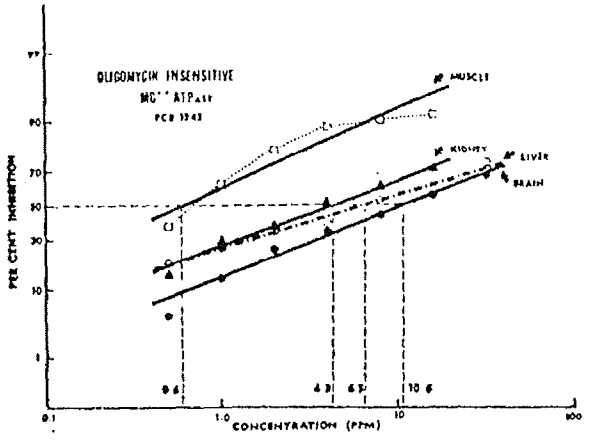

FIGURE 1. Aroclor 1242 inhibition (Probit Units) of oligomycin—insensitive Mg^{2+} ATPase from fish homogenates. ATPase activity of untreated in μmoles P_i mg^{-1} protein hr^{-1} for brain 14.5±0.03; kidney 16.1±0.02; liver 10.4±0.5; muscle 46.2±9.6. Unbroken lines computed; dotted line connects actual points.

activities. Four tissues of fish (brain, muscle, liver and kidney) were selected as enzyme sources.

Results and Discussion

Those PCBs tested in vitro were generally most effective as inhibitors of oligomycin-insensitive Mg^{2+}ATPase, particularly from muscle homogenates. Aroclors 1242 and 1254, which

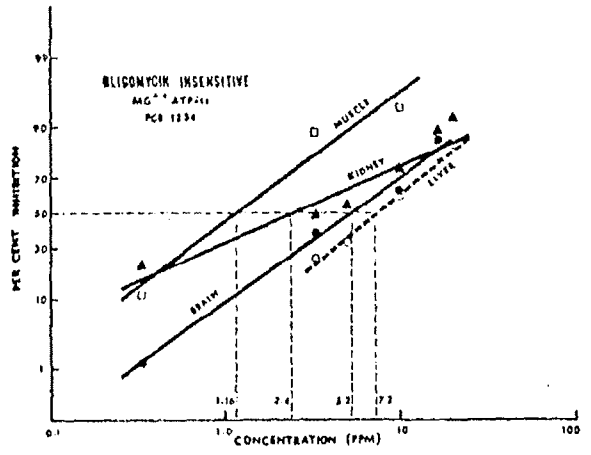

FIGURE 2. Aroclor 1254 inhibition (Probit Units) of oligomycin—insensitive Mg^{2+} ATPase from fish homogenates. ATPase activity of untreated in μmoles P_i mg^{-1} protein hr^{-1} for brain 14.2±0.4; kidney 17.3±3.0; liver 10.0± 0.05; muscle 63.7±5.5.

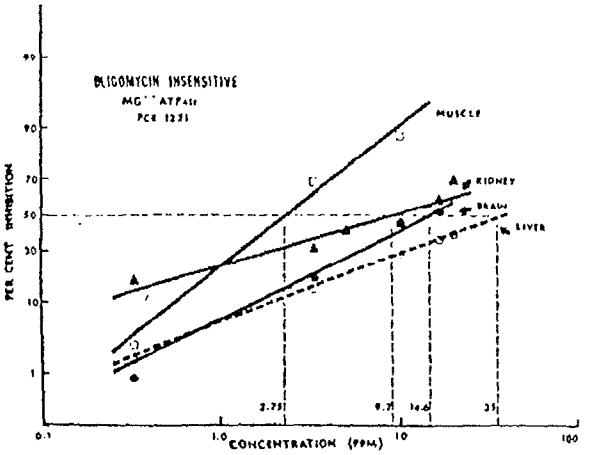

FIGURE 3. Aroclor 1221 inhibition (Probit Units) of oligomycin—insensitive Mg^{2+} ATPase from fish homogenates. ATPase activity of untreated in μmoles P_i mg^{-1} protein hr^{-1} for brain 14.2±0.4; kidney 17.3±3.0; liver 10.7± 0.07; muscle 63.7±5.5

are in the intermediate range of chlorination, were more effective than 1221 and 1268. Compared at 50% inhibition, the values were 0.6 ppm for Aroclor 1242 (Fig. 1), 1.2 ppm for Aroclor 1254 (Fig. 2) and 2.3 ppm for Aroclor 1221 (Fig. 3), the smallest value indicating the most effective. Mg^{2+}ATPase from mitochondria (oligomycin-sensitive) was not affected as much by the PCBs as by DDT-type compounds. The I_{50} for Aroclor 1242 was 2.0 ppm on mitochondrial Mg^{2+}ATPase from muscle, 4.0 ppm from kidney (Table 1 and ref. 2) and 3.5 ppm from brain

Table 1. Percent inhibition of mitochondrial Mg^{2+} ATPase activity by AROCLOR 1242 in fish kidney and muscle homogenates.

Concentration (ppm)	Per cent inhibition*	
	Kidney	Muscle
0.5	+13.7	+26.5
1.0	+10.4	25.9
2.0	17.4	67.8
4.0	62.0	69.4
8.0	66.2	77.9
16.0	66.0	76.6
Untreated	27.2	5.6
Sp. Act±S.E.	±2.5	±1.1

* (+) Values represent the per cent enzyme activation.

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Table 2. Fifty percent inhibition values (in ppm) of ATPases determined from homogenates of blue gill fish brain. Summarized from references 2 and 8. (Lower values denote greater effectiveness.)

ATPases	*In vitro* tests DDT	Aroclor 1242
Total Mg^{2+}	15.0	8.3
Mitochondrial Mg^{2+}	0.5	3.5–5.6 (max. 64%)
Oligomycin-Insensitive Mg^{2+}	>7.5 (30%)	10.8
Na^+–K^+	>15.0 (30 %)	18.5

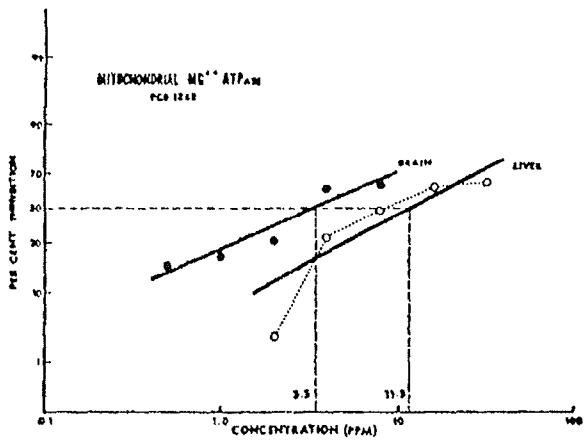

FIGURE 4. Aroclor 1242 inhibition (Probit Units) of mitochondrial Mg^{2+} ATPase from fish homogenates. ATPase activity of untreated in μmoles P_i mg^{-1} protein hr^{-1} for brain 13.2±1.1; liver 29.1±0.004. Unbroken lines computed; dotted line connects actual points.

