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PCBs *and the* ENVIRONMENT

Volume II

John S. Waid

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PCBs and the Environment

Volume II

Editor

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Professor Waid's research interests at present are root biology (in particular the decomposition of grass and legume roots and release of legume-nitrogen to the soil, a study which has been supported by ARGC grants since 1977) and polychlorinated biphenyls in the environment and their degradation. The PCB work has been supported by grants from the Rural Credits Development Fund (1976-1978), the Victoria Ministry for Conservation (1979-1989), and the Australian Marine Sciences and Technologies Advisory Committee (1980-1981). He has also had contracts with the Victoria Ministry for Conservation, and the Federal Department of Science and the Environment to review problems associated with PCBs and the monitoring of hazardous chemicals in the environment. He has made surveys of PCBs in soils and waterways on behalf of a government department, an urban authority, and a large industrial organization (all unnamed), and is consulted regularly on matters pertaining to PCBs by the Victoria Environment Protection Authority.

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Chapter 1

POLYCHLORINATED BIPHENYLS: ACCUMULATION AND EFFECTS UPON PLANTS

H. K. Mahanty

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I. INTRODUCTION

Polychlorinated biphenyls (PCBs) are an important group of chemicals which have become dispersed in our environment mainly as a result of industrial uses.[1] Following detection of their presence by Jensen[2] in 1966, in water and soil, it has been considered that PCBs represent a major environmental pollutant. Thereafter, extensive studies followed to determine their widespread persistence[3] in the environment and to evaluate their toxicological and physiological effects on the biota.[4] This chapter summarizes the information available on the accumulation and effects of PCBs on higher plants and algae including diatoms.

II. TOXICITY OF PCBs TO HIGHER PLANTS

A. Absorption of PCBs from the Atmosphere

The first comprehensive report on the presence of PCBs in a diverse flora was made by Klein and Weisberger.[5] The samples were taken from two selected areas — one from an area of densely growing vegetation near a highway which was presumably an industrial area containing higher levels of PCBs and another from a remote area in the European Alps. The levels of PCBs in various samples are shown in Table 1.

The plants sampled from the motorway contained 0.09 to 0.95 ppm of PCBs, whereas from the remote alpine region a minimal value of 0.05 to 0.15 ppm was recorded and the authors concluded that PCBs were transported by air to the remote area. It is also interesting to note that the different plants sampled showed variable amounts of PCBs. For example, raspberry leaves from both areas contained approximately the same level of PCBs, whereas larch leaves near the highways contained a much higher level than those from the alpine region. Further study would be needed to determine the rate of absorption and retention of PCBs by terrestrial plants in high and low polluted areas. However, this study does establish the fact that PCBs are absorbed preferentially by different species.

B. Translocation of PCBs from Soil into Plants

Because of their numerous applications, including possible usage in pesticide formulation, it is possible that these compounds could be present in soils of agricultural or forest areas. Furthermore, the application of municipal and industrial sewage sludge is becoming an inexpensive method of fertilizing agricultural lands. Factors controlling the transport of PCBs and estimates of the amounts present in various ecological reservoirs are detailed in other chapters of these volumes. At this point, I would like to summarize the evidence for translocation of PCBs from soil to plants and the effects on plants when grown in environments contaminated with PCBs.

1. Absorption

Several studies have shown that these compounds can be translocated from the soil to various parts of the plants. Furthermore, the PCBs can accumulate selectively in particular tissues in higher quantity than in others. For example,[9,10] it was shown that of the residue applied over 97% was present in the peel of a carrot, thereby indicating little translocation to other tissues. Similar preferential absorption was also recorded in soybean sprouts.[8] Using a ^{14}C-2,2'-dichlorobiphenyl, Moza et al.[11] showed, in two aquatic plants, concentration factors of 814 and 289 when compared to the amount of radioactivity present in water. Thus, it can be concluded PCB can be translocated into plants not only from soil but also from aquatic environments.

2. Toxicity

The effects of PCBs on the growth and development of plants, especially higher plants,

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Table I
DISTRIBUTIONS OF PCBs IN TWO DIFFERENT LOCATIONS[9]

Near motorway		Remote alpine areas (Tegelberg or Hotensee)	
Plant	Dry weight (ppm)	Plant	Dry weight (ppm)
Moss	0.13	Moss	0.06
Heather	0.28	Heather	0.08
Raspberry leaves	0.15—0.27	Raspberry leaves	0.11
Larch leaves	0.95	Larch leaves	0.17
Rushes	0.12	Rushes	0.07
Hay	0.13	Fern	0.08—0.15
Grass	0.20—0.46	Juniper	0.07
Grass silage	0.40—0.42	Dwarf fir	0.09—0.10
Clover	0.33	Alder leaves	0.09
Mint	0.20		
Strawberry leaves	0.16—0.46		
Blackberry leaves	0.49		
Fir apex	0.31		
Oak foliage	0.50		
Pine leaves	0.11		
Water hemlock	0.23—0.57		

are not as well documented as for animals (see other chapters). It is feasible that since PCBs are lipophilic and less mobile chemicals their effects are difficult to monitor in the whole plant situation. However, a few studies have shown that these chemicals can interfere with the overall growth of the plant albeit, when applied at a much higher concentration than the available concentration in natural environments.[12,13]

These results which have been reviewed in a recent publication[47] are important in connection with the agricultural practice where soil is fertilized with industrial sludge which may contain very high levels of PCBs.[14] As mentioned earlier, root crops such as carrots and beets do tend to accumulate PCBs or their derivatives in the tissue without any visible damage and are thus more likely be used for animal consumption. It would be desirable to have information on other crops grown in fields fertilized with raw sewage in many countries of the Third World.

In a comprehensive study, Strek et al.[12] showed significant reductions in height, fresh top weight, and inhibition of cumulative water use in soybean and beet root at 1000 ppm Arocior® 1254, but not in corn and sorghum. They concluded that less phytotoxic damage is likely to result from application of sludge containing moderate levels of PCBs (1 to 50 ppm), but the above contention is subject to a major criticism. Industrial sludge is usually a mixture of PCB compounds, with a variable chlorine content and perhaps, derivatives of PCB isomers resulting from microbial action. The effects of these chemicals on plants could be totally different from those of laboratory-simulated studies in which a pure isomer (Arocior® 1254) has been used.

For example, Mahanty[13] showed in a free floating aquatic angiosperm, the duckweed *Spirodela oligorrhiza*, that a significant reduction in growth as measured by an estimation of fresh weight, occurred in the presence of less than 20 ppm of Arocior® 1242. The treated plants had smaller fronds and a characteristic chlorotic pattern.[13] This observation was later confirmed as interference by PCBs in photosynthesis.[14-16] However, the effects of PCBs on photosynthesis and cell division will be discussed later. This particular plant has added advantages in monitoring the effects of environmental pollutants in laboratory or simulated

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conditions. These duckweeds can be easily maintained on a simple medium contained in vessels which are resistant to PCB adsorption and growth can be determined visually by an increased number of colonies and simply counting the number of fronds in each colony.

The above discussion on PCBs in relation to higher plants suggests that PCBs when distributed by air could be transported to remote areas and can be absorbed and accumulated preferentially in different plants. They may be absorbed from the soil by roots and become accumulated either in the metabolized or original form. This ability to move within or outside the plant depends on the structure and degree of chlorination.

Higher chlorinated compounds are metabolized to lesser chlorinated biphenyls and other derivatives after absorption. These products are more easily translocated within the internal tissue of plants than in the form originally applied. So far, no toxicity studies have been made using these metabolites. This particular information could be a contributory factor in deciding whether PCBs, as available commercially, present a potential hazard to plants or not. It is well known from animal studies that chemicals which are apparently harmless externally may be converted to potential carcinogens by enzymes present in the liver. Similar mechanisms may exist in plants. It is also important to note that these intermediary compounds are also fairly stable and therefore are likely to be dissipated through the food chain to other ecosystems[9] where decomposer microbes could further modify these compounds for easy absorption and bioconcentration in higher trophic levels.[10] Production of crops will be reduced if PCBs are present in the field at a high concentration and this effect might be reduced by the use of activated carbon to adsorb excessive pollutant from the soil. In laboratory studies PCBs inhibited photosynthesis in higher plants at lower concentrations. This is a serious interference when photosynthesis is considered as a primary process for the fixation of the energy of the sun.

III. EFFECTS OF PCBs ON PHYTOPLANKTON

PCBs are known to accumulate in aquatic environments as a result of dumping sewage and industrial effluents, runoff from terrestrial ecosystems, as well as atmospheric fallout and rainfall.[10] Separate chapters in this publication are devoted to reviewing our knowledge of the distribution of PCBs. Phytoplankton form the basis of many aquatic food chains and consequently numerous studies have been made, both in laboratory and in natural ecosystems, to evaluate the likely influence of these chemicals on phytoplankton. In a separate chapter the accumulation and effects of PCBs on phytoplankton communities will be considered. Here, I wish to discuss the work done mainly on algae including diatoms as representatives of lower plants. The majority of these are laboratory studies, the results of which seem to reflect the effects of PCBs on plants in general.

A. Laboratory Studies of Effects of PCBs on Algae Including Diatoms

As mentioned earlier there are advantages in using algae and diatoms as test organisms since they are present in almost every aquatic environment and act as primary producers as well as a direct food source to animals inhabiting these ecological niches. Experiments can be designed to test all aspects of plant growth, and to test plant-environment interactions which are not possible on a large scale using higher plants. More importantly, sensitivity to PCBs can be studied at the very low concentrations which are present in our ecosystem. These results should reflect the likelihood of damage, if any, by the continuous presence of these chemicals. All our knowledge of the sensitivity of higher plants to PCBs comes from very high levels of the compound — ranging from 20 to 200 ppm of PCBs, whereas phytoplankton have been shown to be sensitive at 10 ppb or less.

1. Environmental Factors Affecting the Sensitivity of Algae and Diatoms to PCBs

The sensitivity of phytoplankton has been shown to vary with temperature,[20] geographic

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location,[21] interspecific competition,[22,23] cell density,[24] osmotic pressure,[25] and the presence of other toxicants.[26] Consequently, the health, abundance, and distribution of selective phytoplankton feeders on other animals in the food chain could be indirectly affected by the presence of PCBs. Laboratory experiments have shown that PCBs can be taken up from the growth medium and accumulated to higher concentrations by the marine diatoms, *Cylindrotheca closterium*[27] and *Thalassiosira pseudonema*[28] and the unicellular green algae *Chlamydomonas reinhardii*,[29] *Chlorella pyrenoidosa*,[30] and *Dunaliella* sp.[31] A similar preferential absorption has also been noted in higher plants. Thus, environmental factors coupled with the preferential absorption, retention, and tolerance of high levels of PCBs by individual species,[32] could confer a selective advantage over sensitive species. These laboratory studies also provide information useful for assessing the ultimate consequences of PCBs present in the environment, however low these concentrations may be. They still could have an impact on species composition in natural environments.

a. Effects on Growth

Studies on the growth strategies of algae and diatoms indicate the following patterns.

Most marine diatoms tested so far are found to be sensitive to PCBs at a concentration ranging from as low as 0.1 part per thousand million (10^9 or ppm)[26] and higher.[27,33,34] The highest concentration used to show the inhibition of cell division was at 100 $\mu g/\ell$, which is still far below the concentration tested in any higher plants. These levels of PCBs are certainly present in some waters near estuaries or in agricultural fields fertilized with industrial sewage sludge. It is possible that the extreme toxicity of PCBs to diatoms is due to their intricate cell wall development during which silica particles are absorbed from the media and secreted in a biologically active form to build the cell wall. It is also known that glass surfaces which contain silica can absorb considerable amounts of PCBs from the media.[35] Therefore, in contaminated water, silica particles are likely to be modified by PCBs and thus available to the diatomous communities in an altered form which could affect normal developmental processes of the cell wall.

The effects of PCBs on the growth of algae are somewhat different from those of diatoms. Unlike diatoms, certain algae can absorb and accumulate PCBs to a greater concentration than available in the growth media[29,30,36] and like marine diatoms certain algae show extreme sensitivity to PCBs. For example, Larsson and Tillberg[37] showed that the growth of the unicellular green alga *Scenedesmus obtusiusculus* was inhibited at 300 ppb PCBs and above but its viability was only affected at a concentration of 1000 ppb. By contrast, *S. quadricauda* was inhibited at 5 ppb and above,[36] and *S. obliquus* at 0.25 ppm.[26] Similarly, Mahanty and Grosshoff[32] showed the toxicity of PCBs as variable in two closely related species of *Chlorella*. One species was found to be extremely resistant while the other was sensitive. Sensitivity or resistance to PCBs was also dependent on the chlorine content of the PCB compound used. Thus, Craigie et al.[26] showed different responses by the same alga *Dunaliella tertiolecta* to different PCB mixtures. Lower chlorinated forms were more toxic than higher chlorinated forms.

b. Effects on Photosynthesis

There has been much speculation on the exact nature of the inhibition of growth of phytoplankton caused by PCBs in sensitive species. Nevertheless, there is general agreement that the observed inhibition of growth is symptomatic expression of some interference in phytoplankton physiology. One of these physiological processes is photosynthesis which appears to be disturbed by PCBs in almost all the studies made so far. Once again, it should be emphasized that the disruption of the photosynthetic process or the overall inhibition of growth was only recorded for sensitive species. Thus, the nature of sensitivity or resistance is not known clearly or is as yet a matter of speculation. Resistance could be inherited as

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a genetic factor as has been shown in *Scenedesmus obliquus*.[15] In any case, for the disruption of physiological processes such as photosynthesis or respiration, PCBs have first to enter through the phospholipid membranes of cellular systems.[40] Therefore the nature of the phospholipid membrane could determine species specificity to PCBs but more detailed knowledge is needed to prove this speculation. However, in sensitive species, if the molecules are capable of penetrating the outer membrane then it is likely that they also penetrate organelle membranes and interfere with major processes in photosynthesis and respiration. Thus, the most frequently reported physiological effect of PCBs on phytoplankton is a reduction of ^{14}C-bicarbonate uptake which suggests inhibition of the photosynthetic process.[24,29,34,41,42] However, some authors have reported a slight stimulation of ^{14}C-bicarbonate uptake and stimulation of respiration in certain species of algae.[18,34] One of these was *Chlorella vulgaris*[18] which appeared to be highly resistant to PCBs even at 200 ppm concentration.[32] Inhibition of photosynthesis, as measured by oxygen evolution, was recorded with isolated spinach chloroplasts (*Spinacia oleracea*). Decreased amounts of chlorophyll in PCB-treated phytoplankton[19,28,44] and characteristic chlorosis in *Spirodela oligorrhiza*[16] also suggested disruption or interference with the photosynthetic process in both sensitive phytoplankton and higher plants. Detailed knowledge of the effects of PCBs on photosynthesis of higher plants is still very limited and further information is needed.

c. Changes in Ultrastructure

Inhibition of photosynthesis by disruption of chloroplast membranes has been substantiated by a number of electron microscope studies.[17,34,45] In one of these reports, Mahanty et al.[45] showed that when sensitive species were treated with PCBs they showed disruption of the internal membranes of chloroplasts but not for *Chlorella vulgaris*, a freshwater alga which was treated with a high concentration of PCBs. Apart from inhibition of photosynthesis, another interesting abnormality was recorded in a sensitive species of *Chlorella*;[45] this was the loss of cleavage of the cytoplasm following nuclear division. This failure of daughter colonies to separate from parent colonies in the duckweed, *S. oligorrhiza*[13] could also be an interference in the cytokinetic process. Thus, the overall inhibition of growth, as discussed earlier, by the measurement of cell density in phytoplankton might possibly be attributed to interference in the process of cell division.[40]

IV. CONCLUSION

Terrestrial plants including certain crops show no general phytotoxicity symptoms caused by PCBs, either in natural habitats or in field studies. Uptake of PCBs from soil and water and accumulation to a higher concentration are possible, especially with lower molecular weight chlorinated hydrocarbons. Thus, higher plants could act as a temporary reservoir for PCBs or their derivatives for future dissipation.

Algae and diatoms are comparatively more sensitive than higher plants; however, the phenomenon of sensitivity or resistance appears to be species specific. Most marine diatoms are extremely sensitive to very low levels of PCBs, whereas algae may be sensitive and resistant. The resistant species are able to accumulate PCBs to a higher concentration than present in their environment. For sensitive phytoplankton, a lack of cell division, lower chlorophyll index, disruption of chloroplast membranes, and inhibition of photosynthetic processes are the recorded features of PCB interference.

Thus, preferential absorption and accumulation in certain phytoplankton and the deleterious effects on others could lead to an alternation on species composition even in a very low-level contaminated environment.

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Chapter 2

ACCUMULATION AND EFFECTS OF PCBs IN MARINE INVERTEBRATES AND VERTEBRATES

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I. DYNAMICS AND PATHWAYS

The accumulation of any anthropogenic substance by an organism from its environment involves two opposing processes, those of uptake and loss. Uptake occurs either by direct contact between the contaminant and the body of the organism or respiratory surfaces or through ingestion of contaminated food. Loss may occur with or without metabolism of the contaminant through the general body surface, through excretion of metabolic waste, through defecation of undigested material, through production of young, or voiding of other body tissues. The extent of contamination of an organism or a population at any time depends on the dynamic equilibrium between the processes of uptake and loss; these processes are illustrated thus:



The rate of adsorption of PCBs by marine organisms depends mainly on the amount and availability of PCBs present in sea water. Open ocean values for subsurface waters during the 1970s ranged up to 10 ng/ℓ with values as high as 40 ng/ℓ reported for the surface film.[1-8] Some continental seas were more contaminated, with levels up to 20 ng/ℓ in subsurface water and up to 2 μg/ℓ in surface films.[9-11] The worst cases of pollution have been reported near shore, as is to be expected, near point sources of industrial or domestic discharges; such values may approach 275 μg/ℓ.[12-14] These levels exceed the experimentally determined solubilities of commercial PCB formulations in sea water.[15-17] Marine biota from various habitats are therefore exposed to a wide range of PCB concentrations, depending on whether they are open ocean, neustonic, neritic, or benthic dwellers.

A brief digression is required to discuss the availability of PCBs to marine organisms at concentrations reported in sea water. First, more highly chlorinated PCB components are less soluble in sea water[15,16] yet the quantification of concentrations in sea water, sediments, and biota is usually carried out by reference to the best matching commercial formulation.[17,18] Our present estimates of PCB contamination in nature are therefore less precise than are analyses of discrete compounds, and intercomparisons between regions or laboratories are more difficult. Second, PCB availability to organisms depends not only on their size and activity but on the quality, quantity, and size of competing inert particles because organochlorine compounds are very hydrophobic and adsorb readily to other surfaces.[19,20] The extensive surface area associated with marine particles of less than 1-μm diameter implies that most organochlorines should occur in particulate form.[21] Organochlorines are also incorporated into colloidal micelles believed to be formed by the humic substances which comprise more than 90% of the dissolved organic matter in sea water.[22-24] "Solution" is defined operationally in oceanography as passage through a 0.5-μm pore-size filter,[25] and this definition would not include the micellar structures; the point is illustrated by the fact that filtration does not remove experimentally added organochlorines from humic solutions.[24]

All the reported concentrations of PCBs in sea water have been determined either by the direct solvent extraction approach, or by concentration of the samples on resins such as Amberlite® XAD-2, polyurethane foam, activated carbon, or other absorbent.[2,7,9,26] Obviously, the direct extraction approach combines PCBs in solution with those bound to particulates, both living and inert, whereas the sorbent methods allow an unknown fraction of particulates and associated organochlorines to escape sampling.[27] As our estimates of PCBs in sea water include a fraction adsorbed onto particles, it is important to know

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something about the dynamics of PCBs between particles and larger organisms. Several authors have attempted recently to evaluate the uptake of PCBs by organisms directly from particulate fractions. Fowler et al.[19] have examined the accumulation of Phenoclor DP-5 by the sandworm *Nereis* separately from contaminated sediments and from sea water. They assessed the relative importance of both these contaminant sources in the Mediterranean by applying their experimentally determined concentration factors to ambient PCB levels measured in both sediments (110 ng/g) and the overlying water (1.5 ng/ℓ). They concluded that 99% of the total uptake by sandworms in nature would be derived from sediments. However, it is not clear from this study whether PCBs were taken up directly from the sediment through contact with the cuticle, indirectly via the pore water, or through ingestion of sediment and subsequent absorption through the gut. It is likely that the sediment pore waters contain higher PCB levels than the overlying waters through the chemical partitioning process, because sediments have in general PCB concentrations at least three orders of magnitude higher than those in overlying water.[19] Goerke[20] has experimentally determined that the sandworm retains on average 79% of ingested PCBs so this avenue can be important especially if growth and metabolic activity are high. Haller and Johnson[21] exposed fathead minnows to PCB-contaminated sediment, with and without direct access to the sediment. Although the rate of PCB uptake was greater in fish allowed to contact the sediment, the chromatogram patterns in both groups of fish were similar, despite the presence of more highly chlorinated components in the sediments. These results implied that PCB uptake was essentially a two-step process, from sediment to water and then to fish. Lynch and Johnson[22] exposed freshwater amphipods directly and indirectly to PCB-contaminated sediment with very similar results; however, they found that *Gammarus* accumulated the least amount of PCB when exposed to the finest organic sediments; thus, organic content was more important in limiting desorption of PCBs from sediments than was particle size. Harding and Phillips[23] found that PCBs associated with natural particulates were rapidly transferred to several species of phytoplankton, with equilibria being reached in a matter of hours. They concluded that either a two-step process occurred, or that PCBs were transferred to diatoms during a transient association with the particulate matter. The important point is that a dynamic equilibrium was established rapidly between particulate material, phytoplankton, and sea water.

In conclusion, the reported values of PCBs in sea water overestimate what is truly dissolved, but these values may be realistic estimates of the contaminant pool available for uptake by larger planktonic and pelagic organisms. Organisms living in sediments are exposed to much higher PCB concentrations in their surroundings then are pelagic biota, but we cannot distinguish whether PCB uptake by benthic infauna is through direct contact with sediment, or through pore water. Organisms living close to the bottom are probably exposed to some level of contamination intermediate between that of the water column and of sediments.

The literature contains many descriptions of the accumulation of waterborne PCBs by aquatic organisms, but unfortunately the results are rarely intercomparable. The mathematical basis of first-order kinetic models was formulated many years ago for radionuclide research.[24,25] Only recently have investigators begun to use single compartment models to describe and predict the accumulation of organochlorines by aquatic organisms from their environment.[26-30] These models assume that the rates of uptake and clearance are proportional to the organochlorine concentration in sea water [W] and in or on the organism [Ø], thus:

$$\frac{d[Ø]}{dt} = k_u[W] - k_l[Ø]$$

where k_u and k_l are the rate constants onto and/or into, and out of, the organism, respectively.