Table 3. Percent inhibition of ATPases in homogenates of mitochondrial fraction of kidney of 4-month Aroclor 1242 treated fat-head minnows

ppb of 1242	ATPases		
	Mg^{2+} ATPase		
	Mitochondrial	Oligomycin-insensitive	Na^+–K^+ ATPase
0.9	56	23	21
2.8	75	21	36
8.3	45	35	18

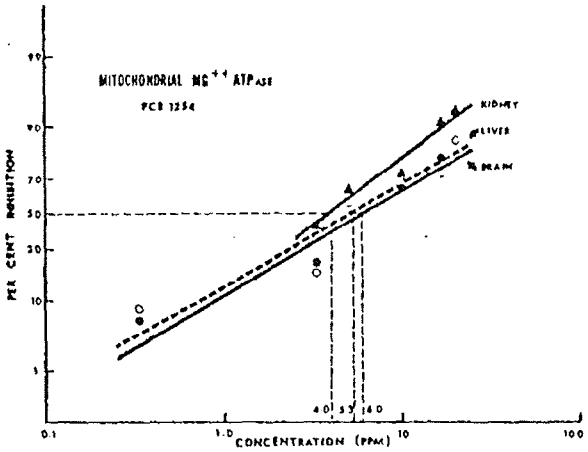

FIGURE 5. Aroclor 1254 inhibition (Probit Units) of mitochondrial Mg^{2+} ATPase from fish homogenates. ATPase activity of untreated in μmoles P_i mg^{-1} protein hr^{-1} for brain 13.5±0.35; kidney 28.6±0.85; liver 39.0±1.25.

(Fig. 3). The I_{50} for DDT was 0.5 ppm in brain (Table 2). In contrast to the effective inhibitors, two of the poorest inhibitors, Aroclor 1221 and Aroclor 1268 (see ref. 2), showed some stimulation of Mg^{2+}ATPase.

Na^+-K^+ATPase from fish brain homogenates was inhibited by Aroclor 1242, but the concentration required was about 5× that for mitochondrial Mg^{2+}ATPase. Aroclor 1242 is compared with DDT in Table 2, showing this PCB to be a better inhibitor of Na^+-K^+ATPase, but poorer on mitochondrial Mg^{2+}ATPase.

The in vivo studies were conducted with fat-head minnows, *Pimephales promelas*, which were chronically exposed to Aroclor 1242 and 1254 for four months following hatching. Comparisons of ATPase activity showed considerable inhibition and some stimulation over a concentration range in which fish were exposed to 0.31, 0.93, 2.8 and 8.3 ppb. Aroclor 1242 compared with Aroclor 1254 showed the greatest inhibition of Mg^{2+}ATPases, as it had in in vitro studies. The inhibition in the chronic in vivo study was greatest on mitochondrial Mg^{2+}ATPase from kidney (Table 3). Inhibition of mitochondrial Mg^{2+}-ATPase was also greatest in brain and liver homogenates. Comparisons with muscle homogenates were not feasible in the small, chronically exposed minnows. The result contrasted with the inhibitory effect on oligomycin-insensitive

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Mg^{2+}ATPase which occurred in vitro. A maximum inhibition by Aroclor 1242 in treated fish (in vivo) was 75% on mitochondrial Mg^{2+}ATPase and 35% on Na^+-K^+ATPase. In contrast, Aroclor 1254 did not inhibit mitochondrial Mg^{2+}ATPase in kidney but gave 30% Na^+-K^+ATPase inhibition in the group of fish treated with 8.3 ppb (3). Results from brain tissues showed more consistent inhibitory effects than did other tissues.

Overall, PCB-treated fish showed more variation of enzymic effects than in vitro determinations. Thus, although the precision of the results was greater in vitro than in vivo, both approaches seem desirable for assessing the mode of action. In both cases the greatest effects were on Mg^{2+}-ATPase, with lesser effects on Na^+-K^+ATPase. Of the Aroclors studied, those in the intermediate range of chlorination appeared to have the greatest inhibitory effect on the ATPase system. Aroclor 1242 was a particularly good inhibitor.

Conclusions

The ATPase enzyme system in fish is adversely affected by all PCBs studied. Mg^{2+}ATPase is inhibited to the greatest extent in muscle, but effects in kidney, brain, and liver also occurred. Fifty per cent inhibition occurred with less than 1 ppm up to a few ppm. The greatest effects, in vitro, were on oligomycin-insensitive Mg^{2+}-ATPase, but on oligomycin-sensitive (mitochondrial) Mg^{2+}ATPase in vivo (chronically-exposed fish). The most effective PCB studied, Aroclor 1242, required a somewhat higher concentration than DDT in comparative in vitro studies.

Acknowledgements

Research support by grant of FWPCA, Environmental Protection Agency, 16030 ELZ.

Chronically exposed fish were obtained from experiments from the National Water Quality Lab., Environmental Protection Agency, Duluth, Minn. through the courtesy of Dr. Alan Nebeker. Monsanto Company kindly supplied the Aroclor preparations.

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Studies on the Mechanism of Toxicity of DDT and Polychlorinated Biphenyls (PCBs): Disruption of Osmoregulation in Marine Fish

by W. B. Kinter,* L. S. Merkens,† R. H. Janicki,† and A. M. Guarino‡

In 1970 studies were initiated at the Mount Desert Island Biological Laboratory to explore mechanisms of toxicity underlying the high sensitivity of bony fish, teleosts, to organochlorine pollutants. The starting point was several reports that DDT [1,1,1-trichloro-2,2-bis (p-chlorophenyl) ethane] inhibited Na,K-ATPase (Na^+, K^+-activated adenosine triphosphatase), as well as the knowledge that this enzyme appears to play a central role in osmoregulation by marine teleosts. In the face of a desiccative environment, these fish maintain body fluid hypotonicity (Fig. 1) by drinking sea water, absorbing water coupled with salts across the intestinal epithelium, and eventually secreting the NaCl across the gill epithelium while retaining the free water. The primary driving mechanism in both intestine and gill is the Na pump with which Na,K-ATPase appears to be intimately involved. For fuller discussion, see (2) and (3).