Both coefficients, as experimentally measured, incorporate a contaminant dilution factor which reflects growth of the organisms during the experimental exposure. We have already noted that the reported open ocean concentrations of PCBs have been consistently in the low nanogram per liter range during the past decade; thus, the assumption that sea water concentrations [W] are constant is justified. Many experimenters have shown independently that an exponential function best describes the clearance of organochlorines from aquatic organisms.[28,38,40,61] Both the basic assumptions in this model therefore appear to be reasonable.

In Table 1, we have compiled rate constants for organochlorine uptake and loss from various sources in the literature. We have included data pertaining to the DDT group of insecticides because of the limited data available for PCB dynamics in aquatic organisms. This is entirely justifiable, because of the close similarity of partition coefficients and bioconcentration factors among these organochlorines.[32,33] The most striking feature in this tabulation is the near constancy of the clearance rate coefficient, in spite of the range of compounds used and the other variables such as organism type and size and experimental conditions of temperature, salinity, and so forth. Even the route of uptake, either through ingestion of contaminated prey or direct uptake from water, appears not to be an important factor in governing the clearance rate constant. Laboratory studies show that clearance occurs mainly through excretion into the digestive tract, followed by elimination with fecal material in fish,[26] crustaceans,[37] and polychaetes.[38]

The values of the uptake rate constant range over three orders of magnitude (Table 1). In Ruzic's[39] formulation of the model, a surface-area term was used which is a prerequisite for describing the uptake of known hydrophobic substances. Harding and Vass[40] could not justify its use when modeling planktonic crustacea differing in surface area by an order of magnitude; however, the organisms listed in Table 1 represent a weight range of seven orders of magnitude. It is therefore worthwhile considering the extent of surfaces available for contaminant adsorption in the marine environment. Sheldon and his colleagues[59,60] have hypothesized that biomass is distributed equally between logarithmic size intervals in aquatic food webs:

$$[B] = a D^{-1},$$

where [B] is the concentration of particles in sea water (vol/vol), D is the equivalent spherical diameter of these particles, and a is a fitted constant. This hypothesis is applicable to organisms ranging in size from phytoplankton to commercial fish stocks.[61,62] The surface area present per unit volume of sea water [S] can also be expressed as a function of size:

$$[S] = bD^{-1},$$

where b is a proportionality constant. Organisms in motion are exposed to a continuously renewed supply of organochlorines. This point is important as organochlorines are believed to be adsorbed on contact. However, organisms ranging in size from zooplankton (where D is about 0.5 mm) to pelagic fish (where D = 100 mm) have been found to swim at speeds between 0.5 to 3.0 body lengths per second; the greater speeds occur during active foraging.[63,64] Thus, [S] per unit time is also proportional to D^{-1}, which means that the surface areas exposed per unit time of all pelagic fish weighing about 100 g would be about nine orders of magnitude less than that of all nannoplankters. Now, this difference is obviously overestimated because larger organisms have a greater surface area exposed than that assumed from a spherical shape primarily because of the convoluted respiratory surfaces. Respiratory surface is thought to be proportional to metabolic rate.[65,66] Metabolic rate is not directly proportional to weight,[66] and is described by the 3/4 allometric law

$$M \propto W^{0.75}$$

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Table 1

RATE CONSTANTS TAKEN OR DERIVED FROM LITERATURE FOR UPTAKE AND CLEARANCE OF PCBs AND DDT BETWEEN ORGANISMS AND THEIR ENVIRONMENT

Compound	k/day	k/day	Sources of OC	Temp (°C)	Species	Ref.
2,5-di CB	920	0.066*	Freshwater	23	Goldfish, *Carassius auratus*	42
2,2',5-tri CB	950	0.048				
2,4',5-tri CB	890	0.021				
2,2',5,5'-tetra CB	740	0.015				
2,3',4',5'-tetra CB	420	0.010				
Clophen® A50	—	0.018	Freshwater	21—23	Goldfish	43
Aroclor® 1254	510	0.017	Sea water	23—32	Spot, *Leiostomus xanthurus*	44
p,p'-DDT	1,147—1,206	0.024—0.026	Freshwater	19—23	Bluegill, *Lepomis macrochirus*	43
p,p'-DDT	—	0.015	Freshwater	18.5	Goldfish, *Carassius auratus*	46
		0.013			Bluegill, *Lepomis macrochirus*	
p,p'-DDT	—	0.004	Food	16	Rainbow trout, *Salmo gairdneri*	47
2,5,2',5'-tetra CB	—	0.008	Freshwater	11	Rainbow trout	48
2,5,2',5'-tetra CB	—	0.003	Freshwater	10—11	Rainbow trout eggs and sac fry	49
		0.047			Rainbow trout swimming fry	
2,2',4,4'-tetra CB	286	0.030	Freshwater	10—12	Rainbow trout muscle	38
p,p'-DDT	—	0.025	Food	20	Menhaden, *Brevoortia tyrannus*	50
Phenoclor® DP-5	25	0.032	Sea water	17—19	Sandworm, *Nereis diversicolor*	28
2,2'-di CB	—	0.027	Food	10	Sandworm, *Nereis virens*	41
2,4,6,2',4'-penta CB	—	0.010				
Aroclor® 1254	—	0.025	Sea water	24	Oyster, *Crassostrea virginica*	51
p,'-DDT	7,600—12,100	0.043	Sea water	5—6	Euphausiid, *Thysanoessa raschii*	40
	10,400—25,100	0.048			Copepod, *Calanus finmarchicus*	

* Poor fit.

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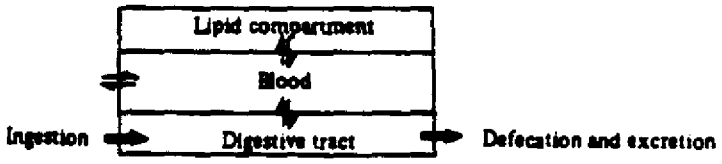
Thus, a 1-kg lobster, for example, has only about 5% of the weight-specific respiratory surface of a euphausiid weighing 10 mg. These considerations taken together, strongly suggest that direct adsorption from sea water must be a progressively less important route for the accumulation of organochlorines with increasing organism size. Marine mammals are a notable exception because their respiratory (adsorptive) surfaces do not come in direct contact with ambient sea water. The spherical model is the best approximation we have to predict organochlorine sorption through adsorptive surfaces until consistent techniques are developed to measure surface area directly.[65]

The importance of considering surface area available for adsorption on a unit-time basis is further illustrated by experimental studies which show that dead invertebrates and fish accumulate very low levels of organochlorines compared to their healthy counterparts.[67,68] Trout fry accumulated four times the concentration of tetrachlorobiphenyl than did sac fry, which in turn, accumulated seven times more than eggs;[69] these observations can be explained in terms of the greater surface area exposed by a swimming organism. Several studies have shown[33,60,70,71] that fish accumulate organochlorines at a faster rate at higher temperatures, within the normal thermal range of the species. In most cases, a doubling of contaminant levels in tissues is found with a 10°C rise in temperature. This corresponds to the "Q_{10}" law whereby a rise of 10°C doubles metabolic rates[72] and presumably, respiratory surface area exposed per unit time. Edgren et al.[71] found no measurable effect of temperature on organochlorine elimination, which corroborates the lack of variation found in the measured k_1 values (Table 1). Several authors[73,74] have shown that reduced salinity increases the uptake of organochlorines by fish from water. This too is probably related to the amount of water that comes in contact with body surfaces due to increased osmoregulatory activity. In general, then, the experimentally determined, organochlorine uptake rate-constants are a complex function of the exposed surface area; this explains why the measured k_1's are so variable between species and environmental conditions.

If we know little about the initial processes by which PCBs are taken up by marine biota, we know even less about how they are transported within the organism. It seems likely that organochlorines adsorbed to the external surfaces of marine organisms will partition into the hemolymph or blood plasma lipoproteins[75] and then would be transported to lipid-rich tissues, analogous to the experimental results following intravenous administration to invertebrates[76] and fish.[75,77-79] Several of these authors have reported blood plasma clearance curves of more than one phase, indicating the presence of more than one "compartment" in the organisms, clearing at different rates. Grzenda et al.[80] have shown in a thorough analysis that different tissues and organs in the goldfish have a different rate of DDT loss, but they could not prove this statistically.



See Ruzic[35] for mathematical formulation of all possible scenarios concerning the above structure. Our current level of experimental sophistication has not yet warranted the use of multicompartmental models.

Another important process by which organochlorines can be maintained at high levels in animal populations is through the transfer of residues accumulated over a lifetime from mother to offspring. (In this context, we should mention the largely unproven possibility that the known deleterious effects of organochlorines on developing embryos[81-83] could result in greater mortality of the first brood and therefore serve as a mechanism to clear populations

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of residues.) The documented high concentrations of PCBs and ΣDDT in the egg and developing embryos of starfish,[44] shrimp,[45] fish,[46-48] and marine mammals[49] are not surprising considering the affinity of these compounds for lipids. Butler and Schutzmann[50] present data which imply strongly that the female dogfish accumulates both ΣDDT and PCBs over an 18- to 20-year generation span, and then transfers these compounds to the ovarian egg and and mature fetus. Another illustration of this point is that whole body elimination of a PCB from experimentally exposed trout was more rapid in both sexes during the spawning season, and this appeared to be primarily due to the voiding of PCB-containing eggs and sperm. The great barracuda loses most of its abdominal fat during the development of gonads and is reported to lose most of its ΣDDT residues during the spawning period.[47] This internal redistribution of organochlorines has been observed experimentally in elevated organochlorine levels in eggs of both water-[51] and food-exposed[50] fish.

In a preliminary model the long-term importance of generation transfer of organochlorines was explored using the extensively studied marine copepod *Calanus*.[51] This work emphasizes the effect that increased generation length may have on the accumulation of organochlorines in animal populations. Slow developing populations in cooler climates are predicted to equilibrate at low bioconcentration levels over a longer period than tropical populations, which indirectly reflect differences in metabolic rate. Temperate populations with warm and cold water generations are also expected to equilibrate at different bioconcentration levels.

Organochlorines are also incorporated into organisms through feeding on contaminated food. A feeding term can be added to the kinetic model:

$$\frac{d[\emptyset]}{dt} = k_i[W] - k_j[\emptyset] + \alpha\, I_o[F]$$

where α is the organochlorine assimilation ratio from food, I_o is the weight-specific ingestion rate, and [F] is the concentration of organochlorine in the food.[42,93] This equation can be rewritten to span the maturation period from birth to adulthood, using the size dependency of ingestion rate as a function of time:

$$\frac{d[\emptyset]}{dt} = k_i[W] - k_j[\emptyset] + \alpha\beta t^{-\gamma}[F],$$

where t is time and β and γ are proportionality constants which depend on environmental temperature.[93] Several models have recently been described in the literature which use variations of this approach to simulate organochlorine residue dynamics in aquatic food webs.[94,95] In Table 2, we have collated all the best estimates of assimilation efficiencies of ingested PCBs and DDT by invertebrates and fish. These values are generally high and variable throughout the range of organisms and compounds studied. There appears to be no relationship between the amount of DDT or PCB retained and size of organism or trophic level. However, one study found that the assimilation of organochlorine decreases as food consumption increases.[93] When more than one level of contaminated food was offered to separate batches of experimental organisms, the results of different studies are conflicting with no clear overall relationship apparent between assimilation and the degree of contamination of food.[41,47,50,97,102,103] This result is to be expected unless the contaminant has a toxic effect on the organism at higher concentrations. In conclusion, it is not surprising that most of the modeling studies listed above have shown that food is the most important source of organochlorines to invertebrates and fish.

A lively controversy has persisted over the past decade about the relative importance of diet and direct uptake from water in the accumulation of organochlorines by aquatic organisms. Early field studies showed that organochlorine concentrations were greater at higher

Table 2
THE ASSIMILATION (α) OF INGESTED ORGANOCHLORINES BY INVERTEBRATES AND FISH

Compound(s)	Species	Av. size	Experimental: Temp (°C)	Experimental: Duration	Food conc (ppm) wet wt	Ingestion rate	α %	Ref.
3,4,3',4'-tetra CB	Coho salmon	2.9 g	11—14	108 days	10	1 % bd wt /day	56	96
2,4,5,2',4',5'-hexa CB	(*Oncorhynchus kisutch*)						39	
2,4,6,2',4',6'-hexa CB							35	
p,p'-DDT	Chinook salmon	3.2 g	10	65 days	6.2	—	56	97
	(*O. tshawytscha*)	2.5 g			25		19	
Tech. DDT	Coho salmon (*O. kisutch*)	6.5 g	10	39 days	6.2	—	48	
		5.5 g			25		46	
Aroclor® 1254	Rainbow trout	0.8 g	11—12	224 days	15	—	68	98
	(*Salmo gairdneri*)							
p,p'-DDT	Brook trout	15 cm	14	120 days	3	1.5% bd. wt/day	35	99
	(*Salvelinus fontinalis*)							
p,p'-DDT	Rainbow trout	15 g	16	168 days	0.2	2% bd wt/day	20	47
	(*Salmo gairdneri*)				1.0		24	
p,p'-DDT	Lake trout	30 g	10	152 days	2.5	6% bd wt/day	20	100
	(*Salvelinus namaycush*)							
Phaseolus® DP6	Grey mullets	56 g	—	30 days	50	—	71	101
	(*Chelon labrosus*)							
2,5-di CB	Goldfish	5 cm	23	150 days	10	1% bd wt/day	56	42
2,2',5-tri CB	(*Crassius auratus*)						49	
2,4',5-tri CB							60	
2,2',5,5'-tetra CB							53	
2,3',4',5-tetra CB							48	
p,p'-DDT	Atlantic menhaden	64 mg	20	48 days	0.00058*	2—3% bd wt/day	8—41	50
	(*Brevoorta tyrannus*)				0.009*		4—24	
					0.09*		8—26	
Clophen® A50,	Perch	12 g,	27	4 days	1.7	8% bd wt/day	75	102
p,p'-DDT	(*Perca flavescens*)	17 g			0.1	5.5% bd wt/day	80	
Aroclor® 1242	Crab	1—5 g	20	36 days	0.017	—	26	103
	(*Uca pugnax*)							

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2,5,4'-tri CB	Sandworm	5 g	10	21 days	≈1.5	3% bd wt/day	58	41
2,2'-di CB	(*Nereis virens*)				≈3		70	
2,4,6,2',4'-penta CB					≈4		91	
p,p'-DDT	Krill	2—10 mg	10	24 hr	—	21—49 *Artemia*/day	62	104
	(*Euphausia pacifica*)						76	
p,p'-DDT	Copepods	0.6 mg	1—6	24 hr	100—200	< 200 cells/hr	66	91
	(*Calanus finmarchicus*)					> 200 < 600 cells/hr	32—94	
						1000 to 2000 cells/hr	7—33	

* Dry weight basis.

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trophic levels and these observations were used as evidence for "food chain magnification".[106,107] Population turnover times, however, are closely related to organism size,[39] thus, this apparent biological magnification at higher levels in the food chain could also be explained by increased exposure times to aqueous organochlorines. Experimental field studies in which organochlorines were added to a pond or marsh have shown either that fish continue to accumulate residues, presumably through ingestion, long after aqueous concentrations have declined below detection limits,[36,108] or conversely, that an equilibrium is reached before residue levels disappear from the water column.[109] Laboratory studies have shown that fish and crustaceans accumulate proportionally more organochlorines from water than from feeding when their prey was previously equilibrated to the same water concentrations.[110-112] However, when laboratory fish were exposed to water and to prey contaminated at levels similar to ambient levels in Lake Michigan, direct uptake from water was found to be of minor importance.[99,100] Similarly, analyses of laboratory feeding and water sorption studies with pelagic copepods,[91] euphausiids,[99] and benthic polychaetes[28] have all indicated that the "food" source of contamination is the more important.

These contradictory results can be explained if uptake from water is unrealistically high in laboratory studies. This is entirely possible, because organochlorines are either added with a surface-active carrier[113] or maintained at near-saturation levels by desorption from sands.[43,114] In either case, organochlorines are more biologically available for uptake by adsorptive surfaces than is the case in adventitiously contaminated systems, where organochlorines are present in low "solution" concentrations and are strongly associated with particles or micellar structures. Scura and Thielacker[115] tried to evaluate the relative importance of these routes by exposing a three-level food chain to organochlorines which had been prepared in a physical state similar to that in natural sea water by bubbling air through cleansed sea water. They interpreted their results as indicating direct partitioning of PCBs from sea water to all three levels of the food chain, but their measured values were so close to detection limits, particularly in sea water, that the problem remains unsolved.

It appears that we have gone from one extreme to the other in ascribing organochlorine contamination of marine organisms first to food chain transfer, and more recently to direct partitioning between sea water and the lipid pool of the biota. A growing number of papers demonstrate the usefulness of the octanol-water partition coefficient in predicting equilibrium concentrations of organochlorines in natural populations.[32,35,116,117] However, we feel that it is an oversight to ignore the laboratory studies which clearly show the importance of the "food" route in favor of the attractive simplicity of a partition model. The equilibrium concentrations observed in nature can be reached in a much shorter time through feeding[118] and may explain why some fish[119] and marine mammal[120] populations have higher organochlorine concentrations on a lipid weight basis than have lower links in the food web.

II. EFFECTS OF PCBs ON MARINE BIOTA

In this section, we consider the effects of PCBs on invertebrates, fish, and mammals. In many cases, marine organisms have not been studied specifically, and to draw an integrated picture of the effects of PCBs we have had to infer effects on marine biota from studies of terrestrial or freshwater biota. We have considered effects under the general topics of biochemical responses and whole organism effects, i.e., in terms of different levels of biological organization.

A. Biochemical Effects: Mixed Function Oxidase Induction

One of the most intensively studied effects of the PCBs is their induction of mixed function oxidase (MFO) enzymes. Since MFO induction in marine biota has been studied mainly from a comparative biochemical viewpoint, we introduce the subject with a description

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(probably oversimplified) of MFO systems in general; this information is derived mainly from studies on the laboratory rat.

The MFOs are a group of membrane-bound enzymes located in the endoplasmic reticulum. They are usually studied in, but are not exclusive to, liver and they sediment at about 100,000 × *g*. They require a characteristic electron transfer cytochrome P-450, oxygen and NADPH and they are inhibited by carbon monoxide. They catalyze various reactions which usually convert a lipophilic substrate to a more polar product; the substrates may be endogenous (such as steroid hormones) or exogenous (such as certain pollutants). The MFOs are inducible especially by some organic pollutants such as polynuclear aromatic hydrocarbons (PAHs), certain organochlorine compounds including PCBs, and barbiturate drugs; this has led to the conclusion that one of their roles may be to act as a kind of latent defense mechanism which remains at a low level under normal conditions but which springs into action when the organism is stressed by (among other chemicals) certain pollutants, including the PCBs. Until recently, MFO inducers and MFO systems could be classified into two groups: (1) those systems which were inducible by compounds such as phenobarbitone, which required cytochrome P-450 and which catalyzed a specific suite of reactions including aniline hydroxylation and aminopyrine demethylation, and (2) those systems which were inducible by PAHs, which required a modified cytochrome P-448 and which catalyzed a different series of reactions typified by benzpyrene hydroxylation. It now seems that this classification was an oversimplification, as some compounds, including some PCB components, function as "mixed" inducers and induce both types of MFO system. In any case, this description applies to the rat, and MFO systems in marine biota may differ.

The phyletic distribution of MFO systems in marine biota has attracted some interest. MFO systems appear to be present in phytoplankton[121] but is not clear that they are inducible by, e.g., biphenyl, which is a component of the simpler PCB mixtures.[122] (Biphenyl hydroxylase activity is present in tissues from various marine invertebrates,[123] but we do not know whether or not this is a MFO-catalyzed process.) Some invertebrates such as crustacea and polychaete worms[124] have MFO systems which share many of the characteristics of mammalian systems; these features include similar cytochrome P-450-carbon monoxide difference spectra, a requirement for oxygen, and localization of the system in the microsomal fraction. The only report of MFO induction by PCBs in invertebrates refers to the worm *Nereis*, and induction is indicated only by increases in cytochrome P-450 concentrations and not changes in enzyme activity.[126] In bivalves, whose MFO systems have been studied as possible monitors of the effects of organic pollutants (a biochemical "mussel watch") it now seems probable that MFOs are present only in certain tissues;[125] whether they are inducible or not remains to be seen.

The induction of MFO systems in fish by PCBs has been more intensively studied. Most information has come from studies on the trout because it is a convenient experimental animal, but the limited data from marine fish generally support those from trout studies. The characteristics of MFO systems in fish differ from those in the rat: cytochrome P-450 has different spectral binding features[126] and neither phenobarbitone[127] nor the insecticide *p,p'*-DDT[128] (which is a "phenobarbitone-type" inducer in the rat[129]) induce MFO systems in trout. Furthermore, some PCB components can be identified as either specifically P-450- or P-448-inducing compounds in the rat[130,131] but this classification does not hold for trout.[131] PCBs, however, induce MFO systems in several species of fish including trout,[132-139] carp,[136,137] catfish,[138,139] and marine species such as sheepshead.[140] MFO systems in other marine fish are induced by PAH[141-144] and since in trout both PAHs and PCBs induce MFOs rather similarly[132,145] it seems probable that PCBs would induce MFOs in the additional marine species. However, the details of MFO induction vary considerably from species to species: Aroclor® 1254 will induce a dramatic increase in ethoxycoumarin-*O*-de-ethylase in cod[146] but only a moderate increase in trout,[147] whereas it will not change ethoxyresorufin-*O*-de-

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ethylase activity in cod, but induces it dramatically in trout. In general, however, one consistent sublethal effect of the PCBs seems to be to induce various components of the MFO systems in fish.

There is no evidence that MFO systems are present or are inducible in marine mammals. However, since MFO systems are ubiquitous in the terrestrial mammals so far studied, it seems probable that they are also present in marine mammals. MFO systems have been reported in sea birds.[148]

Finally, although PCBs induce MFO systems in at least the higher aquatic organisms, the adaptive significance of this is not clear. For example, it has not yet been shown — even in the relatively intensively studied field of MFO induction by PAH components of petroleum oils — that MFO induction enhances the ability of the organism to degrade or excrete a foreign compound. We do not know whether MFO induction leads to disruption of the steroid hormone system (as we might expect from considerations of the natural role of MFO systems) although low levels of PCBs are reported[149] to disrupt the normal steroid hormone biochemistry of cod.