After demonstrating that DDT did, in fact, inhibit Na,K-ATPase activity in homogenates of intestinal mucosa and gill filaments from several marine teleosts (2), attention was focused on the sea water-adapted eel (*Anguilla rostrata*), in which osmoregulation has been well investigated. This eel can also adapt to fresh water and can be used for future DDT studies.

* Studies conducted at Mt. Desert Island Biological Laboratory, Salisbury Cove, Maine 04672.

† SUNY Upstate Medical Center, Syracuse, New York 13210.

‡ To whom reprint requests should be sent: Dr. A. M. Guarino, Bldg. 10, Rm. 6N119, National Cancer Institute, Bethesda, Md. 20014.

Materials and Methods

Eels, *Anguilla rostrata*, and killifish, *Fundulus heteroclitus*, were captured in estuaries along the Maine coast. They were adapted to and maintained in sea water for about three weeks before use. Non-radioactive puriss grade p,p′-DDT was procured from Aldrich Chemical Co., Milwaukee, Wis. ^{14}C-DDT was obtained from Amersham/Searle, Arlington Heights, Ill. and had a specific activity of 24 mCi/mM.

Aroclor 1221 was donated by Monsanto Co., St. Louis, Mo. Plasma milliosmolarity was determined by a standard freezing point depression technique, while Na^+ and K^+ were analyzed by means of a flame photometer. Levels of ^{14}C-DDT were determined as described previously (4). The fractional ^{22}Na space of isolated gills was measured according to the procedure of Kamiya (5) where eel gills are incubated for 1 hr at 15° in oxygenated sea water. Control gills were incubated with the solvent 0.5% N,N-dimethylformamide (DMF), and experimental gills were exposed to 50 ppm DDT in 0.5% DMF. Gill homogenates were assayed for Na,K-ATPase activity as described previously (2). Acute toxicity and osmoregulatory studies were conducted in killifish using a simple static system. For each experiment ten sea water-adapted fish (5 males and 5 females, each weighing about 5 g) were placed in an aluminum or enameled metal container holding 2 liters of sea water and maintained at 14–16° throughout the exposure time. In control containers 2 ml of ethanol were added to the sea water; in experimental containers Aroclor 1221 (mixture of PCBs

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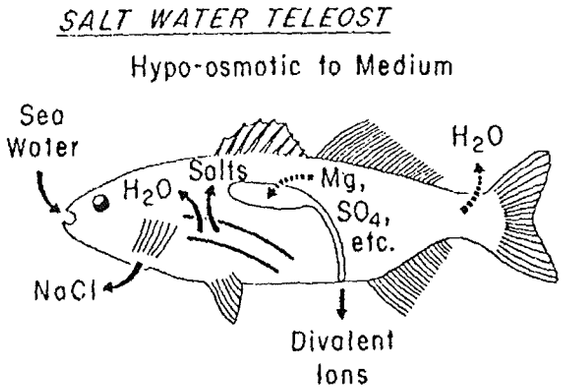

FIGURE 1. Water and salt transport in a salt water teleost. Typically the milliosmols/L are 1,000 for sea water and 350 for fish body fluids. Adapted from (1).

with 21% chlorine content) or p,p'-DDT was first dissolved in the ethanol. These solutions formed a cloudy suspension when added to the sea water, and in addition some separation of the Aroclor was noted, making the actual concentrations unknown (Aroclor 1248 was not tested due to much greater separation). Blood was drawn by cardiac puncture with lightly heparinized glass capillary tubes. Whole blood from 3 to 5 fish was pooled and centrifuged. The 0.05–0.1 ml of serum was diluted, and the osmolarity and concentration of Na and K ions were measured as for the eel.

Results

Ability to osmoregulate was followed in individual eels (about 100 g) placed in 15° sea water (2 liters) containing 0.1% ethanol and 1 ppm DDT, a level which is fatal by 10 hr. In Table 1 it is seen that, at 6 hr, plasma milliosmolarity of

Table 1. Effect of p,p'-DDT on plasma osmolarity, sodium and potassium in sea water adapted eels (*Anguilla rostrata*).*

Treatment	Osmolarity (mosmols./L)	Na^+ (mEq/L)	K^+ (mEq/L)
Control	358±10	163±2	2.4±0.3
p,p'-DDT	462±10†	192±3†	5.4±0.1†

* Mean±SE for 5–6 animals exposed to 1 ppm of DDT for 6 hr.

† Significantly different ($P<0.01$) from controls.

Table 2. ^{14}C-DDT levels in sea water adapted eels (*Angullia rostrata*).*

Tissue	DDT levels, ppm
Plasma	17.2±3.0
Gill	22.2±2.2
Gut mucosa	6.8±0.8
Brain	11.2±1.0

* Mean±SE for 6 animals exposed to 1 ppm of ^{14}C-DDT for 6 hr.

Table 3. The effects of DDT on sodium space and Na,K-ATPase in gills from sea water adapted eels (*Angullia rostrata*).*

Treatment	Na space†	ATPase activity‡
Control	0.112±0.010	0.37±0.07
DDT, 50 ppm	0.162±0.010§	0.23±0.03§

* Mean±SE for 15 gills in each group for Na space determination and 5 duplicate assays per group for ATPase assays.

† Fractional Na space after incubation at 15° for 1 hr expressed as ^{24}Na gill filament to media ratio.

‡ μmoles Pi/mg protein×hr.

§ Significantly different ($P<0.01$) from controls.

Table 4. Summary of DDT effects on osmoregulation in sea water eels.

Study	Results: Gut	Results: Gill
In vivo at 6 hr (1 ppm in water)*	7 ppm DDT Plasma osmolarity	22 ppm DDT increased 29%
In vitro (50 ppm in media)	H_2O absorption from sacs decreased 47%†	Na content of isolated gills increased 45%
Homogenate (50 ppm)	Na,K-ATPase inhibited 63%†	Na,K-ATPase inhibited 38%

* DDT solubilized with ethanol (0.1% in sea water) or N,N-dimethylformamide (0.5% in media and 5% in homogenate).

† Calculated from ref. 3.

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Table 5. Acute toxicity of Aroclor 1221 and DDT in killifish.

Compound	Initial level* in sea water	Total number of fish	% Dead (cumulative)			
			Day 1	Day 2	Day 3	Day 4
Control (ethanol)		10	0	0	0	0
Aroclor 1221	7.5 ppm	10	0	0	0	0
	25 ppm	10	50	80	80	80
	75 ppm	50	88	96	98	100
DDT	0.025 ppm	10	0	0	0	0
	0.075 ppm	10	40	50	50	50
	0.25 ppm	10	60	80	90	90
	0.75 ppm	10	100	100	100	100

* See description of static system in text.