B. Biochemical Effects: ATPase Inhibition

The ATPases are a group of membrane-bound enzymes which contribute to ion transport across membranes.[150] They may therefore assist in osmoregulation and excretion. Following the observation[151] that rabbit brain ATPases were inhibited in vitro by organochlorine insecticides there have been several studies of the effects of related materials, including the PCBs, on these enzyme systems.

The addition of various PCB mixtures to ATPase preparations from sunfish (*Lepomis*) tissues in vitro resulted in an appreciable ATPase inhibition, especially by Aroclor® 1254 and generally in brain, liver, and kidney tissue.[152] Similar effects occur in ATPase preparations from rainbow trout.[153] In both studies, PCB concentrations in the tens of parts per million were required to produce an effect. However, attempts to reproduce these effects in the minnow (*Pimephales*) exposed for 4 months to aqueous concentrations in the parts per billion range of Aroclor® 1242 and 1254 yielded results not as clear as those from in vitro studies.[154] Although some inhibition of ATPase was described, especially in kidney tissue of fish exposed to Aroclor® 1242, no statistical analyses of the data were presented nor were any analyses of the putative target tissue for PCB residues reported. More recent studies have shown that feeding of PCBs to rats can cause inhibition of ATPases in brain, liver, and kidney.[155]

ATPases are inhibited by a wide variety of lipophilic products which may include pyrethroid insecticides,[156] chlorinated diphenyl ether derivatives,[157] phthalate esters,[158] toxaphene,[159] and mirex and kepone derivatives.[160,161] This implies that the inhibition of ATPases may be rather nonspecific, and Sharom and Mellors[162] have proposed that PCB inhibition of ATPases arises from a physical interaction between the toxicant and the phospholipid component which is required by the enzyme.[163] Most reports describing the effects of PCBs on ATPases have dealt with concentrations in the enzyme preparation of around 10^{-3} *M*: i.e., in the parts per million (w/w) range. It is at these concentrations that PCBs could act as a physical toxicant by interference with membrane lipid components.[162] Such concentrations are reported in some lipid storage tissues (e.g., depot fat) of adventitiously contaminated marine biota, though whether they are reached in the enzymatically active tissues remains to be established.

One possible result of inhibition of ATPase activity is interference with ion transport and a change (at the tissue or whole organism level) in osmolality or osmoregulatory capacity. In practice, this may be difficult to detect, because osmoregulation may not depend exclusively on ATPase activity. Many of the studies of this subject deal with the insecticide *p,p'*-DDT rather than with the PCBs, but since both materials probably act similarly, we shall discuss them together.

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Exposure of grass shrimp (*Palaemonetes pugio*) to PCBs at the parts per billion level in sea water did not affect chloride ion levels in hemolymph or chloride exchange kinetics, at least in adults, but juveniles showed some disruption of these features which might have been associated with acutely toxic effects.[164] Exposure to PCBs did not affect hemolymph free amino acid levels[165] which in this organism contribute to intracellular osmoregulation. *p,p'*-DDT at sub-parts per billion levels in water reduced sodium and potassium levels in ashed hepatopancreas from a related penaeid shrimp.[166] Another genus of penaeid shrimp exposed to 3 ppb Aroclor® 1254 for 7 days and then exposed to salinity stress showed some response in hemolymph ions: at low salinity, total hemolymph cations were lower in treated shrimp than in controls but total hemolymph serum osmolality was not affected.[167] Taken together, the data suggest some interference of organochlorines, including PCBs, in ion transport, but this need not be reflected in tissue osmolality. It should be noted that although aqueous organochlorine concentrations in these studies were higher than those reported in "natural" circumstances, exposures were short and the tissue residue concentrations (where these have been reported) seem similar to those found in adventitiously contaminated samples.

ATPases of the rock crab (*Cancer irroratus*) were inhibited in vitro, and in vivo, following an injection of *p,p'*-DDT to yield hepatopancreas concentrations of about 1 ppm.[168] However, this crab is not an osmoregulator; even so, rather surprisingly, the possible effect of *p,p'*-DDT on serum osmolality was not described in this paper. In the blue crab *Callinectes sapidus*, which is a more effective osmoregulator, similar *p,p'*-DDT treatment produced a transient effect on serum osmolality after salinity stress, but 28 hr after transfer to low salinity water both ATPase and osmolality values had returned to levels similar to those in controls.[169] In other crabs, a similar general effect of organochlorines on ATPase activity, though not necessarily on hemolymph osmolality, has been observed.[170] The crab *Uca pugilator* exposed to Aroclor® 1254 and 1016 appeared not to be any more susceptible to salinity stress (in terms of acute lethality) than did unexposed animals; whether or not this crab osmoregulates is not specified.[171]

In fish, the impact of *p,p'*-DDT on ATPase inhibition is more clearly related to osmoregulatory effects. Marine teleosts drink continuously to replace water; intestinal mucosa and gill are both active in controlling ion transport. *p,p'*-DDT inhibits intestinal mucosal ATPase in *Fundulus heteroclitus* adapted to sea water and this is reflected in the increased (though variable) levels of plasma Na^+ and K^+; however, these increases resulted from a short and sharp exposure to *p,p'*-DDT.[172] Rainbow trout which had been fed *p,p'*-DDT showed reduced gill ATPase activity at three salinities, but in fresh water neither serum osmolality nor Na^+ was affected; at 33 and 100% sea water, both variables were significantly increased.[172]

Salmon injected with Aroclor® 1254 before smoltification showed no change in gill ATPase activity, even though appreciable concentrations (approximately 1 ppm) of PCB were deposited in the liver.[174] Since these analyses were carried out over a 6-week period, any transient effect due to sudden high concentrations of PCBs may not have been detected; alternatively, PCB concentrations in target tissues may have declined to the point where they were ineffective.

In summary, organochlorines in general, and PCBs in particular, affect ATPase activity, probably by acting as a physical toxicant. Although these effects may occur at environmentally realistic concentrations, ATPase inhibition may not be reflected in whole organism responses in lower biota, such as hemolymph osmolality changes; however, such transient changes may sometimes be detected in fish.

C. Miscellaneous Biochemical Effects

As well as inducing MFOs and inhibiting ATPases, PCBs have other biochemical effects which have been studied in less detail and which are more difficult to interpret.

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Relatively large doses of Aroclor® 1242 (8 ppm aqueous suspensions) reduced the dispersal of tissue melanophores[175] in the crab, *Uca pugilator*, through interference with eyestalk neurohormone release.[176] The net result was that the crabs became paler in appearance. PCBs may affect hormones in fish. Trout exposed to high dietary levels of Aroclor® 1254 showed no changes in serum thyroid hormone levels, although the organochlorine insecticide mirex induced such changes.[177] Cod (*Gadus morhua*) fed environmentally realistic doses of Aroclor® 1254 showed some disruption of steroid hormone biochemistry; the site of action of these effects was not identified, but it could presumably have been associated with MFO activity.[149]

The potential interaction of PCBs with lipid biochemistry has been reviewed;[178] in general, changes in lipid metabolism or in the fatty acid composition of lipids can be produced experimentally[179-180] though usually only with PCB doses far above those expected to be found in nature.

D. Whole Organism Responses to PCBs

The most commonly studied whole organism response has been death, in the form of measurements of acute toxicity (lethality). Acute toxicity data have been collated and reviewed elsewhere;[181-182] the main conclusions can be summarized as follows:

1. Acute toxicity varies considerably with the PCB preparation, with the species of organism tested, and with its age, sex, and condition
2. For what the generalizations are worth, acutely toxic concentrations (96 hr LC_{50}s) are usually in the microgram per liter range (parts per billion)
3. LC_{50}s in longer tests are usually lower than in short-term tests, which implies that PCBs are cumulative toxicants
4. For some species, especially marine invertebrates, the long term LC_{50}s may approach environmentally realistic concentrations (e.g., 0.01 $\mu g/\ell$); however, adventitious PCBs may not be as available biologically as those deliberately introduced in toxicity tests

Despite the potential lethality of some environmentally realistic PCB concentrations to some species, there is no unequivocal evidence that biota from adventitiously contaminated environments have been killed by PCBs.

There is more convincing evidence that adventitious levels of PCBs in some biota may cause sublethal reproductive effects. In freshwater fish, high PCB concentrations are often associated with abnormal fry development[183] and there is a high correlation between PCB concentrations in salmon eggs and fry mortality. However, the structure of many marine ecosystems is so variable (for natural causes) that it is not clear whether similar effects induced by adventitious PCB levels could be reliably detected.

Minnows fed PCB-contaminated food showed an enhanced growth rate, possibly as a result of hormonal disturbances.[184]

Probably the best documented evidence for an effect of adventitious PCB concentrations comes from studies of marine mammal reproduction. Very high levels of both the DDT group of insecticides and of PCBs have been found in Californian sea lions[185,186] and premature births are associated with the higher levels; it is not possible to assign these effects to any specific organochlorine. The affected animals had blubber organochlorine concentrations in the 1000 ppm (wet weight) range. In ringed seals from the Baltic (Bothnian Bay), uterine occlusions which lead to resorption of the fetus are correlated with high DDT group and PCB levels;[187] pregnant females had lower residue levels than had females with uterine occlusions; normal nonpregnant females had levels similar to those with uterine occlusions. The residue concentration differences between animals with successful and unsuccessful pregnancies were relatively small (around 100 ppm) but were statistically significant. A

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more recent report[148] suggests that reproductive failure is becoming more frequent, but this report does not cite any organochlorine analyses to which these effects could be attributed. In related studies, Reijnders[149,150] has suggested that the higher juvenile mortality and lower pup production in Dutch than in Danish or North German harbor seals could be attributed to the higher PCB (but not higher DDT-group) levels in the former group. (High levels of PCBs occur in the Dutch seal population as a result of riverine input from the Rhine.) As in the Californian sea lions, PCB concentrations in the apparently affected group were around 700 ppm, whereas DDT group concentrations were about one tenth of that.

In short, there are suggestions that the adventitious levels of PCBs (possibly aggravated by those of the DDT group) may interfere with marine mammal reproduction. However, symptoms vary from place to place and this may indicate different modes of action.

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Chapter 3

ACCUMULATION AND EFFECTS ON BIRDS

D. B. Peakall

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I. ACCUMULATION OF PCBs IN BIRDS

The accumulation of PCBs in birds can be studied both under field and laboratory conditions. The former studies have the advantage of applying to the real world but the relationship between prey and predator is usually assumed rather than known. Most species of fish-eating birds will shift to other food sources if available. This is particularly true of gulls. In any case, the sampling of the prey may well not be typical of the total food intake. In laboratory studies the input of PCBs can be carefully controlled. Few studies on birds are available on accumulation of specific isomers, although there is growing evidence that this is of importance.

Laboratory data for the accumulation of PCBs from known diets are given in Table 1. It is clear that there are wide differences between species. The commonly used laboratory species — chicken, quail, dove, and mallard show little bioaccumulation. In contrast, high coefficients of bioaccumulation were found for the two fish-eating birds studied (herring gull, white pelican); the one raptorial bird studied (American kestrel) shows intermediate values.

Isomer specific studies appear to be limited to those of Bunyan and Page.[1] The residue levels given by these workers are for the heart, an organ rarely studied by others so that comparisons are difficult. What is clear is the wide difference between isomers. The dichloro isomers show low residue levels as would be expected; however, there are marked differences between the four tetrachloros and two hexachloros studied. A further point of interest is the residue levels of those isomers that do accumulate, viz., 3, 4, 5, 3', 4', 5' hexachloro and decachlorobiphenyl are some tenfold higher than the overall figure for Aroclor® 1254.

II. PHARMACODYNAMICS OF PCBs

The uptake of Aroclor® 1254 at a high dose (500 ppm) into four organs (muscle, liver, kidney, and brain) has been studied in the chicken.[20] An essentially linear uptake over a period of 10 to 60 days was found. The ratios of the tissue are given in Table 2. Lincer and Peakall[3] studied the levels in eggs of ring doves exposed to 10 ppm Aroclor® 1254 and found a linear rise for approximately 100 days, after which the values began to approach an asymptote value.

The distribution of PCB on an organ basis is largely governed by the lipid content of the tissue, although the values obtained both in the laboratory[3,21] and field[22] (Table 3) indicate the levels in the brain are lower than other organs and that fat serves as the main depot.

The mobilization of organochlorines in birds exposed to food stress has been considered to be a factor in deaths of wild birds.[23] The "wreck" of guillemots in the Irish Sea in 1969 was linked to high PCB levels.[16] Subsequently, Parslow and Jefferies[24] studied the relationship between liver levels and total body burden in this species. They found a marked increase in the percentage of PCBs found in the liver of guillemots involved in the wreck (and having no body fat) compared to those shot. The increase in the load in the liver was from 0.9 to 22.5%. In both groups there was a strong correlation between liver levels and total body burden. In ring doves, Lincer and Peakall[3] noted a 70-fold increase in liver residue values when the birds were food stressed to the point that they had no body fat. No differential mortality due to PCB mobilization was observed in this experiment.

Anderson and Hickey[19] studied the seasonal variation of PCB levels in herring gulls and observed a diphasic response in juveniles and a triphasic response in adults.

III. ACUTE AND SUBACUTE TOXICITY

PCBs have a low acute toxicity to birds. The 5-day LC_{50} for four species is given in

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Table 1
ACCUMULATION OF PCBs IN BIRDS FROM LABORATORY EXPERIMENTS

Species	Aroclor*	Dose	Duration (days)	Residue level (ppm, wet weight)						Ref.
				Whole body	Fat	Muscle	Liver	Brain	Eggs	
Chicken	1260	400	60				210—340			2
(*Gallus gallus*)	1242	100	28		283					3
		200			427					
		400			1686					
	1254	100			515					
		200			783					
		400			1557					
	1260	100			482					
		200			1427					
		400			2159					
	1248	0 5	56		3 1				0 22	4
		1 0			6 6				0 41	
		10			57				3 1	
		20			83				7 0	
Japanese quail	1254	5	84	5			1 9			29
(*Coturnix coturnix*)		25		20			12 5			
		100		88			72			
Ring dove	1254	10	200		736	8 1	15 3	3 5	15 5	5
(*Streptopelia risoria*)										
Mallard	1242	25	28	55 (♀)					21	6
(*Anas platyrhynchos*)				64 (♂)						
Screech owl	1248	3	56	12 8					1 1	7
(*Otus asio*)			approx. 250						7 1	
American kestrel	1254	5	approx. 100						40	8
(*Falco sparverius*)										
White pelican	1254	144	90		2130		290	110		9
(*Pelecanus erythrorhynchos*)										
Herring gull	*	1	322	200						10
(*Larus argentatus*)										

* Fish diet averaging 2 ppm PCB.

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Table 2
TISSUE DISTRIBUTION OF PCBs

Species	Dosage	Levels in organs (ppm)						Ref.
		Muscle	Liver	Kidney	Brain	Heart	Fat	
Chicken	500 ppm Aroclor® 1254 Measurements at 50 days	108	285	470	145	—	—	20
Ring dove	10 ppm Aroclor® 1254	8	15	—	5 5	—	736	5
	Measurements at 200 days	172	1120	—	293	—	—	
Japanese quail	Aroclor® 1242	—	17	—	7 8	3 2	123	21
	Aroclor® 1254	—	28	—	7 7	7 0	304	
California g... (*Larus calif...icus*)	Field	—	0.79	—	0.29	—	18 87	22

Table 3
ACCUMULATION OF PCBs IN BIRDS FROM FIELD EXPERIMENTS

Avian species	Residue level in prey	Residue level (ppm, wet weight)					Ref.
		Whole body	Muscle	Liver	Brain	Eggs	
Great-crested grebe (*Podiceps cristatus*)	0.17 (0.16—0.18)	—	—	11	—	—	11
	0.12 (0.06—0.35)	32	—	—	—	—	
	0.8—(.3	—	—	—	—	13	12
Brown pelican (*Pelecanus occidentalis*)	0.19 (0.17—0.23)	—	—	—	—	2.2	13
White pelican	0.05—0.11	2.3	3.1	4.5	—	1 7	14
Double-crested cormorant (*Phalacrocorax auritus*)		3.6	2.3	2.0	—	5 7	
White-crested cormorant (*Phalacrocorax carbo*)	1.6	6.2	—	—	4 4	2.9	15
White-tailed sea eagle (*Haliaetus albicilla*)	0.03—0.18	—	150—240	130	29—70	—	16
Common guillemot (*Uria aalge*)	0.27 (0.01—1.0)	—	—	—	—	7.9—21	17
	0.01—2.0	3.4	—	0.4	—	—	18
Herring gull	2.2	—	—	—	—	124—157	19

Table 4.[25] There is steady increase in toxicity as the degree of chlorination increases, although the percentage of chlorine alone does not determine the toxicity at least for the Japanese quail.[26] There are substantial differences between the LC_{50} values for the bobwhite and Japanese quail, with the former species being some three- to fourfold more sensitive. But even the lowest LC_{50} given (604 ppm for Aroclor® 1260 in bobwhite quail) is high compared to organochlorine pesticides.

Subacute experiments also show that PCBs have low toxicity (Table 5). The data on the chicken indicate that the middle chlorinated Aroclor® 1242 is more toxic than either the lightly or heavily chlorinated mixtures. Nevertheless, levels of 200 ppm are required to cause appreciable mortality.

Hurst et al.[21] showed the cold stress potentiated mortality and that, under cold stress, there was a sex-related difference (males being more susceptible than females). Nevertheless, the dosage level was high (500 ppm causing mortality starting at day 20 under cold conditions). Lincer and Peakall[5] found no differential mortality when food stress was applied to ring doves.

The report on the seabird wreck in the Irish Sea in 1969[18] concluded that one of the most striking findings was the presence of unusually high levels of PCBs in the livers of dead

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Table 4
5-DAY LC_{50} FOR FOUR AVIAN SPECIES (ppm)[28]

Aroclor[a]	Bobwhite	Japanese quail	Ring-necked pheasant	Mallard
1221	6,000	12,000	5,000	5,000
1232	3,002	5,000	3,106	6,000
1242	2,098	6,000	2,078	3,172
1254	1,175	4,844	1,312	2,798
1260	604	2,898	1,091	2,699
1262	747	2,186	1,260	1,975

Table 5
MORTALITY ASSOCIATED WITH FEEDING EXPERIMENTS

Species	Aroclor[a]	Dosage (ppm)	Duration (days)	Mortality	Ref.
Chicken	1221	500	38	None	27
	1242	38	28	None	38
		75		Increased from 4 to 21% in chicks; no effect poults	
		150		Increased to 75% in chicks; no poults	
		500	38	40% mortality	27
		100	28	No mortality	29
		200		40% mortality	
		400		100% mortality	
	1254	100		No mortality	
		200		20% mortality	
		400		60% mortality	
		500	30	No mortality	27
	1260	100	28	No mortality	29
		200		No mortality	
		400		No mortality	
	1268	500	30	No mortality	27
Japanese quail	1242	250	20	No mortality	21
		5000	21	No mortality mentioned	30
	1254	250	20	No mortality	21
		1250	21	No mortality mentioned	30
	1260	1000	21	No mortality mentioned	
Bobwhite quail (*Colinus virginianus*)	1260	500		Mortality started at 20 days, complete by 40 days in cold; in heated building, mortality of males complete in 60 days, females in 120 days	31
Pigeon (*Columbia livia*)	1242	500	28	No mortality	21
Ring dove	1254	100	56	No mortality	32
Mallard	1242	150	84	No mortality	33
White pekin	1254	144	70	No mortality	9
American kestrel	1254	5	100	No mortality	

guillemots. High levels would be expected due to the mobilization of body fat and the levels reported (mean 56 ppm, range 10 to 200 ppm), while high, are not outside the levels measured in apparently healthy herring gulls on the Great Lakes (50 to 200 ppm).[34] Levels in the early to mid 1970s were, presumably, even higher. While the data on the effects on target species under environmental realistic conditions are poor, there is little evidence that toxicity of PCBs has been the primary cause of mortality in the field.

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Table 6
EFFECT OF PCBs ON REPRODUCTION IN THE CHICKEN

PCB	Dosage (ppm)	Duration (weeks)	Egg production	Hatchability	Ref.
1221	20	9	No effect	No effect	35
1232			765 69	94 57	
1242	2	9	No effect	No effect	
	5	6	No effect	No effect	36
	10		No effect	No effect	
	20		No effect	91 75	
	20	1	—	55 44 (Leghorns)	37
				59 43 (Broilers)	
		6	—	69 7 (Leghorns)	
				65 6 (Broilers)	
		9	76 66	94 18	33
	40	6	No effect	91 50	36
	50	1	—	55 15 (Leghorns)	37
			—	59 40 (Broilers)	
		6		69 0 (Leghorns)	
				65 5 (Broilers)	
	80	6	No effect	91 0	36
1248	1	8	No effect	No effect	4
	2	9	No effect	No effect	35
	10	8	74 69	91 50	4
	20	8	74 65	91 2	
	20	9	76 66	94 12	
1254	2	9	No effect	No effect	
	5	14	83 72	No effect	38
	20	9	76 66	94 81	35
	50	14	83 62	Reduced to near zero	38
	50	6	No effect	Reduced to zero	39
1268	20		76 71	No effect	25

IV. REPRODUCTION

The effects of PCBs on the overall reproduction of birds are summarized in Tables 6 to 8. By far the most detailed information is available for the chicken (Table 6). It will be seen that hatchability is affected to a much greater extent than egg production. There is appreciable variation in the effects of the different Aroclors®. Taking the 20 ppm data, one sees that 1221, 1254, and 1268 have only minor effects, whereas this dosage level causes complete hatching failure when 1242 or 1248 is used. Other members of the Galliformes (Table 7) are less sensitive; hatchability of the Japanese quail was not affected by 20 ppm Aroclor® 1248, nor was bobwhite quail affected by 50 ppm Aroclor® 1254. Both these dosage regimes reduced the chicken to near zero hatchability.

A. Eggshell Thickness

The bulk of the evidence shows that PCBs do not affect the thickness of eggshells. The following studies found no significant eggshell thinning: mallard,[6,41,43] American kestrel,[8] screech owl,[7] ring doves,[40] bobwhite quail,[41] Japanese quail,[40] ring-necked pheasant,[42] and chicken.[1,33,36] The only study reporting eggshell thinnings appears to be that of Call and Harnell[39] on Japanese quail using high dosages of three Aroclors®.