DDT-treated fish had increased about 30% compared to ethanol controls. Furthermore, in this table one can see that, while much of this increase was due to plasma Na, plasma K also doubled in these animals. In additional experiments with 1 ppm ^{14}C-DDT, the fish had taken up about half the radioactivity by 6 hr. The levels of radioactivity for some tissues are indicated in Table 2 and ranged from 7–22 ppm. Using isolated eel gills the fractional ^{22}Na space was found to be increased significantly (Table 3) by 50 ppm DDT. Data in Table 3 also shows that Na,K-ATPase activity was significantly inhibited (38%) by 50 ppm DDT.

Considered all together (Tables 1, 2, and 3 and summarized in Table 4), our eel data provide circumstantial evidence that disruption of osmoregulation is a primary toxic effect of DDT, *i.e.*, the DDT levels in gut and gill of fatally-dosed fish were high enough to have affected osmoregulatory Na transport under in vitro conditions. Both water absorption from gut sacs and Na content of isolated gills are directly dependent on Na pump activity and can be altered similarly by obtaining a highly specific Na,K-ATPase inhibitor. Moreover, from the published (3) dose-response curve for gut homogenate, 7 ppm DDT in mucosal tissue should still inhibit Na,K-ATPase activity

Table 6. Effects of Aroclor 1221 and DDT on serum osmolarity, Na and K in killifish.

Compound and initial level	*mosmols*/liter	Na^+ mEq/liter	K^+ mEq/liter
Untreated	352±2 (18)	173±2 (16)	3.6±0.2 (16)
6-Hour exposure			
Control	365±3 (6)*	171±2 (6)	4.3±0.2 (5)*
Aroclor 1221			
75 ppm	396±7 (9)†	173±3 (9)	4.4±0.3 (9)
DDT			
0.25 ppm	389±9 (8)†	178±4 (9)	4.4±0.3 (9)
24-Hour exposure			
Control	361±7 (6)	176±2 (6)	5.3±0.5 (6)*
Aroclor 1221			
25 ppm	374±7 (6)	179±4 (6)	4.5±0.3 (6)
75 ppm	407±10 (4)†	189±5 (4)†	5.0±0.4 (4)
DDT			
0.075 ppm	368±3 (7)	180±5 (5)	4.1±0.4 (6)

* Significantly different ($P<0.05$) from untreated controls.
† Significantly different ($P<0.05$) from ethanol-treated controls.

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by 45%, provided, of course, that tissue content on a wet weight basis is equivalent to homogenate content based on added DDT.

The data in this same curve also indicate that Mg-ATPase is less sensitive to DDT than Na,K-ATPase. Certainly, until more information is available, the present data would appear to provide a partial explanation as to why a whole body burden of less than 10 ppm DDT is often lethal to fish. It is also recognized that DDT may exert a primary toxic effect on the central nervous system; by 6 hr our fatally-dosed eels were hyperactive and easily stimulated to convulsions by touch. Such symptoms, however, could be secondary to alterations to osmolarity of plasma and cerebrospinal fluid.

Last summer, because of the chemical similarity between the insecticide DDT and the industrial PCBs (polychlorinated biphenyls) pollutants in the environment, acute toxicity and osmoregulatory effects were compared using killifish. The toxicity data, Table 5, show that, with respect to the acute lethal level in sea water, the Aroclor 1221 was less toxic than DDT by a factor of about 100. There was a "dose-response" in terms of the theoretical concentration in sea water and number of deaths for both Aroclor 1221 and DDT, but the actual dose which the fish received is unknown since the tissue levels were not measured.

The osmolarity and Na concentration of the serum (Table 6) consistently increased (toward sea water values) in fish exposed to lethal levels of either Aroclor 1221 or DDT. The Aroclor and DDT levels selected for these serum-composition studies were those that were potentially lethal to most of the fish, but low enough to have killed only some at the time (6 or 24 hr) samples were taken from those still surviving. With the higher levels tested, most of the increases in serum osmolarity and Na were statistically significant when compared to ethanol controls.

Also, serum osmolarity and K were elevated in all ethanol-treated controls ($P < 0.05$, except for 24-hr osmolarity); this elevation may have been due to the ethanol or to confinement of the fish in the experimental containers. Additional data (Table 7) show that in the extreme cases, when blood was sampled before the fish died but after they had completely lost their righting ability and remained sideways, the serum osmolarity and electrolyte concentrations were all increased well above normal. It should be noted that the osmolarity changes (Tables 6 and 7) cannot always be accounted for by changes in measured electrolytes.

Table 7. Osmolarity, Na and K in individual pooled samples of killifish serum.*

Compound and level	Exposure hr	*mosmols* / liter	Na^+ mEq/liter	K^+ mEq/liter
Aroclor 1221				
75 ppm	23–32	550	208	8.1
		428	216	7.3
DDT				
1 ppm	9–10	430	207	8.3
		440	210	8.9

* Each sample represents serum from about 3 fish.

Discussion

Overall, these results suggest that lethal levels of Aroclor 1221 and DDT both decrease the ability of killifish to osmoregulate to about the same degree. Also killifish and eels seem about equally sensitive to DDT. Thus, inhibition of Na,K-ATPase by appropriate levels of Aroclor 1221 was anticipated. Using a homogenate of King O'Norway (*Hemitripterus americanus*) intestinal mucosa 250 ppm of Aroclor 1221 inhibited the Na,K-ATPase by 51% and 22% in two experiments. Moreover, recently-published work by Yap et al. (6) showed that several of the Aroclor mixtures inhibited the enzyme from fish brain and kidney.

Concerning the molecular basis for Na,K-ATPase inhibition by DDT and PCBs, one may speculate that these lipophilic compounds interact with the phospholipid-activating component of this lipoprotein enzyme (7, 8). It is well known that many lipophilic compounds, e.g., cyclohexanone, inhibit the enzyme and evidence of proton interaction between DDT and a phospholipid, lecithin, recently has been reported (9). Moreover, other organochlorine insecticides, including endrin, also inhibit Na,K-ATPase from fish brain (10), and endrin has been observed to disrupt osmoregulation in both a marine and a fresh water teleost (11, 12). Thus, it seems reasonable to predict that many organochlorine

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pollutants will be found to disrupt osmoregulation in fish.

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Polychlorinated Biphenyls (Aroclor 1242): Effects of Uptake on *E. coli* Growth

by Julian E. Keil, M.S.*, Charles D. Graber, Ph.D.†, Lamar E. Priester, Ph.D.‡, Samuel H. Sandifer, M.D.*

Introduction

Polychlorinated biphenyls (PCBs), similar to DDT in structure and physiological effects (7), are of concern to the scientific community because of their ubiquity (8), persistence (11) and possible toxicity. The alleged lack of biodegradability of this environmental pollutant has caused alarm and curtailment of this useful chemical. Work as early as 1963 by Cope (3) indicated that DDT, another chlorinated hydrocarbon, might be metabolized by several species of bacteria. Subsequently, other workers (2, 4, 9, 10) reported degradation of this insecticide by bacteria, fungi, and marine diatoms. Unpublished work performed in our laboratory (5) also indicated possible metabolism of certain PCB components by *Cylindrotheca closterium*. With the thesis established that DDT was indeed biodegradable and with evidence that PCBs may be biologically converted, the authors postulated that human flora may play an important part in the metabolism of DDT, PCBs, and other ecological contaminants.