B. Effects on Males

Platonow and Funnell[7] fed cockerels Aroclor® 1254 at a dietary level of 250 ppm. A

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Table 7
EFFECT OF PCBs ON REPRODUCTION OF OTHER GALLIFORMES

Species	PCB	Dosage	Duration	Egg production		Hatchability	Ref.
Japanese quail	1242	100 ppm	9 weeks	0.89	0.75	Not studied	40
		312 ppm	3 weeks	0.55	0.45	Not studied	30
		5000 ppm			0.24		
	1248	20 ppm	8 weeks	Not reported		No effect	4
	1254	78 ppm	3 weeks	0.55	0.29	Not studied	30
		1250 ppm			0.18		
	1260	62.5 ppm			0.48		
		1000 ppm			0.12		
Bobwhite quail	1254	50 ppm	2 seasons	No effect		No effect	41
	1260	500 ppm	14 weeks	Significant decrease for first 8 weeks, significant increase thereafter			31
Pheasant (*Phasianus colchicus*)	1254	12.5 mg/kg/day	17 weeks	0.62	0.46	No effect	42
		50 mg/kg/day			0.29		

marked reduction in comb size was noted within 6 weeks, and the weight of the testes was reduced after 9 weeks. No direct studies of the reproductive performance of these birds were made. Lillie et al.[39] fed cockerels Aroclor® 1248 at 10 and 20 ppm for an 8-week period without finding any effect on semen or fertility.

In contrast, Ahmed et al.[40] found that 10 to 40 ppm of Aroclor® 1254 in the diet caused decreased semen volume, sperm concentration, and testes weight. Biessman[41] found that Clophen® 60 decreased testicular weight in Japanese quail. Bird et al.[30] found that 33 ppm Aroclor® 1254 fed to American kestrels caused a significant decrease in sperm concentration but did not affect semen volume.

C. Teratogenic Effects

Carlson and Duby[31] found that 5 ppm of Aroclor® 1242 injected at day zero caused several malformations including development of only one eye, hydrocephalus, incomplete abdominal closure, and nervous tremors. Tumasonis et al.[29] noted leg, toe, and neck deformities in many chicks that hatched from eggs with a yolk level of 10 to 15 ppm Aroclor® 1254 or more. Cecil et al.[32] examined the effect of a diet of 20 ppm of a range of Aroclors® on the production of abnormal embryos. These workers found a marked increase of abnormal embryos as a percentage of fertile eggs for Aroclors® 1242 and 1248, a slight effect with Aroclor® 1232 and 1254, and no effect for Aroclors® 1221 and 1268. In all the treated groups, there were 843 dead embryos of which 286 were abnormal. The most common abnormality was edema, including edematous cysts and subcutaneous edema. Other abnormalities, in decreasing order of occurrence, were unabsorbed yolk, small body, external hemorrhage, skinny legs, rotated ankle, and deformed beaks.

Dahlgren and Linder[42] noted decreased chick weight in their studies on the pheasant, but no mention is made of abnormalities. Peakall et al.[33] found an increase in chromosomal abnormalities in embryos of ring doves exposed to 10 ppm Aroclor® 1254. Blazak and Marcum[34] injected Aroclor® 1242 into chicken eggs so that the final concentration was 10 or 20 ppm. They found increased mortality (64 and 67%, respectively, compared to 30% for controls), and a few abnormalities (2/64 at 10 ppm and 3/57 at 20 ppm). However, in contrast to the study on ring doves, no chromosomal breakage was observed.[34] In the dove

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Table 8
EFFECT OF PCBs ON REPRODUCTION OF NONGALLIFORM BIRDS

Species	Aroclor®	Dose	Time	Incubation	Clutch size	Survival to			Ref
						Fertility (%)	Hatch (%)	3 weeks	
Mallard	1242	150 ppm	12 weeks	Artificial and natural	No effect	No effect	No effect	No effect	11
	1254	25 ppm	2 seasons	Artificial	No effect	No effect	No effect	No effect	41
		25 ppm	4 weeks	Natural	No effect	No effect	No effect	No effect	6
		60 ppm	16 weeks	Natural	No effect	No effect	Not studied	Not studied	43
Puffin (*Fratercula arctica*)	1254	30—35 mg/bird	1 season	Natural	No effect		No effect	No effect	45
Screech owl	1248	3 ppm	2 seasons	Natural	No effect	No effect	No effect	No effect	47
Ring dove	1254	10 ppm	12 weeks	Natural	No effect		Reduced	Reduced	
				Artificial	No effect		86 60	—	

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study the birds were fed PCBs over a prolonged period and alterations may have occurred in the body of the parent. Whether experimental design or species difference is responsible, it is clear that increased embryonic mortality was associated with chromosomal breakage in one case and not in the other.

D. Behavioral Effects

Delayed reproduction has been noted with DDE[illegible] but the effect of PCBs is either slight[illegible] or nonexistent.[illegible] Behavioral abnormalities leading to marked reduction of reproductive success were found in ring doves fed 10 ppm Aroclor® 1254.[illegible] Monitoring of egg temperature suggested that increased mortality was due to decreased parental attentiveness, which was confirmed by comparison of natural and artificial incubation. McArthur et al.,[illegible] feeding ring doves a mixture of Aroclor® 1254, DDE, and mirex found effects on breeding synchrony, nest construction, incubation attentiveness, and parental care and were able to relate them to hormonal changes. Decreased reproductive success was marked in the higher dose (28 ppm PCB, 4.6 ppm DDE; 0.9 ppm mirex) groups. In contrast, the Custer and Heinz[illegible] study feeding 25 ppm Aroclor® 1254 to mallard did not reveal any behavioral abnormalities nor any reduction of breeding success.

V. OTHER PHYSIOLOGICAL EFFECTS

In this section, physiological changes not directly associated with reproduction are discussed (although indirect effects are possible).

A. Hepatic Enzyme Induction

There are considerable data on the induction of hepatic enzymes in birds by PCBs starting as far back as 1968.[illegible] It is clear from the data in Table 9 that the phenomenon is found in many species and that a number of different enzyme systems are involved. The studies of Bunyan and Page[illegible] show that the response is markedly different for different specific isomers and they relate these differences to degree of retention in the body. Elevated activity of hepatic enzymes has been found in the livers of herring gulls collected on the Great Lakes[illegible] and the possibility of using this system for monitoring purposes has been put forward.

These hepatic enzymes (often called mixed function oxidases or MFO) have the role of converting liposoluble foreign compounds into water-soluble metabolites that may be more readily excreted. Thus, these systems are a defense against xenobiotics and a knowledge of the activity of MFO systems can be used to determine the half-lives of some xenobiotics. In general, the activity of MFO is mammal > bird > amphibian > fish.[illegible] Within the order Aves, considerable variation has been found, for example, the puffin shows much higher MFO activity than the cormorant.[illegible]

In addition to metabolizing xenobiotics, the MFO system also metabolizes endogenous compounds, especially steroids. The possible interrelationship of these changes with altered reproductive behavior has been discussed earlier.

B. Thyroid Function/Vitamin A Levels

Thyroid hormones play a major role in regulating oxidative metabolism, growth, and development and are interrelated with vitamin A metabolism.

Jefferies and Parslow[illegible] dosed lesser black-backed gulls with Aroclor® 1254, at 50, 100, 200, and 400 mg/kg/day for 8 weeks. An increase in thyroid weights (30 to 40 mg) was noted between the controls and dosed birds, but no dose-dependent increase was observed. Analysis of the colloid area showed a small increase. The major histological change was larger follicles well filled with colloid. In a subsequent experiment[illegible] on guillemots, dosages of 12 and 25 mg/kg/day caused increased thyroid weight, follicle size, and colloid area. Increasing doses (up to 400 mg/kg/day) caused dose-related decreases in these parameters.

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Table 9
EFFECT OF PCBs ON HEPATIC ENZYME INDUCTION

Species	PCB	Dosage	Duration (days)		Ref.
Chicken	1254 1260	1 mg/day, chicks	7	3—4-fold increase in rate of metabolism of testosterone, androsterone, & estradiol	58
	1254	10, 100 ppm	14	Marked increase of benzopyrene hydroxylase at both levels, no dosage dependence	59
	1254	0.1, 1 ppm 10 ppm 100 ppm	7	No effect Doubling of cytochrome P450 4-fold increase of cytochrome P450	60
	1221	5, 500 ppm 50 ppm	30	No effect Decrease of AHA and APD activity	27
	1242	5, 50, 500 ppm		No effect	
	1254	5, 50, 500 ppm		No effect on AHA; nondose related effect on APD activity	
	1268	5 ppm 50 ppm 500 ppm		No effect ADP increased AHA and APD increased	
Japanese quail	1242	250 ppm	20	4-fold increase of cytochrome P-450	21
	1254	250 ppm	20	8-fold increase of cytochrome P-450	
	1016 1242 1254 3,3'- and 4,4'-dichloro 3,4'-dichloro 2,4,6,2'-tetrachloro 2,4,6,4'-tetrachloro 2,3,3',4'-tetrachloro 3,3,3',5'-tetrachloro 2,4,6,2',4',6'-hexachloro 3,4,5,3',4',5'-hexachloro Decachloro	50 ppm	20	No effect 3-fold increase of cytochrome P-450 3-fold increase of cytochrome P-450 No effect Small increase of EMD activity No effect Doubling of EMD; 3.5-fold increase of cytochrome P-450 Doubling of EMD and cytochrome P-450 4-fold increase of cytochrome doubling of EMD and AHA activity No effect 3-fold increase of cytochrome P-450, 2—3-fold increase of EMD & AHA No effect	1
Pigeon	1262	20 mg/kg, single i.p. injection	7	3-fold increase in metabolism of estradiol	57
	1242	500 ppm	28	6-fold increase of cytochrome P-450	21
American kestrel	1254	0.5, 5.0 ppm	14	Increased metabolism of estradiol	61
	1260	0.5, 5.0 ppm	14	Increased metabolism of estradiol	
Barn owl (Two other)	1254	30 mg/kg injected into liver	3	Results difficult to interpret due to toxic effects on liver	62

Jefferies and Parslow considered that in the pituitary, the decrease of weight of which was significantly correlated with increasing dose, is one of the target sites for Aroclor® 1254.

Hurst et al.[21] studied the effect of Aroclor® 1260 at 5, 50, and 500 ppm for 1 to 4 months on bobwhite quail. Significantly different thyroid weights were found for all dose levels after 4 months, but this effect appears to be due to the abnormally low control value for

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this period. There was no actual rise in thyroid weights when compared to measurements made at 1, 2, or 3 months. There was a tendency for ^{131}I uptake for the total thyroid to be higher in quail exposed to the highest dose (500 ppm), but this increase was significant for only one time period and did not show up when expressed on a per 6 milligram of thyroid basis.

Cecil et al.[66] studied the effect of 100 ppm Aroclor® 1242 on liver vitamin A levels in quail. After 2 months on the diet, Aroclor® 1242 caused a marked reduction (approximately halved) in vitamin A in male quail, but the females were unaffected.

Spear[67] found that leghorn chicks receiving a low vitamin A diet developed hypothyroidism following two 10 mg/kg injections of 3,4,3',4'-tetrachlorobiphenyl (TCB). Plasma vitamin A concentration and growth rate were not affected by the toxicant. On a low iodine-low vitamin A diet, chicks exposed to TCB were subject to an alteration of thyroid hormone metabolism distinct from that occurring at the higher dietary level of iodine. Total T_4 decreased commensurate with a decrease in the binding of thyroid hormones in plasma such that the free thyroid hormone index remained constant. The result was interpreted to mean that the toxicant did not alter the concentration of unbound thyroid hormones at the lower level of dietary iodine.

Tori and Mayer[68] feed mourning doves (*Zenaidura macroura*) 40 ppm Aroclor® 1254 and found a significant effect on body temperature. The metabolic rates of PCB-fed doves increased more slowly with decreasing ambient temperatures than did the controls.

C. Hydropericardium, Generalized Edema, and Porphyria

McCune et al.[69] discovered hydropericardium when chickens were placed in a battery treated with unhardened paint. Additional studies revealed that Aroclor® 1242 caused hydropericardium at dosages, and in the abdominal and subcutaneous areas Aroclor® 1242 was pronounced in the chicks exposed to 400 ppm for 3 weeks, but was much less marked at 200 ppm. Rehfeld et al.[71] observed general edema in chicks when the diet contained over 20 ppm Aroclor® 1254. Chicks exposed to diets containing 50 to 150 ppm Aroclor® 1254 had extensive generalized edema. This included serious effusion into the pericardial sacs, muscles, subcutaneous tissues, and abdominal cavity.

Bayer and Bird[71] studied structural changes in the epicardium of chickens exposed for 11 weeks to a diet containing 20 ppm Aroclor® 1254. They found that all the birds exposed to PCB showed morphological changes in the pericardium and three out of four birds showed large quantities of ascites fluid. Vos and Koeman[2] studied the effects of 400 ppm of three different preparations of PCBs containing 60% chlorine. These were Phenoclor® DP6, Clophen® A60, and Aroclor® 1260. The chickens were exposed to this diet for 60 days. Hydropericardium was common in the Phenoclor® and Clophen® group. Similarly, abdominal and subcutaneous edema was observed only in the first two groups. These differences were considered to be due to the presence of chlorinated dibenzofurans in the first two preparations.[73] Nevertheless, there are considerable differences between Vos and Koeman's results on Aroclors® and those of other workers. These differences may be due to the fact that other workers have used lower chlorinated Aroclors® (1242 and 1254) which may themselves act differently or may contain more chlorinated dibenzofurans than Aroclor® 1260. Firestone[74] reviewed the etiology of chick edema disease and concluded that the chlorinated dioxins and furans were the chemical agents responsible. In view of the studies of Rehfeld et al.[71] and those of Bayer and Bird[72] which show effects at moderate levels, (about 20 ppm) it would be useful to examine the effect of pure PCBs to clear up the question of the agent causing chick edema.

In contrast to their findings on edema, Vos and Koeman[2] found that porphyria was induced by all preparations of PCBs studied. They found that the chicks increased fecal excretion of coproporphyrin, protoporphyrin, and fluorescence (indicating the presence of porphyrins)

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in the liver, bile, small intestine, and bone. Subsequently, Vos et al.[74] made a more detailed study using Japanese quail. Using Aroclor® 1260 at 50, 100, 250, and 500 ppm in the diet for 7 days on male quail they demonstrated a tenfold increase of mitochondrial δ-aminolevulinic acid synthetase in the liver of birds receiving 50 ppm, and a further increase at higher levels. Increased fluorescence of the liver only became marked at higher dosages. Lower levels were studied on female quail. A slight but statistically nonsignificant increase of δ-aminolevulinic acid synthetase was noted at a dosage of 0.1 mg/kg/day for 7 days, but the increase was significant at a dosage of 1 mg/kg. δ-Aminolevulinic acid synthetase catalyzed (ALA synthetase) the first step in porphyrin synthesis, for example, the reaction of succinyl CoA with glycine to form α-amino-β-ketoadipic acid. Metabolic control of porphyrin formation is excreted through a feedback mechanism operating at the first two steps of the pathway (ALA synthetase and δ-aminolevulinic acid dehydrase). ALA is also the precursor of the porphyrin moiety of chlorophyll and of the corrin ring of vitamin B_{12}.

Chick edema disease and hepatic porphyria have been described in herring gull embryos collected from Lake Ontario colonies in the early 1970s.[75] Substantial levels of PCBs were known to have been present, but also a wide variety of other compounds including 2,3,7,8-tetrachlorodibenzo-*p*-dioxins.[76]

D. Immunosuppressive Activity of PCBs

Friend and Trainer[78] examined the effect of Aroclor® 1254 on mortality of young mallards exposed to duck hepatitis virus. Three dose levels were used, 25, 50, and 100 ppm, in the diet. At all dosages, the mortality was increased markedly over control values, but the greatest increase was at 50 ppm. The actual values were control 14.3%, 25 ppm 35.05%, 50 ppm 65%, and 100 ppm 44.4%.

The importance of the effects of PCBs on the immunosuppressive mechanisms is hard to evaluate. The lack of clear dose dependence is one problem, and interrelation with other stresses is another. The possibility of the burden of PCBs and other organochlorines[79] increasing susceptibility to disease should be borne in mind.

E. Other Physiological Effects

Combs et al.[80] found that dietary PCBs (Aroclor® 1254) added to the diet of breeding hens increased the susceptibility of progeny to vitamin E-selenium deficiency when the ducks were deficient in vitamin E and contained only marginal concentrations of selenium. No effects were seen when the birds were maintained on normal diets. Subsequently, studies[80] indicated that PCBs did not affect the physiologic function of vitamin E in protecting biological membranes, but may interfere with the absorption-retention of the vitamin.

Srebocan et al.[81] studied the effect of Aroclor® 1254 on the levels of gluconeogenic enzymes in embryonic and growing chicks. In one experiment, Aroclor® 1254 was injected into chicken eggs so that the final concentrations were 0.05, 0.1, 0.5, or 5 ppm. At 14 days of incubation, modest decreases (values reduced to 80% of controls by highest dosage) were seen for phosphoenolpyruvate carboxykinase, phosphoenolpyruvate carboxykinase and fructose-1,6-diphosphatase but not glucose-6-phosphatase. In growing chicks, the levels of all of these enzymes, except phosphoenolpyruvate carboxykinase were severely reduced over dietary range of 0.1 to 200 ppm. The authors consider that effects on the energy utilization mechanism could be responsible for embryonic mortality and death at time of pipping. The pattern of enzymatic change, i.e., moderate in the embryo, marked in chicks, is the inverse of the pattern of mortality. However, it could be that energy demand is critical at the earlier life stages and much less so later.

Dieter[82] demonstrated significant increases of the levels of several plasma enzymes (creatine kinase, cholinesterase, fructose diphosphate aldolase, and lactate dehydrogenase) in quail fed 5 ppm Aroclor® 1254 for 12 weeks. These responses were dose dependent and the plot of enzyme activity against the log of the dose was linear.

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Table 10
PCB LEVELS IN GULLS AND THEIR EGGS

Species	Location	Tissue	Year of collection	Residue level (ppm wet weight)	Ref.
Herring	Norway (west coast)	Egg	1972	3.1—12.6	83
	Baltic (Gdansk Bay)	Muscle	1975—76	22—150	84
	Finland	Muscle	1972—74	0.68—18	85
	East Scotland	Muscle	1971—75	0.2—1.2	86
	Camargue, France	Egg	1972	16—160	87
	New Brunswick, Canada	Egg	1968—72	3.1—41.2	88
	Lake Ontario	Egg	1974—75	74—261	89
	Maine	Egg	1977	10—32.0) 7.76 (30)	22
	Virginia	Egg	1977	(0.13—16.70) 9.06 (28)	90
	East Frisian Is. Germany	Egg	1975	26.5	90
			1971	5.5	
	Denmark (North Sea)	Egg	1971	2.1 (1.3—2.8)	91
	Baltic		1972	92 (21—199)	
California	Alberta	Egg	1969	0.87 (1.1)	92

Heinz et al.[93] examined the effects of 1, 10, and 100 ppm of Aroclor® 1254 on the levels of dopamine and norepinephrine in the brains of ring doves.[93] Significant decreases of both of these compounds were found at both 10 and 100 ppm dosages. It is considered that these changes (40% reduction at 10 ppm) could result in behavioral abnormalities.

VI. PCB LEVELS IN BIRDS

There has been a vast amount of literature on this subject since the initial finding that PCB contamination of birds is essentially global.[17] In this review only the most highly contaminated systems — Great Lakes of North America and the Baltic Sea — are considered. Temporal trends are considered in the chapter by Stout (Volume I, Chapter 9).

One of the avian species considered to be a good monitor of environmental pollution is the herring gull.[83] Some residue levels for that species are given in Table 10. Only in the case of the Lake Ontario birds were affects seen (poor reproduction). Other pollutants were also identified (DDE 23 ppm, mirex 5 ppm, tetrachlorodioxin 1 ppb, all on a wet weight basis) and it is not possible to separate the effects caused by different pollutants. Helander et al.[94] have recently summarized the levels of pollutants in White-tailed Sea Eagles and related them to productivity. They estimate the critical range as 500 to 600 ppm DDE and/or 800 to 900 ppm PCB on a lipid weight basis in the eggs of this species. The mean levels in the poorly reproducing Herring Gulls on Lake Ontario were 1700 ppm (lipid weight) compared to 700 ppm in the eggs from colonies reproducing normally in Lake Huron.[95]

VII. CONCLUSION

Based on laboratory experiments, it is clear that PCBs are not sufficiently toxic in either acute and chronic feeding studies to pose a hazard to birds. Studies on reproduction show marked interspecies variations with chickens highly sensitive. In the wild, galliformes are not at the end of food chains that are likely to accumulate PCBs. Other species tested are less sensitive than chickens, but most target species have not been tested. Behavioral abnormalities also show interspecies variations, with the ring dove affected at modest levels whereas the mallard is not. It is likely, but not proven, that PCBs were involved in the behavioral abnormalities found in gulls on the Great Lakes that led to reproductive failure in the early 1970s. Hepatic enzyme induction occurs at realistic environmental levels, but

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there is no evidence that these changes cause harm to the organism. Little is known about the toxicology of metabolites of PCBs, although phenolic metabolites have been identified in guillemots and seals[91] and sulfone metabolites in seals.[96]

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Chapter 4

PCBs AND THE ENVIRONMENT. PERTURBATIONS OF BIOCHEMICAL SYSTEMS

Wilbert Gamble

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I. INTRODUCTION

Polychlorinated biphenyls (PCBs), as a result of their employment in a number of industrial processes and products, are ubiquitous in the environment.[1,2] Reports of their apparent toxicity to humans appeared as early as 1936[3] and in more recent years.[4] Some of the untoward effects observed after PCBs exposure include chloracne, swollen eyelids, and skin pigmentation. Several enzymes and metabolic systems are altered after PCB exposure. Enzymes of (1) glycolysis and gluconeogenesis,[5,6] (2) microsomal drug metabolism,[6-11] (3) oxidative phosphorylation,[12] (4) phospholipid metabolism,[13,14] (5) triglyceride metabolism,[15] and (6) sterol metabolism[16,17] are significantly perturbed by PCBs. It is evident from the list of enzymes altered that PCBs exhibit a variety of activities. The mechanism of transport and distribution of PCBs has not been described. There is a need to find the underlying mechanism(s) of action(s) of PCBs which permits them to elicit such a diverse number of responses. In this report, a review of some selected enzymes and reactions of metabolic systems altered by PCBs is presented. The results of the action of PCBs on the various enzymes are correlated to physical and chemical properties of the PCBs and affected enzymes in order to delineate a common mechanism of action. A mechanism which is compatible with the reported experimental results is presented.