Experiments were performed to study the effects of PCBs in vitro on a facultative organism, *E. coli*, common to human intestinal flora. This bacterium was also selected because it is the prime indicator of fecal contamination.

Materials and Methods

The study organism, *Escherichia coli*, was selected, because it is a common autochthonous, human intestinal aerobe, easily cultured, and is an accepted standard indicator of fecal contamination

Initial work screened a wide range (0–1000 ppm) of PCB concentrations, against *E. coli* by use of impregnated paper filter discs similar to those used to test antibiotic sensitivity. Impregnated discs with PCB concentrations of 0, .001, .01, .1, 10, 100, and 1000 ppm were placed on tripticase soy broth (TSB) agar plates which had been inoculated with *E. coli*. Experimentation indicated that 6 mm diameter discs cut from number 2 Whatman filter paper would absorb .02 ml of the acetone solvent. The discs were dried prior to use.

For the first experimental series (I), 20 one-liter Erlenmeyer flasks, each with 500 ml. TSB, were inoculated with *E. coli*. Four flasks served as controls, 4 for each of 2 levels of PCB treatments, and 4 as acetone controls since acetone was the suspending vehicle for the toxicants. Additionally, 4 flasks were used as uridine controls. One flask, containing only media, was used as a growth check. Each flask was vigorously agitated every 2 hours during the first and last 8 hours of culture time.

At the conclusion of a 24-hour incubation period, flask contents were centrifuged, the resulting pellet lyophilized, weighed and assayed for PCBs, nucleic acids and tritiated uridine (^3H_U) uptake.

A second experiment (Series II), identical in treatments except for uridine, was conducted to confirm stimulation patterns observed in Series I. Series II measured lyophilized harvest weights only.

A third trial (Series III), identical in design to

* Preventive Medicine Section, Medical University of South Carolina, 80 Barre Street, Charleston, S. C. 29401.
† Microbiology Department, Medical University of S. C.
‡ Environmental Health Laboratories, State Board of Health, Columbia, S. C.

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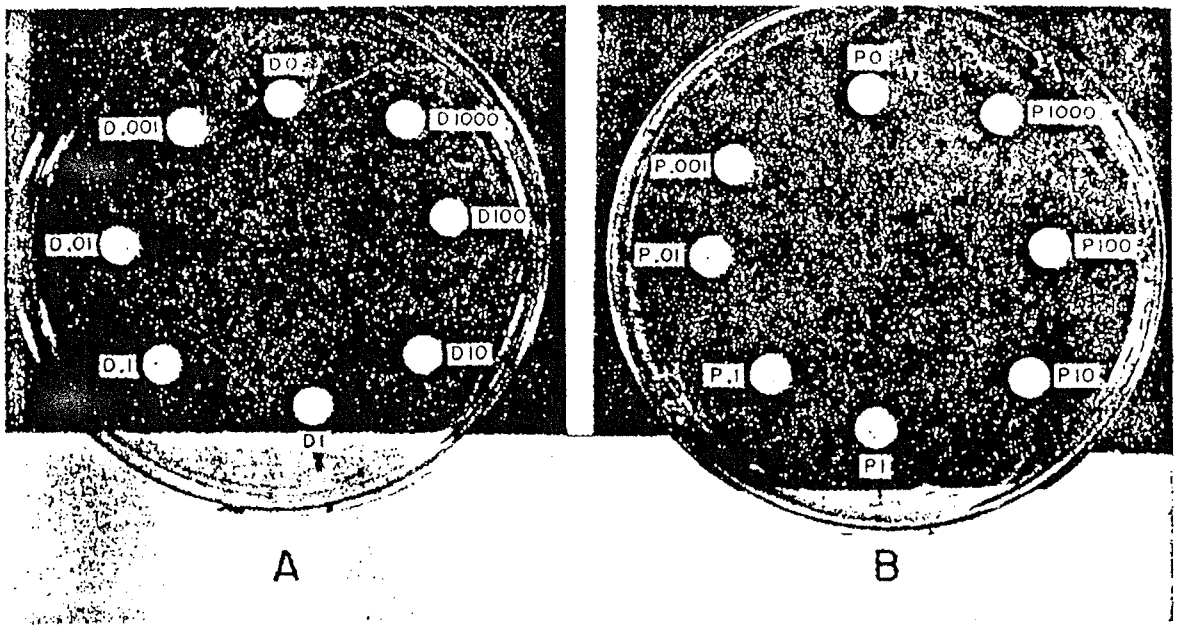

FIGURE 1. Sensitivity screening of *E. coli* on TSB to 0, .001, .01, .1, 1, 10, 100, and 1000 ppm of A, DDT; B, PCB.

Series I, assayed tritiated uridine uptake after 5 hours and 8 hours incubation time to determine if assimulation rates were affected by PCBs during the logarithmic growth phase. Aroclor 1242 (PCB—42% chlorine) was used as the source of active ingredients in these experiments.

Samples of bacteria were extracted with nanograde hexane and a 5 μl aliquot was injected into the gas chromatograph for analysis.

Two-column systems were used: a 6 foot × ¼ inch O.D. glass column packed with 3% SE-30 on 60–80 ABS chromosorb W and 2% QF-1 on 60–80 ABS chromosorb W. The operating parameters were: (1) inlet temperature 235°C; (2) column temperature 200°C isothermal; (3) detector temperature 240°C; (4) flow-rate 70 cc per/min. N_2.

Qualification was accomplished by measurement of the absolute and relative retention times of 5 major peaks of the PCBs. Quantitation was accomplished by determination of the area under the curve of the five major peaks. Utilizing this technique, recoveries of 92.4% of the PCBs were obtained.

RNA and DNA were measured using a modification of Agranoff's procedure (1). Radio assays utilized the Nuclear-Chicago Mark I Liquid Scintillation Spectrometer.

Table 1. Lyophilized Weights and Toxicant Uptake by *Escherichia coli* Cultures Exposed to PCB (Bacterial Series I).

Treatment	Mean[1] harvest weights (mgm)	Mean[1] residue (μg/g)
PCB .1 μg/ml	86.0	13.6
PCB .01 μg/ml	90.4	4.4
Control	44.5	0
Acetone control	27.2	1.2
Uridine control	41.6	0
LSD_{05}[2]	13.6	10.8

[1] Mean of four replicates.

[2] LSD_{05} = Least significant at 95% probability level calculated from one way analysis of variance.

Results and Discussion

As may be seen from Fig. 1, concentrations up to 1000 ppm of PCBs impregnated into filter paper discs failed to inhibit *E. coli* growth on TSB agar.

As shown in Table 1 (Series I), the addition of .01 and .1 μg/ml PCBs to TSB markedly stimulated *E. coli* growth above control levels. This is consistent with other microorganism work (4, 6) showing that DDT and PCBs are concentrated up to 1000 times by marine diatoms. The data also suggest that the concentration factor may be inversely related to dosage.

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Table 2 presents data from a duplicate experiment (Series II), wherein only harvest weights were studied. This second series confirmed apparent stimulatory effects of all levels of PCBs.

There were no differences between treatments in DNA and RNA levels after 24 hours' incubation. The significance of this is not clear, since it had been anticipated that increased RNA levels would accompany growth increases. However, it is thought that differences may have occurred earlier than the 24-hour time.

Data showing uridine uptake by PCB-treated *E. coli* at several phases of growth are presented in Table 3. No significant differences in uptake between treatments and controls were evident except at 5 hours incubation time, when there was a significant diminution of ^3H_U uptake in PCB-dosed bacteria. While not consistent with increased culture yields, the lack of change in uptake between chemically treated *E. coli* and controls does coincide with chemical assays indicating no increased levels of nucleic acids.

Results from these experiments indicate that PCBs stimulate in vitro growth of *E. coli*. If this phenomena occurs in vivo further concern is justified since any environmental contaminant which affects indicator organisms may result in a distorted portrayal of the actual situation.

Acknowledgements

This research was supported by the Community Pesticide Study of the Environmental Protection Agency—Contract #PH21-2017.

Appreciation is also expressed for technical facilities afforded by Training Grant No. AM05078-NIAMD and NIH General Research Support Grant 5420. Monsanto Company kindly supplied the PCB used in this work.

Table 2. Lyophilized Weights of *Escherichia coli* Cultures Exposed to PCB (Bacterial Series II).

Treatment	Mean[1] harvest weights (mgm)
PCB .1 μg/ml	186.4
PCB .01 μg/ml	153.2
Control	160.8
Acetone control	133.6
$LSD_{.05}$[2]	23.6

[1] Mean of four replicates.
[2] $LSD_{.05}$ = Least significant difference at 95% probability level calculated from one way analysis of variance.

Table 3. Tritiated Uridine Uptake by PCB Treated *Escherichia coli* Cultures During Exponential and Decline Growth Phases.

Treatment	Counts per minute[1]		
	5 Hours	8 Hours	24 Hours
PCB .1 μg/ml	4342	2250	2192
PCB .01 μg/ml	4756	2367	2462
Uridine control	8020	2675	2389
$LSD_{.05}$[2]	2800	979	859

[1] Mean of four replicates.
[2] Least significant difference at 95% probability level calculated from one way analysis of variance.

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The PCB Situation in Germany

by H. P. Tombergs*

A PCB problem has been known to exist in Germany since 1969. At the Swedish-German Symposium at Baden-Baden, Dr. Jensen, who first published about this problem in 1966, made a report entitled "PCB—the DDT Problem of Industrial Countries."

In September, 1970, there was a PCB conference in Stockholm. One of the reports given there mentioned that a fatal case of biphenyl intoxication had occurred in Finland. The person was a 31-year-old man who had worked in a plant which used biphenyl (not chlorinated) for impregnation. The exposure level was about 100 mg/m³—10 times the MAC (maximum allowed concentration). He had been working in this industry for 11 years and suddenly developed liver insufficiency—i.e. hepatic necrosis, atrophy, and cirrhosis. General atrophy of the brain cortex was also found. Medical examinations of the other 120 persons working in this plant revealed three additional cases with liver damage (biopsy) and two with abnormal EEGs and impaired peripheral nerve function.

In July, 1970, Acker (University of Münster) was able to find PCBs in human milk at a level of 0.103 ppm, which means 3.5 ppm in the milk fat. In human fat tissue he found an average of 5.7 ppm.

A recent German investigation on rats (Benthe, University of Hamburg) reports the absorption and distribution of PCBs after one inhalation exposure to Pydroul A 200, a mixture of low chlorinated biphenyls. Absorption as well as distribution were studied by measurements of PCB concentration in different organs. By this route very high absorption could be shown. Depending on duration of PCB exposure, a rapid increase in PCB concentration in the liver was observed. After 15 minutes the concentration was more than 50 percent of the maximum concentration attained after two hours (70 μg/g tissue). Concentration in fat after 30 minutes of exposure was 14 μg/g tissue (about 27% of the liver concentration), whereas in brain tissue, only 9 μg/g tissue (17% of the liver concentration) was found at that time.

Depending on the amount of time which passed after the end of aerosol exposure, a rapid decrease of PCBs in the liver during 24 hours was measured and was accompanied by an increase in brain and fat. In the course of two days brain and liver concentrations fell to a minimum value, whereas in fat, a maximum value was attained (260 μg/g tissue) which remained at a constant level in the subsequent period. There was no toxic fatty degeneration of liver under these experimental conditions.

Seven weeks after exposure to low chlorinated PCBs (Tetrachlor), the compounds were found in the fat of rats. After three weeks, 70 percent of the maximum value was still detectable in the fat, and it should be noted that, in a stress situation, PCBs move from fat tissue into the liver.

In addition to the inhalation route, studies were made of other methods of exposure. The results follow:

After intraperitoneal injection of PCBs, a stimulation of the microsomal enzyme systems which are responsible for the metabolism of drugs was found by measuring the O-demethylation, and the increased enzyme activity persisted for a long time. Even after four weeks, the values were still twice the value of the control animals. Because the microsomal enzymes also metabolize adrenal hormones, an effect on reproductive and adrenal hormones can be expected.

After long exposure (3 inhalations for 3 hours),

* West German Ministry of Youth, Family, and Health.

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a toxic effect on liver function was found: transaminase activity (SGOT, SGPT) had increased on an average by 50 percent. Parallel to this increase of transaminase activity, the liver fat increased from a normal 3.9% to 8.7%. We consider this a toxic effect.

After a subclinical dose of tetrachlorinated hydrocarbon (a dose which does not cause a measurable amount of liver damage) was given, the application of PCBs caused a high degree of liver toxicity; that means that, in the case of a subclinical liver damage, PCB absorption may be dangerous by causing liver dystrophy.

All these results will be published in detail in the next issue of the *Archives of Toxicology*.