II. CHEMICAL PROPERTIES OF PCBs

PCBs are chlorinated derivatives of biphenyl. They are sparingly soluble in water[18] and soluble in nonpolar solvents.[19] Thus, by definition they are hydrophobic. The hydrophobic effect of PCBs in water is predominantly due to water per se. Strong attractive forces between water molecules are disrupted or distorted when PCBs are placed in water in contrast to ionic or polar molecules which form strong bonds to water molecules. Consequently, the solution of PCBs is resisted. In physicochemical terms, it is a loss of entropy rather than bond energy that leads to the unfavorable free energy change for the solution of PCBs in water. These properties dictate that PCBs react with hydrophobic compounds and moieties in living systems such as lipids and hydrophobic polypeptide sequences.

PCBs as manufactured for commercial use are a mixture of isomers containing primarily 21, 42, 48, 54, and 60% chlorine by weight. They are sold under a number of trade names including Aroclor® (Monsanto Company, St. Louis) and Kanaclor® (Kanegafuchi Chemical Company Ltd., Osaka, Japan). There are 210 theoretically possible isomers. PCBs are stable compounds and are known to persist in the environment. It will be shown in the following discussions that these properties of PCBs are primarily responsible for the observed responses to PCB exposure.

III. GLYCOLYSIS AND GLUCONEOGENESIS

The metabolism of carbohydrates is a carefully regulated process. Central in the process is the control of the synthesis and degradation of glycogen, as well as the maintenance of glucose concentrations in plasma. Any interference with the control of the process constitutes a metabolic insult and, left uncompensated, leads to metabolic trauma. Several studies have shown that PCBs alter the activities of key enzymes of glycolysis and gluconeogenesis. It has been shown that PCBs decrease blood glucose concentrations in rats.[5,7] The activities of the gluconeogenic enzymes phosphoenol pyruvate carboxykinase, pyruvate carboxylase, and glucose phosphatase were decreased after rats were administered PCBs. Messner et al.[6] observed that a diet containing 0.05% PCB (Clophen® T64, 60% Cl) diminished the activity of phosphoenol pyruvate carboxykinase to about 50% with a feeding period of 7 days. Fructose diphosphatase activity was lowered to a lesser extent. Higher concentrations of

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PCB (Clophen® T64, 0.1 and 0.2%) decreased fructose diphosphatase activity significantly. PCBs decreased the ability of the rats to adapt phosphoenol pyruvate carboxykinase activity to gluconeogenic conditions.

Another important enzyme in the dynamic regulation of glucose metabolism is phosphorylase, the enzyme which catalyzes the breakdown of glycogen to glucose-1-phosphate. Its activities have been shown to be controlled by both covalent and allosteric modifications.[10-12] The enzyme exists in two metabolically significant forms, phosphorylase a and b. Phosphorylase b is converted to phosphorylase a by the action of phosphorylase kinase via phosphorylation of a single amino acid (serine 14) per monomer. Phosphorylase b requires AMP for activity and is inhibited allosterically by ATP, ADP, and glucose-6-phosphate. Phosphorylase a has no significant nucleotide requirement for activity. The amino acid sequence[13] and crystal structure of phosphorylase have been determined.[14,15] This information has been employed to describe the folding of the whole molecule and the loci of several binding sites for substrates and effectors. The AMP binding site of phosphorylase b is hydrophobic in nature.[16] Adenine derivatives substituted with hydrophobic groups with increasing substituent constant π value[17] were the most effective inhibitors. Somen and Philip[18,19] reported that soluble aromatic compounds, such as dinitrophenol, are allosteric inhibitors of phosphorylase b. The binding sites of the compounds were hydrophobic and the relative effectiveness of the inhibitors increased with the degree of hydrophobicity as indicated by the π values. It is widely accepted that the active site of phosphorylase is located in a crevice between two main domains in a hydrophobic pocket. Mead et al.[20] observed and investigated the differential inhibition of phosphorylase a and b by halogenated biphenyls and biphenylols. Polychlorinated biphenyls, polychlorinated biphenylols, and polybrominated biphenyls inhibited both phosphorylase a and phosphorylase b. The degree of inhibition was dependent upon the relative hydrophobicity of the compounds and steric hindrance. 2,4,5,2',4',5'-Hexachlorobiphenyl and 2,4,5,2',4',5'-hexabromobiphenyl did not significantly inhibit phosphorylase a. 2,4,5,2',4',5'-Hexabromobiphenyl and Firemaster BP-6 (a mixture of polybrominated biphenyls) were the most effective inhibitors (K_i, 15×10^{-6} *M*). Kinetic analysis showed the inhibition to be noncompetitive and mixed. It was concluded that the compounds bind to hydrophobic site(s) on phosphorylase, access to which is limited by phosphorylation of serine 14.

One can discern from the results of the studies presented that PCBs inhibit both enzymes involved in the synthesis and degradation of glycogen. The results indicate that the ability of PCBs to bind to hydrophobic domains of peptides is involved in the inhibition of glycogenolysis and suggest that similar interaction might occur with gluconeogenic enzymes.

IV. OXIDATIVE PHOSPHORYLATION

An in vitro study of the action of Aroclors® (1221, 1232, 1242, 1248, 1254, 1260, and 1262) on mitochondrial enzymes has been reported by Pardini.[11] Beef heart mitochondrial NADH-oxidase activity and succinic oxidase activity were inhibited by Aroclors® at 33 μM concentration. The inhibition range was 80 to 100%. There was no apparent inhibition of cytochrome oxidase as evidenced by the lack of inhibition by PCBs in the presence of *N,N,N',N'*-tetramethyl-*p*-phenylenediamine (TMPD). At the level tested, each Aroclor® elicited similar inhibition. The results suggest the inhibition occurs at NADH-cytochrome *c* reductase and succinate-cytochrome *c* reductase. Chesney and Allen[21] reported that Aroclors® 1248 and 1254 fed at 1000 ppm for 3 weeks to rats caused alterations in respiration and oxidative phosphorylation by liver mitochondria. When ADP was limited (state 4, no added exogenous ADP), there was a slight decrease in respiration with glutamate as substrate, but a greater than 75% increase over control mitochondria when succinate was the substrate. In state 3 (exogenous ADP added), there was a 50% increase of the Aroclor® experimental

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mitochondria over the controls with both substrates. The ADP/O ratios increased 11 to 16.6% in liver mitochondria from Aroclor®-treated rats. No significant differences in respiration and oxidative phosphorylation by hepatic mitochondria from rats fed 100 ppm PCBs for 20 weeks were observed. Mitochondria from rats fed low levels (100 ppm) of PCBs had 0.3 μg PCBs per milligram mitochondrial protein, whereas mitochondria from rats fed 1000 ppm had 2 to 3 μg PCBs per milligram mitochondrial protein. In vitro studies with mitochondria showed that concentrations of 50 μg/mg protein and above caused decreased respiration and oxidative phosphorylation in states 3 and 4 with both substrates. It was concluded that the amounts found in the mitochondria after in vivo treatment were insufficient to cause inhibition, since in vitro levels of 1 and 10 μg/mg mitochondrial protein had no significant effect. Aroclor® 1254 administered to rats for 2 weeks in the diet at 500 ppm increased the oxidation of succinate by liver mitochondria and caused a small but significant decrease in the ATP/O ratio (mol ADP used per atom mol O used) according to Garthoff and co-workers.[32] After 3 weeks of treatment, the liver contents of ATP and ADP were increased when expressed as micromoles per gram liver divided by gram DNA per gram liver, but not significantly altered when expressed as micromoles per gram wet liver. The mitochondrial matrix [NAD^+]/[NADH] ratio (glutamate substrate) was increased. The cristae [NAD^+]/[NADH] ratio β-hydroxybutyrate substrate) was slightly decreased. The cytoplasmic [NAD^+]/[NADH] ratio was decreased (32%) and the [$NADP^+$]/[NADPH] ratio was decreased (50%). PCBs of low chlorine content inhibited electron transfer in rat liver mitochondria at all concentrations in vitro in states 3 and 4, whereas PCBs of high chlorine content inhibited electron transfer at low concentration and uncoupled oxidative phosphorylation at higher concentration.[33] Similarly, PCBs were shown to uncouple respiration in cockroach muscle mitochondria.[34]

Yap et al.[35] and Koch and co-workers[36] have shown that PCBs in vitro inhibit Mg^{2+} ATPase and Na^+-K^+ ATPase activity and oligomycin-insensitive Mg^{2+} ATPase activity in fish. Inhibition was observed in brain, kidney, and liver. They observed in vivo inhibition and stimulation of ATPase activity. Stimulation occurred at the lower concentrations. PCBs were shown to inhibit (10 and 100 ppm) and stimulate (500 ppm) kidney and brain Na^+-K^+-ATPase and Mg^{2+} ATPase activities, and to inhibit (10, 100, 500 ppm) liver Na^+-K^+ ATPase and Mg^{2+} ATPase activities in vivo in rats.[37]

The effect of PCBs on oxidative phosphorylation, it is clear, is a function of the mitochondrial concentration. In vivo exposure yields relatively low organelle concentrations even at a 1000 ppm dose. The low levels give rise to increased respiration and increased adenine nucleotide liver content. In vitro studies indicate that high concentrations of PCBs inhibit respiration and uncouple oxidative phosphorylation.

It has been suggested by Chesney and Allen[31] that induction of hepatic mitochondrial activity may have occurred in rats treated with PCBs or the increase in respiration and oxidative phosphorylation may reflect a need for an increase in the amount of cellular ATP. The latter seems to be the more reasonable explanation of the observed results. ATPase activities are altered by PCBs, which no doubt alter cellular ATP balance and contribute to the observed gross responses to PCBs.

V. GLYCERIDES AND FATTY ACIDS

PCBs have been shown to alter the concentration, metabolism, and mobilization of lipids. Oral administration of Kanechlor® 400 induced hyperglyceridemia in rabbits.[13] After 4 weeks of treatment, free fatty acids and triglycerides in skin lipids of the rat were reduced.[13] One percent chlorobiphenyls in olive oil administered orally to rabbits for 3 days increased the content of total lipids and glycerides of liver, but did not alter serum glyceride concentrations.[38] After 11 days of administration of PCBs, the content of total lipids and glycerides

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of liver and the concentration of serum glycerides were increased. Similarly increased triglycerides content after 4 weeks of administration of Aroclor® at 0.5 to 500 ppm was reported by Litterst et al.[39] An investigation of the effect of the administration of PCB (0.1% w/w) in the diet fed to rats for 30 days on glyceride biosynthesis has been reported by Dzogbefia et al.[13] In mitochondria, the incorporation of [^{14}C]sn-glycerol-3-phosphate into monoglycerides (89%), diglycerides (16-fold), and triglycerides (6.8-fold) was increased. Incorporation of [^{14}C]palmitate into monoglycerides (1.6-fold) and diglycerides (35%) was increased by the PCB treatment, whereas incorporation into triglycerides was decreased (70%). Rat liver microsomal incorporation of [^{14}C]sn-glycerol-3-phosphate into mono-, di-, and triglycerides was not significantly altered. Similarly, incorporation of [^{14}C]palmityl CoA by microsomes into mono-, di-, and triglycerides was not significantly changed. There was increased incorporation of [^{14}C]sn-glycerol-3-phosphate by liver homogenates into monoglycerides (2.4-fold), diglycerides (13.8-fold), and triglycerides (10-fold). The incorporation of [^{14}C]palmitate by liver homogenates into triglycerides increased (70-fold), and into monoglycerides and diglycerides decreased 44% and 18-fold, respectively. Dzogbefia et al.[13] found that PCB in vitro increased (50%) the incorporation of [^{14}C]sn-glycerol-3-phosphate into total glycerides by rat liver microsomes. On the other hand, PCB inhibited the incorporation of [^{14}C]sn-glycerol-3-phosphate into triglycerides (76%), diglycerides (28%), and monoglycerides (26%) in homogenates in vitro. 2,4,5,2',4',5'-Hexachlorobiphenyl similarly decreased the incorporation of [^{14}C]sn-glycerol-3-phosphate by liver homogenates into glycerides (28%). Some time (72 hr) after administration of a single oral dose (50 mg/kg body weight) each of three PCB isomers, 3,4,5,3',4',5'-hexachlorobiphenyl, 2,4,5,2',4',5'-hexachlorobiphenyl, and 2,3,5,2',3',5'-hexachlorobiphenyl, it was found[14] that only 3,4,5,3',4',5'-hexachlorobiphenyl altered rat liver triglyceride content (sevenfold increase).

Kling and co-workers[40] investigated the in vivo and in vitro effect of Aroclor® 1254 on fatty acid synthesis. Aroclor® (0.1% w/w — 30 days in the diet) inhibited the incorporation of tritiated water (83%) and acetate (43%) into fatty acids. However, after 60 min incubation in vivo, there was an apparent increase in acetate incorporation into fatty acids while incorporation of tritiated water remained depressed. Aroclor® (50 ppm) inhibited (73%) the in vitro incorporation of acetate into fatty acids. Citrate cleavage enzyme which provides precursor for fatty acid synthesis was inhibited in vitro[40] and in vivo[41] by PCBs. However, no significant effect of PCBs on fatty acid synthase and acetyl CoA carboxylase, the rate limiting enzyme in fatty acid synthesis, was observed.[40] Yeast cells incubated with Aroclors®[42] exhibited decreased synthesis of fatty acids after 15 hr, whereas prolonged treatment up to 42 hr resulted in no significant change.[42] The biosynthesis of unsaturated fatty acids (C16:1 and C18:1) was decreased the most. Aroclors® of lower chlorine[43] content were more effective than those of higher chlorine content.[42] It is interesting to note that PCBs decrease synthesis of fatty acids during initial periods and either have no effect or increase synthesis upon prolonged incubation. These results were observed both in yeast[42] and in rat liver.[40]

Although, as can be seen from the literature cited, there are variations in response, PCBs primarily increase the synthesis of triglycerides. The increase appears to be due to a shift of common precursors from phospholipid synthesis to glyceride synthesis. The suggestion that liver lipase activity may be inhibited has not been experimentally substantiated, although serum lipoprotein lipase is apparently inhibited.

VI. PHOSPHOLIPIDS

Several authors[13,14,44-46] have observed both inhibition and stimulation and synthesis of phospholipids by PCBs. Elevated phospholipid levels in livers of laboratory animals following PCB administration have been reported.[13,43] Ishidate and Nakazawa[43] reported that

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rat liver microsomal phospholipid synthesis was decreased, whereas phospholipid content was increased after oral administration of suspensions of PCBs in 2% (w/v) sodium carboxycellulose daily for 3 days at 100 mg/kg/day, followed by intraperitoneal injection of [^{32}P] orthophosphate, (Me-^{14}C]choline, or [^{3}H]glycerol. Decreases in incorporation of the first two substrates into choline-containing phospholipids were observed, but not into other phospholipids. [^{3}H]Glycerol incorporation into total phospholipids was decreased after 15 min, but nor significantly altered after 60 min. They suggested that the observed increased content of microsomal phospholipid was due to inhibition of catabolism of phospholipids.

When rats were administered Aroclor® 1254 by intraperitoneal injection (20 or 50 mg/kg) for 3 or 6 days, decreased synthesis and turnover of phospholipids occurred.[46] [^{14}C]Glycerol incorporation into phospholipids of homogenates and microsomes declined over a 10-min period when reported as disintegrations per minute per milligram phospholipid. The half-life of the radioactivity in the total phospholipid pool after a single injection of [^{14}C]glycerol was prolonged from 10 (control) to 20 hr (PCB) in homogenates and from 8 (control) to 13 hr (PCB) in microsomes.

Inhibition of [^{14}C]ethanolamine incorporation into phosphatidyl ethanolamine and stimulation of [^{14}C]choline into phosphatidyl choline by mouse L5178Y lymphoma cells in vitro was caused by exposure of the cells to 4-chlorobiphenyl.[45] There was no apparent increase in total phoapholipid content after 60 min incubation. It was concluded that the enhanced incorporation of [^{14}C]choline into phosphatidyl choline was due to increased turnover of phosphatidyl choline.

After intraperitoneal injection of 2,4,2',4'-tetrachlorobiphenyl at 50 mg/kg body weight in peanut oil successively for 3 days, Holub et al.[46] found no significant change in the activities of acyl CoA:sn-glycerol-3-phosphate acyl transferase, CDP-choline:1,2 diacyl-sn-glycerol choline-phosphotransferase, acyl CoA:1,2-diacyl-sn-glycerol acyltransferase, and acyl CoA:1-acyl-sn-glycerol-3-phosphorylcholine acyltransferase. However, in vitro addition of 2,4,2',4'-tetrachlorobiphenyl (40 ppm) to rat liver microsomes caused inhibition (44%) of acyl CoA:sn-glycerol-3-phosphate acyltransferase and acyl CoA:1-acyl-sn-glycerol-3-phosphorycholine acyltransferase, whereas CDP-choline:1,2-diacyl-sn-glycerol choline phosphotransferase was stimulated 44%.

The effects of a single oral dose (50 mg/kg body weight) each of 3,4,5,3',4',5'-hexachlorobiphenyl, 2,4,5,2',4',5'-hexachlorobiphenyl, and 2,3,5,2',3',5'-hexachlorobiphenyl for 72 hr on liver phospholipids have been compared by Kohli et al.[14] Significant decreases in total phospholipids of liver were observed when the data were expressed as milligram per gram fresh liver for control and 2,4,5,2',4',5'-hexachlorobiphenyl-treated and 2,3,5,2',3',5'-hexachlorobiphenyl (approximately 12 to 13%), whereas 3,4,5,3',4',5'-hexachlorobiphenyl had no significant effect.

It should be noted that all of the above-mentioned studies employed short periods of treatment with PCBs, 3 to 6 days at the most. An assessment of the chronic effects of PCBs on phospholipid biosynthesis has been made by Dzogbefia et al.[13] The authors treated rats with Aroclor® 1254 (0.1% w/v) in the diet for 30 days. They observed an increase in the incorporation of [^{14}C]glycerol-3-phosphate into total phospholipids by rat liver mitochondria (14-fold), microsomes (1.7-fold), and homogenates (13.8-fold). Incorporation of [^{14}C]palmitate into total phospholipids by mitochondria and homogenates was increased 76 and 56%, respectively. However, microsomal incorporation of [^{14}C]palmityl CoA into phospholipids was not significantly altered. Dzogbefia and co-workers[13] in parallel in vitro experiments showed that PCBs inhibit (34%) the incorporation of [^{14}C]sn-glycerol-3-phosphate into total phospholipids of homogenates. PCBs inhibited microsomal incorporation of [^{14}C]sn-glycerol-3-phosphate into lysophosphatidate (77%), lysophosphatidyl choline, and phosphatidyl ethanolamine (36%). Similarly, 2,4,5,2',4',5'-hexachlorobiphenyl was shown to inhibit phospholipid biosynthesis in vitro.

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Administration of PCBs, it is clear from the literature cited, alters phospholipid metabolism. Inhibition of synthesis and catabolism occurs after acute in vivo and in vitro treatment. Relatively long-term administration of PCBs elicits increased synthesis of phospholipids. The apparent differences are most likely a reflection of the amounts of PCBs present at the locus of synthesis and catabolism at a given time. Chronic accumulation of PCBs appears to elicit several cellular responses which result in increased production of endoplasmic reticulum and lamellar membranes. Increased membrane production requires more phospholipid synthesis. Direct interaction of PCBs with certain enzymes in the process results in inhibition as seen in the in vitro studies. The observed response at a given time period is a result of the several interactions of PCBs with hydrophobic domains of proteins and lipids per se.

VII. STEROLS AND LIPOPROTEINS

After administering PCBs to rabbits, Koller and Zinkl[17] observed increased serum cholesterol levels. Elevated serum cholesterol levels were found in clinical cases of human PCB poisoning[13] and in rats treated orally with 500 ppm Aroclor® 1254.[11] Oral administration of Aroclor® 1254 at a dose of 1 mg/100 g body weight/day to rats for 12 days caused an increase in liver sterols and sterol esters.[13] A single oral dose of 3,4,5,3',4',5'-hexachlorobiphenyl after 72 hr caused an increase in total liver cholesterol (milligram per gram liver), according to Kohli and co-workers.[14] Livers from rats administered 2,3,5,2',3',5'-hexachlorobiphenyl showed a small decrease in total cholesterol compared to controls when results were expressed as milligram per gram liver, whereas those from rats administered 2,4,5,2',4',5'-hexachlorobiphenyl caused an increase in cholesterol ester (milligram per gram liver), but no significant change in free cholesterol (milligram per gram liver). In contrast, expression of the results as milligrams/100 g body weight yielded increases in ester and total cholesterol biosynthesis in rat livers. Kling and Gamble[47] have investigated the in vivo and in vitro effects of Aroclor® 1254 on cholesterol biosynthesis in rat livers. PCB at a dose of 0.1% w/w of the diet for 30 days decreased the conversion of [^{14}C]mevalonate (26%) and [^{14}C]acetate (29%) to cholesterol. After 30 days of PCB treatment followed by intraperitoneal injection of H_2^3O, [^{14}C]mevalonate, and [^{14}C]acetate, cholesterol biosynthesis was decreased with each substrate. In vitro, PCB (500 ppm) inhibited the incorporation of [^{14}C]mevalonate into cholesterol (86%) and [^{14}C]acetate into cholesterol (90%). It was found that cholesterol biosynthesis was inhibited between squalene and cholesterol, primarily at the demethylation and rearrangement reactions.[47] Aroclor® 1232 inhibited sterol biosynthesis in yeast (*Saccharomyces cerevisiae*) with a concomitant accumulation of squalene.[41] There were relatively large decreases in ergosterol and episterol synthesis, accompanied by smaller decreases in lanosterol and zymosterol synthesis. It was suggested that the results may be due to inhibition of squalene epoxidase and 2,3-oxidosqualene cyclase, because squalene accumulates and PCBs inhibit electron transport.

The effect of Aroclor® 1254 administered intraperitoneally (5 mg/100 g body weight) to rats for 6 to 8 days on the lipid composition of very low-density lipoproteins (VLDL) of rat liver microsomes, liver Golgi, and serum has been reported.[48] Although PCB treatment increased the amounts microgram per gram liver) of phospholipids, cholesterol, triglycerides, and total lipids, the percent composition of the lipids of microsomal VLDL did not significantly change. The rate of [^{14}C]glycerol incorporation into the triglycerides and total lipids of microsomes was enhanced by PCB. In contrast, a decreased labeling rate of lipoprotein triglycerides and total lipids of the Golgi apparatus was observed. The authors suggested that there was impairment of transport of VLDL particles from the endoplasmic reticulum to the Golgi apparatus. Since there was cytoplasmic accumulation, it was suggested that the lipids could not be exported from the cells, but were degraded intracellularly. Oishi[49] reported

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that PCBs increase rat serum β-globulin and decrease α_1-globulins. These results suggest that the changes in serum lipid composition observed after PCB administration may be related to alterations in plasma transport protein concentrations.