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Comments on Research Needs

by Norton Nelson*

I will not attempt to summarize all of the excellent work presented at this meeting—such a summarization would presume expertise in all of the varied fields which have been represented here, an expertise I do not possess. I believe it will be more useful for me to attempt a very personal assessment of those gaps which I see as now confronting us, in an attempt to identify those that are significant and merit attack. In short, "Where should we go from here?"

First, a general statement. I was charged with a similar task last August at a conference convened by FDA on work done on PCBs within the federal government. The extent of additional work that has surfaced as the result of the present conference is remarkable. Clearly, many universities and governmental agencies are extremely interested in the PCBs and have been working intensely in the field in the last few years. I will follow the order of presentation in the present conference roughly, but will assume the liberty of regrouping where this seems desirable.

Analysis

Analytical technics have improved immensely in the last three to four years since the disturbing peaks resembling DDT metabolities were identified on chromatograph traces. It now appears that analytical procedures of considerable specificity are now available and quite widely used. The confusions and uncertainty of several years ago appear to have been largely mastered. The procedures are in some degrees overly tedious and have serious defects in respect to quantitation, especially because of the disparate response of some of the detector systems used in the gas chromatographic analysis. The latter has made quantitation of mixed isomers difficult and somewhat uncertain. Clearly then, there is a need for improved quantitation in respect to mixed isomers and equally room for improved efficiency of the analytical technics.

There is a very substantial gap in reliable analytical technics for impurities such as the chlorinated dibenzofurans. It appears fairly definite that such impurities are present in some preparations and may indeed significantly account for some of the toxicity. Accordingly, better means for identification and measurement of these impurities is required. Because they occur only in the part per million range in the PCBs themselves, which in turn occur in the biosphere in the part per million range, extreme and perhaps unattainable sensitivity would be required for their identification in environmental samples. It may, however, be very important to make the attempt. A closer and more attainable goal would be the development of adequate technics for detection and measurement of such impurities in the original PCBs themselves.

In this connection, it may be desirable to look for biological assays as at least an interim approach to this problem. Dr. Vos, in his excellent presentation, has presented work which suggests possibilities in the use of bioassays in the assessment of impurities in PCBs. This appears to be a very promising approach and should be pursued.

It is proposed below that selected purified isomers of the PCBs and their impurities be made available for toxicological studies. These would also be very useful in the further improvement of analytical technics.

Environmental Transport, Distribution and Alteration

Dr. Sarofim and Dr. Nisbet, in their paper, and

* Director, Institute of Environmental Medicine, New York University Medical Center, 550 First Avenue, New York, New York 10016.

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Dr. Risebrough, in his, have undertaken overall assessments of environmental sources, patterns of distribution and alteration of the PCBs. It has already been clear from earlier surveys reinforced by studies presented at this conference, that the PCBs are very widely distributed and are almost ubiquitous in their presence at many levels in the biosphere. It appears, however, that their distribution may be less uniformly global and more representative of local sources than DDT and its analogues. The approaches used in the two papers noted represent a very good beginning and serve to define, albeit perhaps only in an initial way, some of the major features in the overall PCB budget. Equally important, they serve to define gaps in our knowledge which may play decisive roles in developing a sound analysis of environmental distribution patterns. I note a series of items emerging as urgently needing clarification:

1. Further refinement of sources, including:
   a. Patterns of marketing and use. Improvement here will require improved access to such information from producers and distributors.
   b. Dumping practices.
   c. Stability of semisequestered sources, e.g., capacitors in dumps.
2. Better quantitation of discharge amounts by route into water and air.
3. Appropriate analysis to determine the relative role of vapor versus particle borne PCB in aerial transport.
4. Rain out data in selected regions to assess the atmosphere as a transport route, by season and by region. Allied to this is the need for appropriate selective analyses of dated snow deposits for the development of time trends.
5. Clarification of the importance of localized as opposed to generalized sources of PCB. As only one example of the many dilemmas, the Sarofim-Nisbet scheme requires mid-ocean dumping to explain some high PCB values far at sea. Are such sources important?
6. Clarification of the role of particle bound as contrasted to dissolved PCB in water transport.
7. Development of a more efficient monitoring strategy as a means of optimizin analysis and the development of globa distribution patterns of PCB's.

In approaching the global problem, one mus not overlook the importance of more localize systems of contamination. Again a beginnin towards this was illustrated in the paper of Dr Veith. Studies of well defined localities can pro vide not only knowledge relevant to the specifi units examined, but may permit extrapolation t other similar regions as components in the broade global scheme.

These presentations made very clear the seriou gaps arising from our ignorance of environmenta patterns of alteration of the PCB's and thei contaminants. This was brought out in the ex cellent report of Dr. Hutzinger on photolysis o the PCB's. Work such as this needs to be ex tended, and, at an appropriate point in thei development, brought into more realistic rela tionship to the form in which the PCB's are found in the atmospheric and aquatic environments, e.g., photolysis of PCB's as vapor, or particulates, and so forth. It is also very clear that attempts to deal with the transport and distribution problem are frustrated by lack of understanding of biological alteration of PCB's, that is, the metabolism of the PCB's by biological systems at all levels.

Occurrence

The insidious way in which PCB's have found their way into unexpected sources of exposure was illustrated by Dr. Kuratsune's report on carbonless copying paper. Although the statement has been made that substitutes for PCB's in carbonless copying paper have now been found, the completeness of this substitution will require monitoring. It may also be desirable to check body burdens of those routinely using forms with carbonless copying papers.

Similarly, the reports of Dr. Trout and Dr. Kolbye, on the presence of PCB in papers used in food packaging, further illustrate the need for vigilance for detecting and preventing the escape of these materials into unanticipated places. Although it appears that most of the major sources of PCB contamination in paper and cardboard have been identified and intercepted, this

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optimism needs to be tempered by continuing scrutiny of food packaging material.

The reports of Dr. Kolbye and Dr. Berglund, on the presence of PCB's in foods, suggest considerable similarity in the general patterns of food contamination in Sweden and the U.S. In each instance, fish, particularly fresh water fish, appear to be the largest dietary source. Milk has been found heavily contaminated on occasion. Hopefully, this is no more than episodic, resulting chiefly from the contamination of silage from PCB's in silo lining paints as described by Dr. Fries. This mode of contamination clearly needs careful scrutiny and correction. Control will be simpler with major milk producers than with the smaller units, and particular attention should be given to the latter. In a related matter, the possibility that human milk may be a significant source of exposure of nursing infants needs further examination.