PCBs, it appears, primarily inhibit cholesterol metabolism. There may be species differences and differences in results due to acute or chronic exposure to PCBs as well as different doses. Increased serum levels of cholesterol observed after PCB exposure may be due to actions of PCBs on lipoprotein metabolism and transport.

VIII. MICROSOMAL OXIDATION

The interaction of PCBs with drug metabolizing enzymes is one of the most widely studied aspects of PCB toxicity. A detailed discussion of the subject is beyond the scope of this report. Briefly, it can be stated that, in general, administration of PCBs to animals induces drug metabolizing enzymes in the liver. Aniline hydroxylase, aminopyrine *N*-demethylase, cytochrome P-450, benzpyrene hydroxylase,[9] nitroreductase,[10] and *p*-nitroanisole-*o*-demethylase activities have been shown to be induced by PCBs.[11] It has been reported that enzymes induced by PCBs have different kinetic properties from the noninduced enzymes and are inhibited in vitro by PCBs.[10] Only aminopyrine demethylation was inhibited in microsomes from normal (control) rats. PCBs in vitro inhibited *p*-nitroanisole demethylation and aminopryine demethylation in microsomes of PCB-treated rats.

IX. PROPOSED MECHANISMS AND CONCLUSIONS

One can readily conclude from the above review of the literature that PCBs elicit a variety of profound effects on biochemical systems. One organ often affected is the liver. The morphological changes in the liver after PCB treatment of mice, rabbits, and rats serve to illustrate the characteristic alterations. In hepatocytes, the number of lipid bodies and mitochondria per cell is increased in livers from PCB-treated animals.[11] The mean cell volume, mean nuclear volume, and mean cytoplasmic volume are increased. The volumes of cytoplasmic components, liposomes, and rough and smooth endoplasmic reticulum are increased. It appears that the cellular response to PCBs is an enhancement or augmentation of the normal cellular processes which determine the morphological characteristics of cells. Some changes are relatively nonspecific such as the increased lipids and lipid bodies, which occur(s) with other lipophilic drugs and toxicants. Similarly, the proliferation of smooth endoplasmic reticulum is stimulated by drugs and toxicants to permit the cell to metabolize lipophilic compounds. The rough endoplasmic reticulum correspondingly is increased since it is thought to be a precursor of smooth endoplasmic reticulum.[12] It has been suggested[2] that the enlarged cells would have increased energy demands and demands for digestion, resulting in greater numbers of mitochondria and liposomes per cell.

Some of the observed responses to PCB may be due to indirect actions. Consequently, it may not be possible to describe a mechanism which explains all of the effects of PCBs on living systems. However, a mechanism which explains the fundamental alterations in biochemical systems elicited by PCBs is desirable and perhaps more attainable. Perhaps the most outstanding chemical property of PCBs is their hydrophobicity. They have limited solubility and adhere readily to surfaces, including glass and plastics. They vary in their degree of chlorination and positional location of the chlorines. The molecules lacking substitution of chlorine in the 2,2',6, and 6' positions are planar or nearly so, whereas those possessing chlorine in those positions are nonplanar. They are soluble in lipids having larger partition coefficients for oil(s)/water than for phospholipid/water[13] due to the formation of bilayers by phospholipids and concomitant presence of surface charges. They are aromatic and this property has been shown to contribute to their interaction with enzymes.[14] PCBs

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were shown to inhibit phosphorylase, presumably by binding to the hydrophobic site on that enzyme, normally occupied by AMP. PCBs bind to albumin and low density lipoproteins,[14] proteins especially constituted to bind lipophilic molecules. Their capacity to bind to other molecules and alter their activities is a function of their conformations in addition to their hydrophobic properties. This point was illustrated by Poland and Glover,[15] who established the structure-activity relationship for the induction of aryl hydrocarbon hydroxylase activity in chick embryo liver. There are two apparent structural requirements for induction, the presence of at least two adjacent halogen atoms in the lateral positions of each benzene ring (positions 3,4,3',4' or 3,4,5,3',4',5') and the absence of halogen atoms adjacent to the biphenyl bridge (positions 2,6,2',6'). Mead and co-workers[16] observed that the positional location of the chlorine atom determined whether or not polychlorinated biphenyls inhibited phosphylase a. The active site of the enzyme is in a hydrophobic pocket of sufficient dimensions (6 × 8 × 20 Å) to allow entry of the halogenated biphenyls. It was suggested that steric hindrance prevented certain PCBs from entry into the hydrophobic pocket. That there is specificity of binding of PCBs to proteins was shown in the experiments of Poland and Glover[15] in which congeners having chlorine atoms in the 2,2',6,' position did not induce arylhydroxylase and did not bind to the hepatic cytosol binding protein, whereas those having the prerequisite of at least two adjacent halogen atoms in lateral positions of each benzene rings did bind the cytosol binding protein and induce aryl hydroxylase activity.

PCBs, it can be concluded, are lipophilic aromatic compounds which bind to hydrophobic domains of proteins and associate freely, with the nonpolar moieties of lipids. They are distributed in several tissues when administered to animals.[36-38] The rates of excretion are a function of the degree and positional location of the chlorine atoms, both of which dictate the rates of metabolism.[39-41] PCBs and their metabolites are excreted in the urine and feces, the latter being the major route.[36-38] The major storage sites for the nonexcreted PCBs are adipose tissue, liver, skin, and muscle.[38] The mechanism(s) by which they reach tissues and intracellular loci is not known. Environmental exposure to PCBs occurs usually via the diet or topically. Experimentally, PCBs have been administered orally, intraperitoneally, and topically. They have been shown to bind to lipoproteins[36] and albumin.[36] The lipoproteins are the most probable carrier molecules for PCBs. They will bind to any hydrophobic molecule, which suggests that other hydrophobic protein, carbohydrates, or lipid domains can act as carriers.

All of the presented factors considered, it is proposed that PCBs elicit the responses observed with biochemical systems by the following mechanism. A diagramatic description is shown in Figure 1. PCBs bind to the hydrophobic moieties of the carrier molecules lipoproteins, albumin, and other hydrophobic proteins. They form water-soluble "inclusion" complexes. These complexes are transported via the blood to the constituent tissues. PCBs "inclusion" complexes are passed from the blood capillary lumen by interaction of the lipoprotein — PCB inclusion complex (or other hydrophobic protein — PCB inclusion complexes) with the plasma membrane of the endothelial cell. They then pass through the transcellular channels in endothelium to the plasma membrane of the cells. The complex is taken up by the cells via endocytosis through the plasma membrane. The path taken at this juncture is primarily a function of the normal fate of the carrier molecule. For the sake of simplicity, let us assume the carrier molecule is a lipoprotein, either a chylomicron or a low density lipoprotein (LDL). The LDL-PCB inclusion complex enters the lysosome. The LDL is metabolized and the PCB is released. It associates immediately with the hydrophobic molecules of any LDL residual product or other proteins. When the PCB associates with endoplasmic reticulum, a conformational ordering occurs which results in lamellar structures (in some instances). These changes result from an interaction of the complex with water, a phenomenon described by Tanford[39] in his description of the hydrophobic effect. When the released PCBs bind to hydrophobic domains on an enzyme, the activity of the enzyme will

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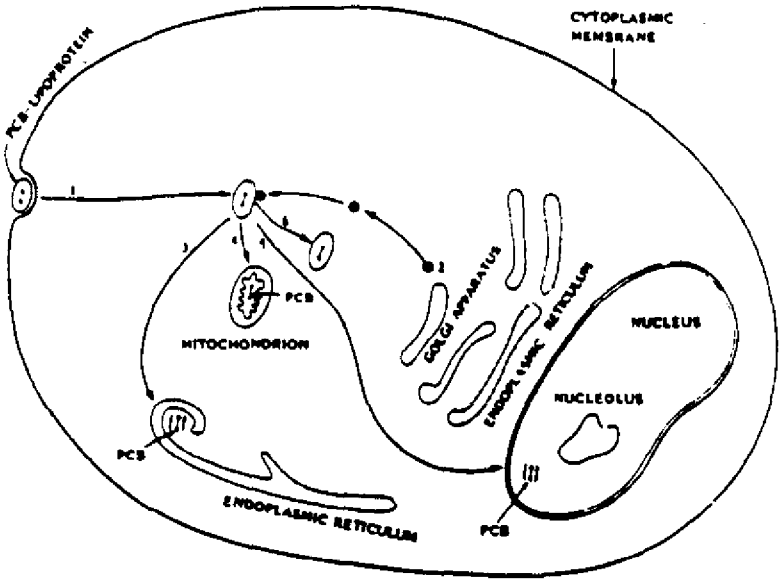

FIGURE 1. A diagrammatic representation of PCB-lipoprotein cellular interaction. (1) Endocytosis of the PCB-lipoprotein complex. (2) A primary lysosome fuses with the PCB-lipoprotein complex. (3) The lipoprotein moiety is metabolized and the PCB-protein remnant associates with the endoplasmic reticulum. (4) Uptake of the PCB-protein complex by the mitochondrion. (5) Uptake of the PCB-protein complex by the nucleus. (6) Formation of a PCB-protein-lipid cytoplasmic vesicle.

be altered in a manner which is a function of the locus of binding and the amount bound. If the hydrophobic site is at, or near, the active site, inhibition occurs. On the other hand, if the hydrophobic site is in some other locus than the active site, a conformational change will occur which may result in either stimulation or inhibition or both, depending upon the amount bound.

When the carrier PCB complex is a chylomicron-PCB inclusion complex, the complex will enter the adipocyte of adipose tissue as described above for cells in general. Metabolism of the chylomicron releases PCBs along with fatty acids. The large quantities of triglycerides present solubilize the PCBs and they remain there until fat is mobilized.

In some instances, PCBs form complexes with proteins and lipids which are not readily metabolized. These cause accumulation of PCB-protein-lipid complexes in vesicular and lamellar structural arrays. As pointed out earlier in this section, PCBs bind to some proteins which participate in gene expression and result in the induction of new enzyme protein. In all of these processes, it is the physicochemical property of PCBs to bind to hydrophobic moieties and form complexes which assemble in ordered arrays such that the PCB-water interaction is minimized that is the driving force.

The gross response observed with PCBs is the vector sums of the interaction with and exchange between the given hydrophobic domains in the biochemical systems. In some cases, the PCBs are covalently modified. The properties of these modified PCBs may be quite different; the observed responses in turn may be different. These are secondary to the initial interaction of the parent compound with the system. Hydroxypolychlorinated biphenyls are the major products of covalent modification.

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Chapter 5

UPTAKE, RETENTION, BIODEGRADATION, AND DEPURATION OF PCBs BY ORGANISMS

M. K. Hamdy and J. A. Gooch

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I. INTRODUCTION

Since 1900, the large-scale use of synthetic compounds (e.g., solvents, propellants, dielectric fluids, plasticizers, flame retardants, pesticides, herbicides) has added greatly to our comfort as well as to increased industrial and agricultural productivity. However, the environmental persistence of some of these compounds, notably DDT, chlorinated dibenzodioxins, chlorophenols, and polychlorinated biphenyls (PCBs) has required curtailment of their use due to possible public health hazards.[1,2] The environmental persistence of such compounds results primarily from the inability of natural aquatic and soil biota to metabolize these compounds at a reasonable rate.[3]

Because aqueous environment (rivers, lakes, estuaries, and oceans) is the ultimate repository for all synthetic chemicals,[3] these contaminants may undergo bioconcentration through organisms in food chain systems or by direct uptake from water[4] and exhibit delayed symptoms of biological activity and toxicity, carcinogenicity, mutagenicity, or teratogenicity.[5]

PCBs are remarkable among organic industrial chemicals for their low solubility in water, their high octanol/water partition coefficients, accumulation coefficients, and their resistance to in vivo degradation.[6] As a result, they exhibit extraordinarily high values for bioaccumulation in animal tissues, especially fish and other aquatic organisms.[6]

There are several factors that affect PCB bioaccumulation. These include concentration in the surrounding environment; duration of exposure; temperature, solubility of the pollutant; species age, weight, feeding habits and lipid content; trophic level variations; and adsorption.[4,6,7]

II. BIOACCUMULATION AND DEGRADATION OF PCBs BY BACTERIA

The large quantities of chlorinated hydrocarbons released into the environment have evidently exerted a strong selective effect on microorganisms to detoxify or utilize these compounds rapidly and effectively.[3] Genetic rearrangements have probably occurred to form a conjugative plasmid with degradative capabilities.[3] One group of researchers found that mono- and dichlorobiphenyls were rapidly degraded by a *Nocardia* spp. and a *Pseudomonas* spp. but that trichlorobiphenyls and tetrachlorobiphenyls were degraded at a much slower rate.[8] It was postulated by these same authors that the mono- and dichlorobiphenyls were degraded with little or no further adaptation of the biphenyl degrading organisms, whereas adaptation would be necessary if the more highly chlorinated components were to be degraded and organisms which were adapted to simple PCBs might also be capable of metabolizing the more complex structures.[8] Maximum uptake of 39.7% was noted for a *Pseudomonas aeruginosa* culture growing in basal salt media after 72 hr with most of the PCB being located in the lipid fraction of the bacterial cells (walls and membranes) as shown in Table 1.[9] Evaluation of PCB destruction using a large volume of waste contaminated with Aroclor® 1248 PCB (150 and 800 mg PCB per liter) showed that the *P. aeruginosa* was able to degrade most of the PCB during 90 to 130 days incubation (Figure 1).[9]

Bacteria capable of degrading PCBs can occur in response to PCB contamination.[10] Seven genera of PCB-degrading bacteria (*Aeromonas* spp., *Pseudomonas* spp., *Micrococcus* spp., *Acinetobacter* spp., *Vibrio/Aeromonas* spp., *Bacillus* spp., and a *Streptomyces* spp.) have been isolated from estuarine samples, whereas sea water and ocean sediment contained only two predominant PCB-degrading genera (*Pseudomonas* and *Vibrio*).[10]

Several studies have been conducted to ascertain the effects of PCB on growth of bacteria. One of these reported that the growth of 26 isolates out of 85 estuarine bacteria was inhibited (in various amounts) by 0.5 mg of either Aroclor® 1016 or 1242 per milliliter solvent on saturated paper disks.[11] The identified genera sensitive to PCBs, as reported by these authors, included *Flavobacterium*, *Bacillus*, *Corynebacterium*, *Pseudomonas*, *Achromobacter*, *Micrococcus*, and *Serratia*, but a slightly greater percent of the sensitive strains were Gram

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Table 1
CHEMICAL DISTRIBUTION OF ^{14}C-PCB AMONG CELLULAR FRACTIONS OF TEST ORGANISMS AFTER GROWTH IN GBSB FOR 72 HR

Cellular fractions	*Serratia liquefaciens*[a] (%)	*Pseudomonas aeruginosa*[a] (%)	*Bacillus sp.*[b] (%)	*Bacillus sp.*[b] (%)
Cold TCA soluble (transient intermediates)	0.7	1.1	3.80	9.87
Alcohol soluble (lipids and proteins)	42	50	67.36	59.93
Alcohol-ether soluble (lipids and proteins)	57	48.5	19.59	17.07
Hot TCA soluble (nucleic acid)	0.1	0.3	5.28	5.95
Residual precipitate (protein)	0.2	0.1	3.05	7.19

[a] Results are average of 4 experiments and computed as percent total activities recovered in all fractions.
[b] Results are average of 2 experiments and computed as percent total activities recovered in all fractions.

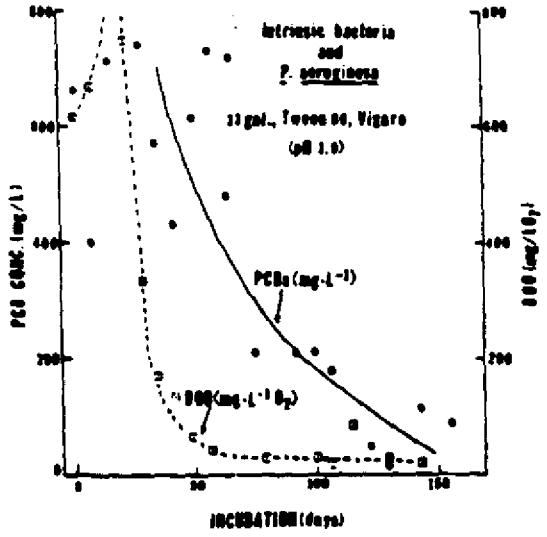

FIGURE 1. Biodegradation of PCB 1248 in reactor system as a function of incubation (37°C) time.

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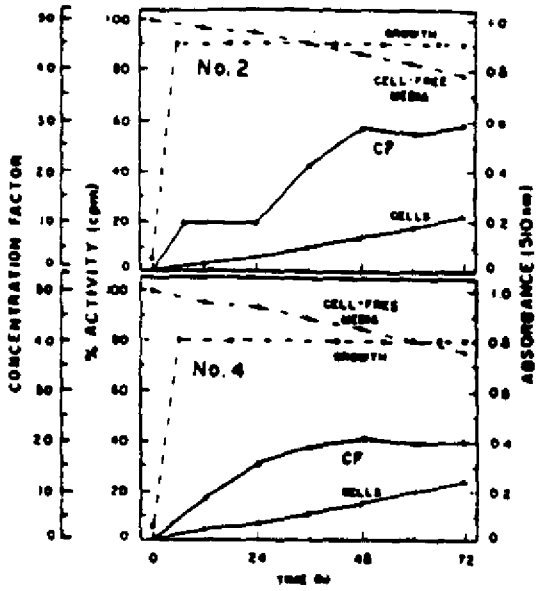

FIGURE 2. Uptake and concentration factor of ^{14}C-PCB by *Bacillus* spp. no. 2 and 4 during growth (at 510 nm). Initial activity of cell-free media = 100%.

positive.[11] The important correlation brought out by this study was that the inhibition by PCBs of specific heterotrophic bacteria could limit the normal turnover of carbon in estuarine sediments.[11] The data for growth, uptake, and CF of ^{14}C-PCB by PCB-resistant *Bacillus* culture nos. 2 and 4, following incubation in glucose basal salt broth (GBSB) containing 200 μg nonradioactive Aroclor® 1254 are presented in Figure 2.[12] Very little effect was noted on the growth of these organisms.[12] Maximal uptake of 22% for culture no. 2 and 24% for culture no. 4 was reached after 72-hr incubation with a CF of 28 and 21 for these bacteria, respectively (CF = specific activity of bacterial cells [cpm/g]/specific activity of medium [cpm/g]).[13] The increase in uptake of ^{14}C-PCB as a function of time during growth was accompanied by a decrease of ^{14}C-PCB in the cell-free media.[12] However, two genera of lake waste bacteria (*Achromobacter* and *Pseudomonas*) were not inhibited by concentrations up to 0.1% of Aroclors® 1221, 1242, and 1254.[13] Stimulated bacterial growth has been noted in the presence of Aroclors® 1242 and 1254.[14-16] Growth was inhibited by Aroclor® 1254 in two open-ocean isolates, one a pseudomonad and the other a tetrad-forming coccus.[17] Growth of the pseudomonad culture in the 10 μg Aroclor® 1254 per liter media had an altered filamentous morphology.[17] Two species of bacteria (*Serratia liquefaciens* and *Pseudomonas aeruginosa*) grew well in the presence of 200 μg PCB per milliliter media (Figure 3).[9] These cultures were also able to resist high levels of PCB in phosphate buffer (Figure 4).[9]

Ten different colony types of aerobic, Gram-negative motile rods were grown in parachlorobiphenyl (pCB) or biphenyl (BP) as the sole carbon source and analyzed for an increase in the number of colony-forming units (CFU) as well as for a concomitant disappearance

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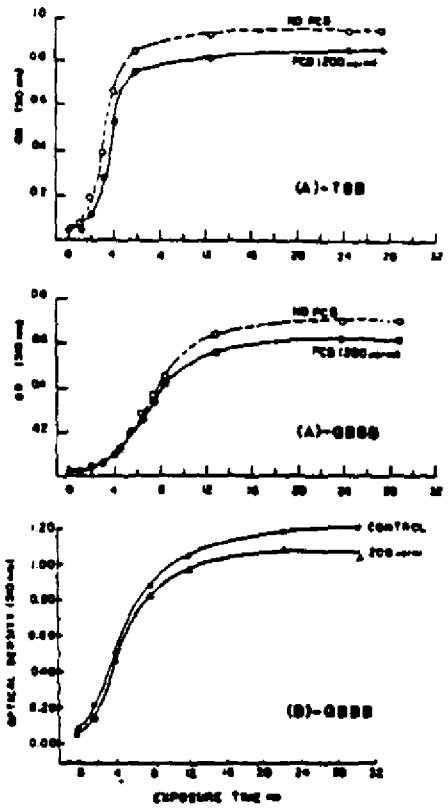

FIGURE 3. Comparative effects of Aroclor® 1254 on growth of (A) *S. liquefaciens* and (B) *P. aeruginosa* as measured spectrophotometrically during incubation (37°C).

of the substrate (pCB or BP).[16] The strains showed an increase of about 2 log counts with an accompanying decrease in extractable pCB or BP from the medium.[16] In a few cultures, the disappearance of pCB was associated with the appearance of possible transient intermediates which were either yellow or gray in color.[16] These observations indicated that the strains isolated were not merely producing a cellular exudate that solubilized pCB, but were able to partially degrade the molecule.[16]

A similar but earlier study yielded more conclusive results on the degradation of pCB and BP by bacteria. Two species of *Achromobacter* were isolated from sewage effluent that used BP and pCB, respectively, as sole carbon sources.[19] The *Achromobacter* spp. were named in accordance to their respective utilizable carbon sources. The *Achromobacter* BP, grown on biphenyl, accumulated a product with a UV absorption maximum at 257 nm which was not identified.[19] Both bacterial isolates produced *meta* cleavage products by fission of the benzene ring and benzoic and *p*-chlorobenzoic acids were produced from the degradation of BP and pCB, respectively, by *Achromobacter pCB*.[19] One of the intermediates in the

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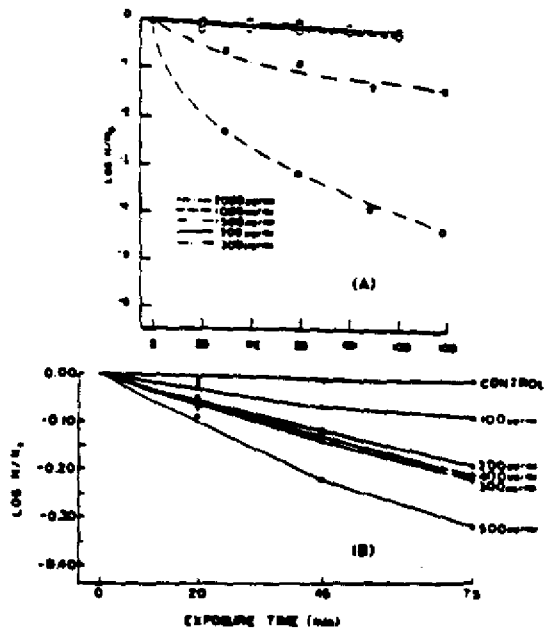

FIGURE 4 Comparative survival of (A) *S. hyperfarsus* and (B) *P. aeruginosa* in phosphate buffer and Aroclor® 1254 incubated at 37°C for various intervals (min).

degradation process leading to benzoic acid is possibly a diol, which means that oxygenation of the parent molecule had occurred.[30] Mixed bacterial cultures isolated from activated sludge when grown with 4,4'-dichlorobiphenyl as the only carbon source present produced 4-chlorobenzoic acid and 4,4'-dichloro-2,3-biphenyldiol as degradation products.[31] However, no hydroxylated or carboxylic acid metabolites could be isolated when 2,4',5-tri-, 2,2',5,5'-tetra-, 2,2',3,4,5'-penta-, 2,2',3,4,5,5'-hexa-, and decachlorobiphenyl were used as the sole carbon sources with the same cultures.[31] Another group of researchers found that a bacterial culture isolated from lake water in Ontario degraded Aroclor® 1242 with the major metabolites being aliphatic and aromatic hydrocarbons.[32] Here, no evidence was found to indicate that any metabolite contained chlorine.[32]

An *Alcaligenes* spp. was isolated that could metabolize various PCB components including a pentachlorobiphenyl through an oxidative route that included the production of colored metabolic intermediates.[33] Tetrachlorobiphenyls were also degraded by three mixed microbial cultures that included *Alcaligenes odorans*, *Alcaligenes denitrificans*, and an unidentified Gram positive bacterium.[34]

When microorganisms are utilizing one compound as an energy source, co-metabolism of another compound may occur.[35] Co-metabolism is described as the process in which a microorganism oxidizes a substance without being able to utilize the energy derived from this oxidation to support growth.[35] Co-metabolism of PCBs in the presence of sodium acetate showed enhanced degradation of higher chlorinated isomers.[36] These authors found that when the microorganisms were allowed to grow on PCBs alone, little additional biomass

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was produced because of the limited amount of carbon present in the medium.[14] However, when acetate or carbohydrates were present, the microorganisms were utilizing acetate for growth and oxidizing the PCBs slightly.[14] This author established that if the number of microorganisms increased greatly, even a slight oxidation of PCBs by each microorganism would lead to a more complete degradation of PCB molecules.[14] Thus, more rapid degradation could be attributed also to the other species of microorganisms (in a mixed culture) removing potentially harmful intermediates.[14]

Mammals oxidize aromatic hydrocarbons to arene oxides which yield *trans*-arenediols by enzymatic hydration.[16] Many researchers assumed that bacteria also oxidized aromatic hydrocarbons through *trans*-arenediols.[16] However, research has shown that *cis*-hydroxylation is a common reaction in bacterial degradation of aromatic hydrocarbons.[17] Unsubstituted biphenyl can be hydroxylated in the 2,3-positions and then a *meta*-type cleavage of the aromatic ring can occur.[18] An acid lactone metabolite was isolated from mixed cultures of near-shore marine bacteria, but no evidence of complete mineralization of ^{14}C-labeled tetrachlorobiphenyl to CO_2 was observed by one group of authors.[19]

III. UPTAKE OF PCBs BY YEAST AND FUNGI

In 1979, Tejedor et al.[20] ascertained the response of *Saccharomyces cerevisiae* grown in fermentable (1% glucose) and nonfermentable (3% ethyl alcohol) substrates in the presence of 25 μg Aroclors® 1232, 1242, 1248, 1254, and 1260 per milliliter media. In fermentable media, all Aroclors® showed an inhibitory effect on yeast growth.[20] The intensity of this inhibition followed an order inversely related to chlorine content of the Aroclor®.[20] In nonfermentable media, the two lesser chlorinated Aroclors® inhibited growth much more than the same in fermentable substrates.[20] The higher chlorinated PCBs stimulated yeast growth.[20] The main conclusion these authors drew was that since PCBs disturb the electron transport system at the cytochrome level of the respiratory chain, if both types of substrates were equally available to a fermentative *Saccharomyces* strain, the presence of PCBs would force the microorganism to use the fermentative pathway for obtaining energy instead of the Krebs cycle.[20] The yeast, *Rhodotorula rubra*, showed bioconcentration factors of 307 ± 63 for biphenyl and 1547 for 4-chlorobiphenyl.[21]

The presence of PCBs can alter the growth patterns of fungi. The dry mycelium weight of *Aspergillus flavus* in liquid cultures decreased as the dose rate rose from 5 to 50 μg Aroclor® 1254 per milliliter medium.[22] The magnitude of this effect was greatest for Aroclor® 1232 and lowest for Aroclor® 1260.[22] In 1980, Schwartz et al.[23] examined 15 species of fungi for their ability to hydroxylate biphenyl. Ten of these fungi produced 4-hydroxybiphenyl and seven of the ten also produced 4,4'-dihydroxybiphenyl.[23] The most efficient strains were *Absidia pseudocylindrospora* NRRL 2770 and *Absidia* spp. NRRL 1341.[23]

IV. UPTAKE OF PCBs BY PHYTOPLANKTON

In 1980, Biggs et al.[24] studied the uptake of ^{14}C-PCB (approximately 54% chlorine by weight) by mixed species of estuarine phytoplankton and naturally occurring particulate matter.[24] The natural particulate assemblages, dominated by the centric diatom *Skeletonema costatum*, had a calculated index of sorption (the ratio of ^{14}C-PCB sorbed onto particles of phytoplankton to that in an equal volume of water) averaged $2 \pm 1 \times 10^6$.[24] Another marine diatom, *Cylindrotheca closterium*, absorbed and concentrated Aroclor® 1242 900 to 1000 times the level in sea water.[25] At 0.1 μg PCB per milliliter sea water, the Aroclor® 1242 inhibited growth and diminished levels of RNA and chlorophyll of this diatom.[25] Other authors found that both Aroclor® 1242 and 1254 were toxic to phytoplankton populations at levels as low as 1 to 2 μg PCB per liter of water.[26] The two Aroclors® showed LC_{50}

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values of 6.5 μg PCB per liter and 15 μg PCB per liter, respectively, for phytoplankton.[16] Another group of researchers investigated the effects of PCB on the growth, photosynthesis, and cell size of estuarine phytoplankton and reported that 5 or 10 μg Aroclor® 1254 added daily to 1 ℓ dialysis cultures consisting of mixed species of phytoplankton reduced algal growth rate and ^{14}C-uptake per unit of chlorophyll a.[17] These authors stated that PCB inhibited growth of large centric diatoms and microflagellates (greater than 8 μm in size) by a reduction in cell division and chlorophyll a synthesis, thus causing community composition to shift in favor of small-size algae.[17]

Lab measurements were obtained concerning the relationship between ambient concentrations of PCBs and the amounts adsorbed and absorbed by phytoplankton.[18] Time-course studies conducted by these authors revealed concentration factors greater than 10^7 on a volume per volume basis.[18] Rapid uptake and the short time required for equilibrium suggested that the uptake process involved adsorption rather than absorption. These researchers also stated that phytoplankton are capable of accumulating substantial amounts of PCBs, and therefore constitute an important means for the introduction of these compounds into marine food webs.[18]

In 1982, a mathematical model of PCB (Aroclor® 1254) bioaccumulation in plankton was devised.[19] In batch experiments exposing individual plankton constituents to Aroclor® 1254, the rate at which the organism approaches partitioning equilibrium appears to be partly size-dependent while the extent of PCB accumulation is species-specific.[19] When ingestion rates exceed desorption and excretion rates, a consuming organism will accumulate PCB above the levels predicted by equilibrium partitioning relationships.[19]

One author found that tetrachloro- and hexachlorobiphenyl isomers were concentrated by dead *Chlorella pyrenoidosa* cells by factors of 6000 to 15,000.[20] These researchers reported that these amounts were greater than that noted in living cells, and pointed out that since concentration of PCBs did occur in dead cells, it was due to a possible chemical partitioning between the water of the medium and the hydrophobic lipids of the cell.[20]

The presence of PCBs reduces phytoplankton biomass and size.[21] These changes could increase the number of trophic levels and divert the flow of biomass from harvestable fish to gelatinous predators.[21] The presence of PCBs can cause other alterations in community composition.[21] Distortion of chloroplast lamellae and increased cytoplasmic vacuole formation plus gross cell morphology changes in algal cultures treated with 50 μg PCB per liter media have been noted.[15]

Aroclor® 1254 can reduce growth rates and population densities in a ciliate, *Tetrahymena pyriformis*.[22] Thus, the availability of these protozoa as food organisms and nutrient regenerators would be reduced and that would permit the toxicant to enter and be translocated through aquatic food chains.[22]

V. UPTAKE AND BIOCONCENTRATION OF PCBs BY OTHER AQUATIC INVERTEBRATES

Young oysters (*Crassostrea virginica*) were continuously exposed to Aroclor® 1254 for 24 weeks by Lowe et al.[15] in 1972. These authors observed that the growth rate was significantly reduced in the presence of 5 μg PCB per liter water with the greatest PCB residue being 425 mg PCB per kilogram (whole body) or 85,000 times the concentration in the water.[15] In oysters exposed to 1 μg PCB per liter water, the greatest residue was 101 mg PCB per kilogram (101,000 times the concentration in the water).[15]

Clams which were fed Aroclor® 1254 adsorbed onto the surface of alumina particles at 1.25 and 12.5 parts/10^6 sea water for 21 days showed a maximal concentration factor of 1.8×10^3 with visceral mass:muscular foot residues less than 5.1 in general.[23]

One study in 1982 compared PCB (Aroclor® 1242) uptake by fiddler crabs (*Uca pugnax*)

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from ingested detritus and from contaminated water.[47] These authors reported that PCB accumulation from detritus was more rapid than from water alone.[47] The rate of accumulation from detritus was approximately linear (1 μg PCB per day per animal).[47] The rate from water was 0.1 μg per PCB per day per animal.[47] Looking at the data from a weight standpoint, the PCB concentration by the animals was independent of the weight of the animal, and PCB concentration of the animals was inversely related to the animals' weight.[47] This second relationship was the result of PCB dilution in larger crabs as compared to smaller ones.[47] Relative differences in gestation rates plus the water concentration of a pollutant control the PCB accumulation and concentration by the fiddler crabs.[49]

The uptake from sediments by a PCB mixture (Phenoclor® DP-5) by the benthic worm *Nereis diversicolor* is dose dependent, attaining equilibrium concentration factors of approximately 3 to 4 after 2 months.[49] In a comparison study, the accumulation of PCBs from water by this worm was much more rapid; concentration factors reached approximately 80 after only 2 weeks.[49] Calculations based on experimentally derived PCB concentration factors and ambient PCB levels in sediments and water suggested that sediments contributed the bulk of these compounds to the worms.[49] After 5 days in sediments containing 1 μg PCB (Aroclor® 1254) per gram, *Arenicola marina* and *Nereis diversicolor* contained 0.24 ± 0.08 and 0.36 ± 0.12 μg PCB per gram worm (wet weight), respectively, and there was significant, although smaller, increase in residues to 0.39 ± 0.2 and 0.49 ± 0.25 μg PCB per gram worm after a further exposure of 5 days.[49]

Nymphs of the mayfly (*Ephemera danica*) were used to study kinetics of uptake and degradation of Clophen® A50.[51] Accumulation of PCB in the insects followed a first order kinetic equation with equilibrium being reached after 4 to 5 days, and no degradation of PCB was observed.[51]

Crayfish, *Orconectes nais*, were observed to be more susceptible to Aroclor® 1242 (7 day LC_{50} = 30 μg PCB per liter water) than they were to Aroclor® 1254 (7 day LC_{50} = 80 μg PCB per liter water).[52] The uptake of PCB was linear, and PCB residues in the crayfish did not reach equilibrium after a 28-day exposure to Aroclor® 1254.[52]

In 1974, Nebeker and Puglisi[53] studied the effect of PCBs on survival and reproduction of *Daphnia*, *Gammarus*, and *Tanytarsus*.[53] Continuous-flow and static bioassays were conducted with survival and reproduction as measures of relative toxicity of the following eight PCBs: Aroclor® 1221, 1232, 1242, 1248, 1254, 1260, 1262, and 1268.[53] These authors reported that Aroclor® 1248 was the most toxic to *Daphnia magna* of the eight forms tested in static tests and that after 3 weeks, the LC_{50} was 25 μg Aroclor® 1248 per liter water.[53] Aroclor® 1254 was the most toxic to *Daphnia* in continuous-flow tests with a 3-week LC_{50} of 1.3 μg PCB per liter water.[53] After 96 hr the LC_{50} values for 1242 and 1248 on *Gammarus pseudolimnaeus* in continuous-flow tests were 73 and 20 μg PCB per liter, respectively.[53] Tissue residues in *Gammarus* ranged from 4 μg PCB (1254) per gram tissue in controls to 552 μg PCB (1248) per gram tissue in scuds held for 60 days in water containing 5.1 μg 1248 per liter water.[53] *Gammarus pulex* was used as an indicator organism in a study on the presence of DDT and PCB in south Swedish streams.[54] These authors stated that there are two ways of attaining a particular level of a persistent substance in an organism: the first by progressive accumulation, the rates of excretion and metabolism being more or less negligible, the second by reaching equilibrium between uptake and excretion (with or without preceding metabolic breakdown) at a certain level.[54] In the first case, the concentration of the residue will rise continuously with age, while, in the second case, no further rise will occur after equilibrium has been reached.[54] The level at which equilibrium is attained is a function of the rates of uptake and elimination of the substance.[54]

When 3- and 5-day-old mosquito larvae, kept at 27°C were exposed to ^{14}C-PCB in water, the percent uptake by 3-day-old larvae gradually increased during incubation to 5.1% after 94 hr.[12] However, 5-day-old larvae showed a maximum uptake of 6.2% at 48 hr followed

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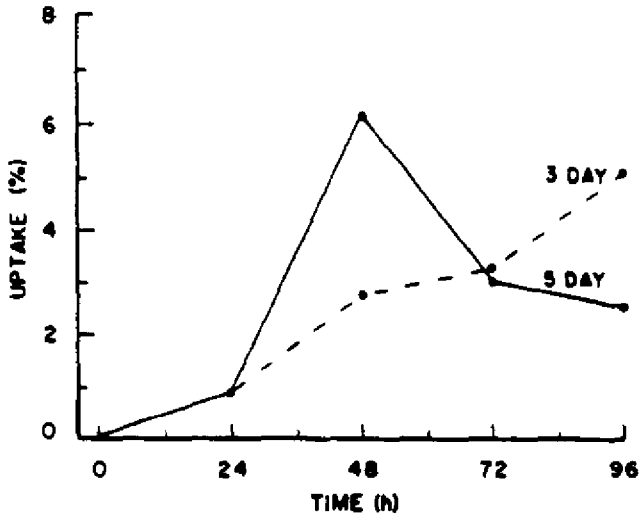

FIGURE 5. Uptake of ^{14}C-PCB by 3- and 5-day-old mosquito larvae.

by a decline thereafter to 2.6% at 96 hr (Figure 5).[11] Similarly, the CF by 3-day-old larvae increased gradually to 553 after 96 hr, whereas that of 5-day-old larvae reached its maximum at 48 hr of 638, followed by a decline at 96 hr (Figure 6).[12] These results indicate that both time of incubation and age of the larvae affected the uptake and CF by the larvae.[13] The decline in rates of uptake and CF by the 5-day-old larvae may be due to accelerated depuration.[13]

In 1976, Khan et al.[35] studied adsorption of Aroclor® 1254 onto unpeeled and peeled shrimp and found that unpeeled shrimp had more PCB adsorption potential than peeled shrimp.[35] These authors stated that PCB are likely to be adsorbed on the outer shell of chitinous material.[36] The toxicity of Aroclor® 1254 to adult, juvenile, and larval stages of the shrimp, *Palaemonetes pugio* was examined and it was observed that larval and juvenile shrimp were more sensitive to 1254 than adults.[36] The authors also reported that at a concentration of 15.6% μg PCB 1254 per liter water, 75% of the larvae died on the 5th and 6th days of exposure and all died within 11 days.[36] Juvenile shrimp showed 96 hr LC_{50} values ranging from 6.1 to 7.8 μg PCB 1254 per liter water.[36]

Fish, shrimp, and oysters were exposed to Aroclor® 1254 in acute (96 hr or less) laboratory experiments.[37] In another test conducted by these authors, juvenile pink shrimp and blue crabs were exposed to concentrations of Aroclor® 1254 for 20 days.[37] No effect was noted on juvenile pinfish exposed to 100 μg PCB per liter water for 48 hr.[37] However, there was 100% mortality of juvenile pink shrimp at the end of 48 hr exposure to 100 μg PCB per liter water.[37] Shell growth of oysters exposed to 100 μg PCB per liter water for 96 hr was completely inhibited.[37] The oysters did live, and after 3 weeks in PCB-free water, their shell growth equaled that of the controls.[37] The chronic toxicity of Aroclor® 1254 to grass shrimp was ascertained and concentration factors were reported ranging from 3000 to 13,000.[38] These investigators also stated that whole body concentrations produced after 5 weeks exposure to 0.04, 0.09, and 0.62 g PCB 1254 per liter water ranged from 200 to 26,000 times the concentration in test water and that the concentrations did not reach equilibrium.[38]

PCB (Aroclor® 1254) concentrations in Atlantic zooplankton on the continental shelf and

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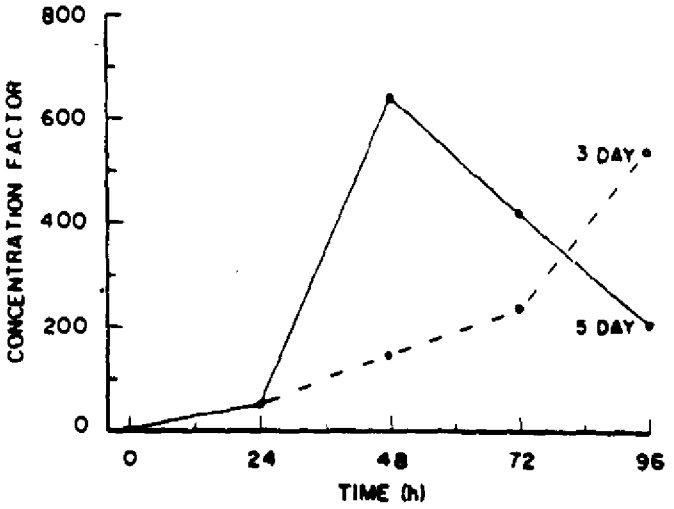

FIGURE 6. Concentration factor of ^{14}C-PCB by 3- and 5-day-old mosquito larvae

slope ranged from 2.4 to 260 µg PCB per milliliter water.[39] The author assumed that the PCB present in the zooplankton was in physical-chemical equilibria with that in the ambient aqueous environment.[39] These results were confirmed in 1977 when Clayton et al.[40] stated that bioaccumulation of PCBs in zooplankton was controlled by equilibrium partitioning of the chemical between the internal lipid pools of the biota and ambient water.[40] Amplification factors of 10^6 were determined in terms of lipid-water concentration ratios.[40] These researchers seemed to think that for pelagic biota at the lower trophic levels, food chain biomagnification was not a controlling factor in the zooplankton residue levels.[40]

The rate of accumulation and biomagnification of ^{36}Cl-Aroclor® 1254 from water by eight species of aquatic invertebrates was examined.[41] The organisms tested were four species of crustacea: scud, grass shrimp, crayfish, and a daphnid, along with four species of immature aquatic insects: dobsonfly larvae, mosquito larvae, stonefly naiads, and phantom midges.[41] The daphnid, *Daphnia magna*, accumulated total body concentrations 48,000 times greater than those in water.[41] Mosquito larvae exhibited a 12,600-fold magnification within 24 hr.[41] This accumulation continued until the 7th day of exposure when the larvae metamorphosed into pupae.[41] At 7 days, the magnification factor was 20,000 in the mosquito larvae.[41] Grass shrimp had a magnification factor of 16,600 after 21 days exposure.[41] Scud showed a magnification factor of 27,000 after 21 days of exposure.[41] PCB residues taken up by scud were characterized by progressively greater uptake of lower chlorinated isomers over time.[41]

There is more evidence to substantiate the theory that the rate of uptake decreases with PCB isomers of increasing chlorine content.[42] However, other data indicate that organisms tend to accumulate the more highly chlorinated PCB isomers since these are much more difficult to metabolize and excrete.[43]

It has been reported that PCBs are carried to ocean waters adsorbed onto suspended organic particles in river water.[4] Sewage sludge as well as polluted dredged spoils being dumped near coastal areas also contribute to the concentration of PCBs in the ocean.[4] Again, it was pointed out that most of the PCBs carried to the ocean either remain in solution or

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are adsorbed onto suspended solids, so it is probable that most of the PCBs delivered to coastal regions are deposited in the sediment.[4] Several studies have been conducted on the flux of PCBs between water and sediment. Particle size distribution and total organic carbon seem to be important factors in determining the extent of partitioning of two tetrachlorobiphenyls to natural sediments.[60-62] The dissolved concentrations of Aroclors® 1016 and 1242 are low, so when highly sorbed to sediments, these PCBs would be transported in association with particulate matter.[60] If the PCBs are not highly sorbed, volatilization does occur.[60] Atmospheric transport and deposition can dominate PCB input to lake sediments.[61] Uptake of PCBs in sediments is governed by equilibrium partitioning between suspended phases and ambient water and the adsorption of Aroclor® 1254 from sea water by sediments is influenced by the sediment type; this also affects desorption from sediment to sea water.[60] The difference between adsorption and desorption isotherms indicates that adsorption of PCBs is relatively irreversible; PCB is firmly bound to the sediment.[60]