The data from FDA is perhaps heavily biased by including a large number of samples secured as part of compliance studies. A more orderly picture of PCB distribution in food will require a more systematic sampling pattern and this should be undertaken. Similarly, food surveillance is now hampered by inadequate technics for detecting the presence of possible impurities in the PCB's. As noted above, resolution of this issue will require either chemical analytic technics many orders of magnitude more sensitive than are now available or bio-assays as suggested above.

Animal Toxicology

The Biotest studies are now complete and were well summarized by Dr. Keplinger. A somewhat parallel study at Chamblee is still incomplete (personal communication, Dr. Renate Kimbrough). Examination of the complete data from the Biotest and the Chamblee work will be required before a full assessment of these toxicity studies can be made. Several issues present themselves. The extreme sensitivity of the mink, both in lethality (1), and in possible reproductive injury (2), raises an alert which will require resolution before we can take too much comfort from results showing lower toxicity in other mammals. Regrettably, this conference did not include an updated report on studies of mink. Although the Biotest report indicates no evidence for PCB carcinogenicity, one (possibly two cases) of bladder cancer may have been identified in the Chamblee study (Personal communication, Dr. Renate Kimbrough). These findings will require that we await the completion of the Chamblee study and examine the Biotest reports in more detail before we can further clarify the question of malignancy.

Reproduction seems to be one of the most vulnerable functions in PCB poisoning. This is true in mammals as well as in other life forms, including both fish and birds. Again, the report on embryotoxicity in the rabbit (3) (not discussed here) suggests that this issue be further examined in mammals. It would also be desirable to relate reproductive effects to PCB burdens in wild populations.

It is a remarkable circumstance that despite the interest in the problem, the kinetics, distribution, metabolism and excretion of PCB in animals are only very scantily understood. There is some data on half time of body burdens in the fat in the cow (Fries) and in the rat (4) but the information is still quite inadequate. To jump ahead, it is unfortunate that such data did not emerge from study of the Yusho victims. These basic questions of metabolism, excretion and distribution need resolution and appropriate research should be put underway immediately.

The range of sensitivity to PCB is enormous, thus invertebrates show effects at levels of a few ppb (5). At the other extreme, E. Coli appear to thrive at thousands of ppm (Keil). Fish, birds and mammals fall between these extremes, with fish and birds appearing to be more sensitive than mammals. Again, reproduction appears to be the most vulnerable function. In respect to birds, and in contrast to DDT, it appears that hatchability may be more important than eggshell thinning.

This area of comparative toxicity from species to species is so large and uncharted that generalities seem out of place. It would thus seem desirable for those involved in the study of interdependence within the biosphere to plan carefully the necessary studies to clarify these issues of comparative metabolic patterns by species. As suggested above, such studies may be of great consequence for determining overall patterns of transport and the ultimate fate of these materials in the environment.

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A problem that persists in confusing our understanding of the toxicity of the PCB's is the unresolved question of contaminants and the different isomeric constituents of the various technical PCB's. This has led me to the strong conviction that it will be very desirable to select a modest number of representative isomers which should be synthesized and highly purified, and then carefully examined toxicologically. The selected isomers should be chosen on the basis of the best judgment available as being likely to typify not only "average" biological properties but also those corresponding to limiting or boundary biological actions in a qualitative as well as quantitative sense. Synthetic capabilities, we are told, are quite adequate to meet this challenge. Included in this should be well defined examples of the chlorinated dibenzofurans. Such an approach should permit a more rational planning of future toxicological work. These pure compounds will also be indispensable in the further development of selected bio-assays.

Mechanism of Action

Vos has described five distinctive patterns of pathologic outcome, acnegenic, liver damage, porphyria, edema and immunosuppression which he believes are differingly contributed to by different chemical components in the crude PCB preparations. As just noted, the availability of purified trace contaminants and isomers would permit an extension and refinement of this important approach to an understanding of pathogenesis.

A number of studies have thrown light on some intracellular actions; however, it can hardly be said, at this stage, that we have good suggestions as to the major underlying mechanisms of toxicity. Rather than to hastily examine the effect of PCB's on a myriad of in vitro test systems, it would seem wiser that these studies be carefully planned as aids to understanding the observed course of PCB poisoning in the intact animal.

Human Body Burden

The reports from Dr. Yobs and Dr. Price and the comments from Dr. Hammer all point to the ubiquity of the presence of PCB's in humans in this country. PCB's occur in the fat of all segments of the population and some gross features of population distribution have been identified. This work needs to be extended. In doing so, particular attention should be given to: 1) more representative population sampling, and 2) standardization on the basis of lipid content of the tissue. This latter appears to be especially important in relationship to plasma concentration. The lack of understanding of the kinetics of disappearance in humans hampers the interpretation of some of this data. Studies on primates could help clarify this issue.

Yusho

Dr. Kuratsune has presented us with a remarkable example of the quick rallying of medical and scientific talents to clarify the unfortunate accident leading to human PCB poisoning. The thorough and thoughtful studies of the staff of Kyushu University have provided excellent data on clinical effects and dose response in the Yusho episode. It is rare that such accidents have been so systematically studied. This data will necessarily play a major role in the assessment of the human hazard from the PCB's. We in this country should be deeply grateful for their skill and thoroughness in studying this episode. It would be extremely desirable to carry out detailed systematic, long term, follow-up studies, perhaps every five years throughout the life of the exposed individuals. We hope that their desire to make such follow-up studies for the assessment of possible chronic effects will be fulfilled. This country should be prepared to lend whatever collaboration it can in this very urgent matter.

General Comments

Extensive deposits of PCB's now exist throughout the biosphere. Even without further discharge, these deposits will remain for some years. It will be very urgent for us to develop information on distribution and degradation in order to reach some predictions as to the probable persistence of these deposits. The manufacturers of PCB in this country are now well alert to the problem and have announced a series of steps intended to restrict uses to those which are controllable. The success in achieving these reductions in usage will need monitoring. In addition,

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owever, the so-called "controlled" patterns of se, such as large scale transformers and capaci-ors, will in turn require monitoring against ccidental discharge and leakage.

Substitution in the less controllable uses is pparently underway. It will be very important n carrying out such substitution, not to replace known hazard with an untried, unknown, pos-ibly greater hazard. Accordingly, considerable are in effecting substitution will be needed.

The approach used in the above discussions of oncentrating on gaps—things not done as op-osed to those which have been done—brings he risk of implying that our ignorance is greater han it actually is. The many detailed and solid apers presented at this conference and as-embled here should serve as an adequate counter-alance against such misunderstanding. Never-heless, our ignorance is still too large. I have thus ried to identify some places where, I believe, we eed additional understanding. Finally, I apolo-gize for the necessity forced upon me by the shortness of time, of omitting from these remarks discussion of a number of important contribu-tions at this conference.

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☆ U.S. GOVERNMENT PRINTING OFFICE: 1972 O—455-263

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