VI. FISH: UPTAKE AND DEGRADATION OF PCBs

Spot fish, *Leiostomus xanthurus*, were exposed to 1 μg Aroclor® 1254 per liter water for 56 days.[70] The maximum concentration in the whole fish was 3.7×10^4 times that in the water with the liver concentrating the greatest amount.[70] The accumulation of Aroclor® 1248 and 1254 by bluegills exposed to 2 to 10 μg PCB per liter water was 26,300 to 71,400 times the exposure levels for both PCBs.[70] Ninety-six-hr LC_{50} values for newly hatched and 3-month-old fathead minnows have been established.[71] The LC_{50} for Aroclor® 1242 in newborns was 15.0 μg PCB per liter water whereas, for 3-month-olds it was 300 μg PCB per liter water.[72] Growth of young fish was affected above 2.2 μg PCB 1249 per liter water, and none survived above 5.1 μg PCB 1248 per liter water after a 1-month exposure.[73] In a contrasting experiment, stimulated growth in adult minnows, *Phoxinus phoxinus*, fed food containing different concentrations of Clophen® A50 has been observed.[74] It was postulated that the PCB might have affected some hormone system in the fish, such as an increase in thyroid or pituitary activity.[74]

Biological accumulation of Aroclor® 1248 and 1254 by channel catfish was 56,370 to 61,190 times the levels in water after 77 days.[75] Coho salmon exposed to concentrations of 1254 up to 14,500 μg/kg body weight per day showed no effects of growth.[75] After 260 days, however, signs of toxicosis appeared and all fish exposed to the highest concentration were dead within 5 days thereafter.[75]

In 1974, Hansen et al.[77] determined chronic toxicity to and uptake of Aroclor® 1016 by pinfish (*Lagodon rhomboides*).[77] The PCB was not toxic to the fish at 100 μg PCB per liter water for 96 hr, but significant mortality occurred when pinfish were exposed to 32 μg PCB 1016 per liter water for 42 days.[77] Guppies exposed to Aroclor® 1254 died after 7 days incubation in the presence of 20 μg PCB per milliliter water.[13] Guppies incubated in beakers containing either 5, 10, or 15 μg Aroclor® 1254 per milliliter water lived longer but all of these died within 3 weeks.[13] Direct uptake of ^{14}C-PCB by these guppies during incubation at 27°C increased gradually to reach 3.6% after 96 hr with a CF of 198 (Figure 7) at that time.[13]

Figure 8 depicts data for the direct uptake and CF of ^{14}C-PCB by cichlid organs.[13] The head and fins accumulated the greatest percentage of ^{14}C-PCB in water.[13] After 72-hr exposure, uptake by the head decreased to 28% probably due to washing and/or depuration, while the uptake by fins remained fairly constant at 15.5%.[13] On the other hand, the liver showed an increasing uptake reaching 18.6% after 72 hr.[13] The CF by various cichlid organs tended to also increase over time.[13] The spleen, fins, liver, and muscle exhibited the highest CF values after 72-hr exposure to ^{14}C-PCB in water with values ranging from 1862 in spleen, 268 in fins, 173 in liver, and 164 in muscle tissue.[13]

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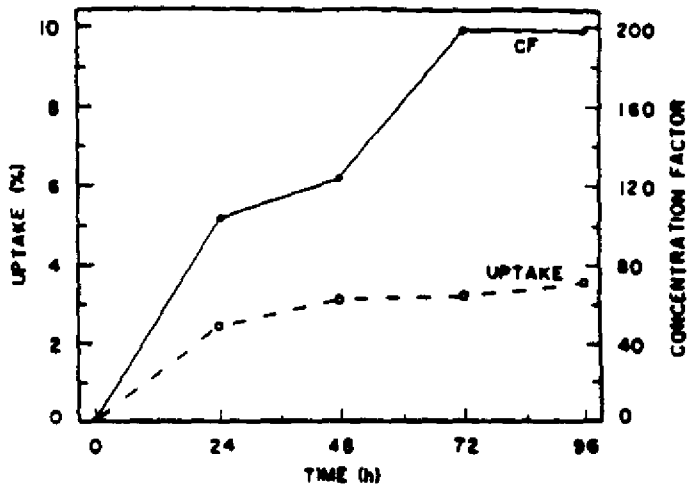

FIGURE 7. Uptake and concentration factor of ^{14}C-PCB by guppies.

Pinfish were exposed to 1 μg PCB per liter water for 56 days. The accumulation factor (maximum whole-body residue) was 17,000 times the nominal concentration in test water.[74] These authors also determined the bioconcentration factor of a tetrachlorobiphenyl isomer in rainbow trout muscle after 5 days exposure was 9550 ± 1610.[74] The average bioaccumulation factor for hexachlorobiphenyl in killifish, *Fundulus similis*, after 11 days exposure was 375.[73]

There are certain factors within the different fish species themselves that determine PCB accumulation factors. Among these are age, weight, percent lipids, and feeding habits.[44] One investigator reported that the concentration of PCBs progressively increased with maturity in a series of lake trout.[76] The effect of age on accumulation of Phenoclor® DP6 in gray mullets showed that DP6 concentrations in 3.5-year-old fish were always higher than those in 2.5-year-old fish when both groups were fed a dry fish diet contaminated with 50 μg DP6 per gram food.[77] PCB concentration in fish can be dependent on the amount of food ingested.[77] Two other studies also indicated that fish accumulate PCBs on a weight-ratio basis.[78,79]

The accumulation of Aroclor® 1016 by fish (Hudson River) in 14 days was 15,000 times the water concentration, and the amount of PCBs accumulated by the different species of fish was closely related to the fat content and weight of the fish.[80] Female fathead minnows accumulated about twice as much PCBs as the males due to a greater amount of lipid in the females.[81] Fish species that contain a greater percent lipid body weight tend to accumulate more PCB.[82] Concentrations of PCB (Clophen® A50) in the fat tissue of goldfish were calculated to be more than 10,000 times greater than the PCB concentrations in the test water.[83]

The concentration coefficient of Phenoclor® DP6 from water and from food in juvenile gray mullets was measured.[84] There was higher accumulation of PCB from water than from food.[84] Other studies supported this premise[12,85,86] although one author stated that in the environment the kinetics of residue uptake from dietary sources could be more important because PCBs have a high affinity for sediments and particulate matter.[12]

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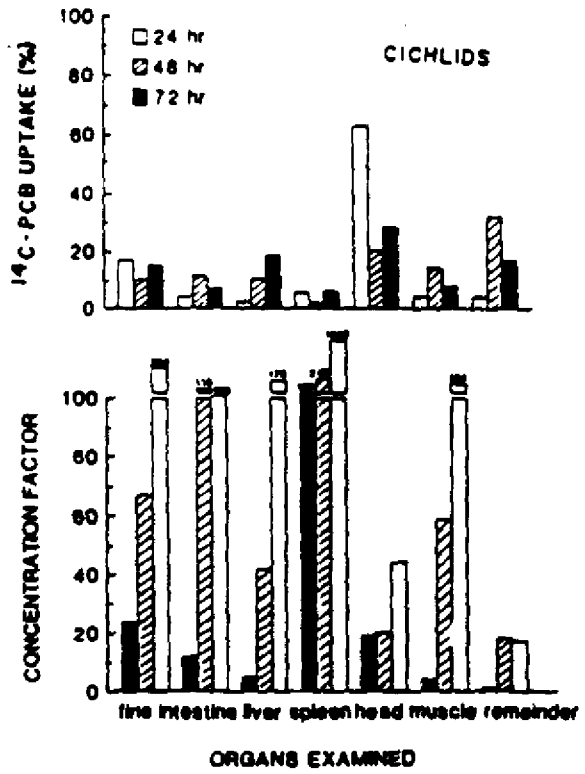

FIGURE 8. Uptake and organ distribution and concentration factor of ^{14}C-PCB by cichlids.

Not many researchers have concrete evidence that fish can metabolize PCBs, but there are a few.[87-89] These different authors were able to isolate hydroxy metabolites from fish (the green sunfish, rainbow trout, and goldfish) exposed to certain tri- and tetrachlorobiphenyls.[87-89]

VII. METABOLISM OF PCBs BY BIRDS AND MAMMALS

Bobwhite quail were fed Aroclor® 1254 for 14 days and significant alteration of certain PCB isomers appeared to have taken place.[90] These authors showed that dechlorination of the molecules was apparent and isomeric transformation was suggested.[90] Pigeons fed wheat contaminated with 500 μg Aroclor® 1242 per gram wheat metabolized the lower chlorinated isomers at a faster rate.[91] A mono-, di-, and tetrachlorobiphenyl was transformed into monohydroxylated derivatives by the pigeon and rat, but no hydroxylated products of a hexachlorobiphenyl were detected from either.[92]

In rats, mono-, di-, and pentachlorobiphenyls can be metabolized to more polar compounds and then excreted in the bile.[93] Two adjacent unsubstituted carbon atoms are important to

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the metabolism of PCBs because their presence facilitates the formation of arene oxides.[94] Other studies also showed that rats were capable of metabolizing di-, tri-, and pentachlorobiphenyls to more polar components via hydroxylation.[95-97]

The biological half-lives of tetrachlorobiphenyls have been studied. It seems that the half-lives of some isomers were determined by the rate of releasing PCB from body fat rather than by the rate of its metabolism.[96] Rabbits were fed food pellets contaminated with 1% hexachlorobiphenyl for 7 days to give a total concentration of 1 mg PCB per kilogram feed.[97] The urine showed the presence of two major metabolites formed from the HCB by hydroxylation and hydroxylation with concomitant dechlorination, respectively.[97] When male and female rhesus monkeys were given either 1.5 or 3.0 mg Aroclor® 1248 per kilogram body weight in a single oral dose, hydroxylated metabolites were formed in the liver and excreted in bile and urine.[98] Again, these authors showed that the lower chlorinated isomers were rapidly metabolized and excreted, while the highly chlorinated isomers remained in the fat tissue for more than 2.5 years.[98] A urinary metabolite (*trans*-3,4,-dihydro-3,4-dihydroxy-tetrachlorobiphenyl) was isolated that suggested metabolism occurs through an arene oxide intermediate.[98]

VIII. BIOLOGICAL HALF-LIFE AND DEPURATION OF PCBs BY VARIOUS ORGANISMS

The depuration of ^{14}C-PCB (Aroclor® 1254) by selected organisms representing a simple aquatic ecosystem (bacteria, mosquito larvae, guppies, and cichlids) was examined.[99] Each system was labeled by growing for 48-hr in ^{14}C-PCB medium at a specified temperature. They were then incubated in an isotope-free environment.[99] Two or three phases of depuration were noted in all systems (Figure 9).[99] Most phases followed first order kinetics but with different magnitudes. In bacteria, rapid depuration was noted on the 7th day followed by a slow rate (Table 2). No evidence of biological half-life (BHL) was observed during the entire experiment.[99] Larvae showed three depuration phases: −0.023/day (0 to 1 day), −0.086/day (1 to 2 days), and −0.0899/day (2 to 6 days) with a BHL of 5.5 days.[99] Guppies had depuration rates (k, slope of line) of −0.131/day for the first phase and −0.137/day for the 2nd with a BHL of 4.7 days.[99] Cichlids had a rapid rate during the 1st day (k = −0.102/day), followed by a slower one thereafter (k = −0.057/day) and a BHL of 6.1 days (Table 2).[99]

Experiments were also conducted to ascertain if depuration was due to excretion of PCB or by merely washing of PCB absorbed onto the cell wall. Data obtained (Figure 10) revealed that after 5 washes in pesticide-grade hexane, a total of 90.6 and 79.5% of the ^{14}C-PCB (based on initial activities as 100%) radioactivities were lost from the fresh and frozen cells, respectively. When the labeled cells were first washed (five times) with 0.05 *M* sodium phosphate buffer, a total of 44.3 and 25.4% of the ^{14}C-PCB radioactivities was lost from the fresh and frozen cells, respectively.[99] After the remaining five washes with pesticide-grade hexane, an additional 39.5% was lost from the fresh cells and 51.9% from the frozen.[99] This indicated that the percent of the ^{14}C-PCB lost by washing is dependent upon the condition of the cells, the solvent, and the number of washes.[99]

The benthic worm *Nereis diversicolor* eliminated Phenoclor® DP-5 at a rate which closely approximated a single exponential phase during the first 2 months following the exposure.[100] Thereafter, the loss rate appeared to decrease. These elimination rates were concentration dependent, with higher initial loss rates in worms containing higher levels of PCBs.[100] However, no significant loss in body load of Aroclor® 1254 by *Arenicola marina* and *Nereis diversicolor* during a postexposure period of 3 weeks was noted.[100]

In 1974, Nimmo et al.[101] studied the concentration and loss of Aroclor® 1254 in grass shrimp (*Palaemonetes pugio*). After several 1-week exposures to a range of concentrations

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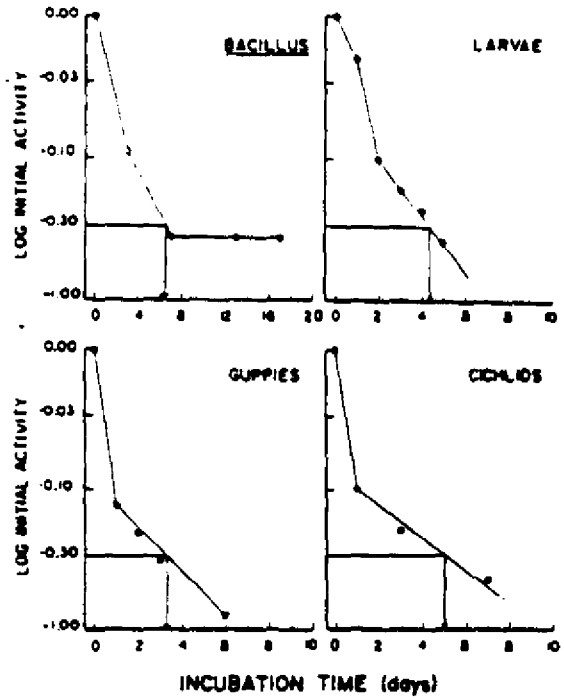

FIGURE 9. Depuration and biological half-life of ^{14}C-PCB in four trophic systems. Initial activity = 1.0.

of this PCB (0.17 to 9.1 μg PCB per liter), the concentration factors ranged from 3000 to 11,000.[58] Whole body levels after 5 weeks exposure to 0.04, 0.09, and 0.62 μg PCB per liter ranged from 200 to 26,000 times the PCB concentration in test water.[58] The concentrations did not reach equilibrium and from 60 to 90% of PCB 1254 was lost from shrimp within 4 weeks after exposure was stopped.[58]

The retention properties of PCBs were examined in the mussel (*Mytilus edulis*) over an 89-day period.[159] These authors reported that when the mussels were transferred from a site of relatively high PCB contamination to a site of relatively low PCB concentration in Puget Sound, Washington, the retention of PCB components increased with increasing chlorine content.[159] It is of interest to point out that the concentrations of PCB decayed logarithmically with calculated half-lives ranging from 3 days for a PCB component with 2 and 3 chlorines to 50 days for a PCB containing 6 and 7 chlorines.[159] Components chosen to best represent the commercial mixtures Aroclor® 1242, 1254, and 1260 were estimated to exhibit half-lives of 8, 23, and 39 days, respectively. The selective retention of the higher chlorinated biphenyls appears to be the result of lower water solubility of these components.[159]

Young oysters (*Crassostrea virginica*) were exposed to Aroclor® 1254.[46] Growth rate was significantly reduced in oysters exposed to 5 μg PCB per liter for 24 weeks.[47] Growth rate was not affected when oysters were exposed to 1 ppb for 30 weeks. When oysters were exposed to 5 ppb, the greatest PCB residue (whole body) was 425 mg/kg (ppm), 85,000

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Table 2
DEPURATION RATE (K, SLOPE OF LINE) IN VARIOUS TROPHIC LEVELS LABELED WITH ^{14}C-PCB

	Incubation time days	k Value[a] (day)	Correlation coefficient	Biological half-life (day) Graph[b]	Biological half-life (day) Calculated[c]
Bacteria	0—3	−0.0305	−1.0	6.4	10.46
(PCB-resistant)	3—7	−0.0863	−1.0		(0—17)
Bacillus spp.)	7—17	−0.0009	−0.8030		55.96
Mosquito larvae	0—1	−0.0229	−1.0	4.4	4.47
(*Aedes aegypti*)	1—2	−0.0859	−1.0		(0—9)
	2—5	−0.0099	−0.9707		4.35
Guppies	0—1	−0.1308	−1.0	3.3	2.47
(*Lebistes reticulatus*)	1—6	−0.1373	−0.9927		(0—6)
					2.54
Cichlids	0—1	−0.1024	−1.0	5.1	4.62
(*Cichlasoma facetum*)	1—7	−0.0574	−0.9988		(0—7)
					4.57

[a] Slopes were calculated from graphs of mutual activities vs. incubation time.
[b] From graph of log values.
[c] Regression analyses.

times the concentrations in the water, and less than 0.3 ppm was retained after 28 weeks depuration in PCB-free water.[45] When oysters were exposed to 1 ppb, the greatest residue was 101 ppm, 101,000 times the concentration in the water, and less than 0.2 ppm was retained after 12 weeks depuration.[45] Shell growth of oysters exposed to 100 ppb Aroclor® 1254 for 96 hr was completely inhibited.[37] However, the oysters did survive and their shell growth equaled that of controls after 3 weeks in PCB-free water.[37] When oysters were exposed to 10 ppb PCB for 96 hr and then placed in uncontaminated water for 4 days, their PCB residues had dropped to 33 ppm.[37] Oysters exposed to 1 ppb for 96 hr and then transferred to PCB-free water for 4 days showed PCB residues of 8.1 ppm.[37]

Aroclor® 1254 adsorbed onto the surface of alumina particles at 1.25 and 12.5 ppb sea water was fed to hard-shell clams, *Mercenaria mercenaria* for 21 days.[46] The clams showed a maximum concentration factor of 1.8×10^3 with visceral mass:muscular foot residues of less than 5:1 in general.[46] Only the muscular foot of clams on a high dosage showed a diminution in PCB content after 6 months in clean seawater.[46] There was no change in tissue residues of clams held in clean seawater after exposure to 1.25 ppb PCB 1254.[46] However, in clams exposed to 12.5 ppb PCB 1254 there was a persistent significant decrease in the residues of the foot so that, after 6 months, the ratio of PCB in the visceral mass to the muscular foot was 10.6:1.[46] Feces collected from clams kept in clean water indicated that PCBs were being lost from the animals at a slow but consistent rate.[46]

Blue crabs (*Callinectes sapidus*) were exposed to Aroclor® 1254 for 20 days.[37] At this time, the crabs contained an average whole body residue of 23 ppm.[37] Residues in five crabs held in PCB-free water for 1 week averaged 22 ppm.[37] Six crabs held in clean water for 4 weeks, following the 20 day exposure, contained an average of 11 ppm — an example of the persistence of Aroclor® 1254 in animal tissue.[37]

Lobsters were fed 2,2',4,5'-tetrachlorobiphenyl (TCB) and 2,2,'4,4', 5,5'-hexachlorobiphenyl (HCB) in a 1:1 ratio applied to shucked mussel tissue at concentrations of 1.7 and 17 mg/kg (ppm) for each chlorobiphenyl.[101] Each lobster was fed every other day for 6 weeks followed by 6 weeks of feeding uncontaminated mussel tissue.[101] The lobsters were sacrificed at 2 week intervals and tissues (hepatopancreas, tail and claw muscle) were

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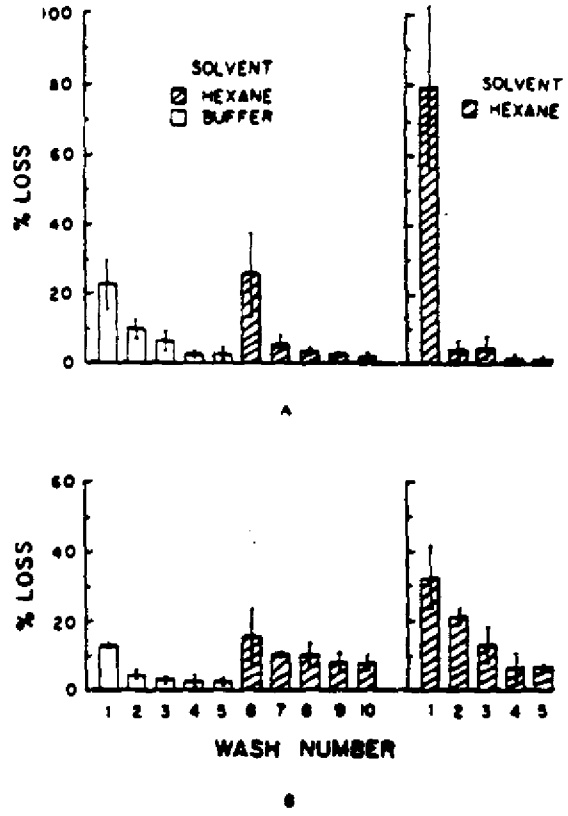

FIGURE 10. Loss of ^{14}C-PCB from bacterial cells by washing. (A) Data for fresh cells and (B) frozen (thawed) cells.

analyzed.[101] The concentrations of TCB and HCB in food were 0.60 and 0.55 mg/kg at low dosage feeds and 4.0 and 4.9 mg/kg at high dosage levels.[101] Data on the uptake and excretion of TCB and HCB in the hepatopancreas indicated that concentrations of these compounds approached an equilibrium within 4 weeks, except that HCB concentrations in high dosage feeds increased throughout the uptake phase.[101] Maximum CFs were 5.1 and 7.3 for TCB and HCB, respectively, at the high dietary level.[101] In all cases there was a decrease in the concentration of chlorobiphenyls in the hepatopancreas during the 6-week clearance period.[101] In low dosage tests, the concentration approached control values after 6-weeks clearance, and in high dosage tests, concentrations of TCB and HCB declined by approximately 60 and 40%, respectively.[101] Maximum concentrations for TCB and HCB in tail and claw muscle were lower than those in the hepatopancreas, and by the end of the excretion phase, concentrations in muscle tissues were approaching control values indicating that most of the residue had been eliminated.[101] In 1975, Bend et al.[102] studied the distribution and excretion of (^{14}C)-2,4,5,2',5'-pentachlorobiphenyl administered parenterally to lobsters.[103] The he-

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hepatopancreas contained much higher amounts of radioactivity (per milligram tissue) than any other organ and accounted for between 91 and 96% of the total radioactivity recovered at all times.[60] The half-life for radioactivity in tissues was estimated from semilogarithmic plots of the specific activity (dpm/mg) vs. time (day).[60] The tissues which had a $t_{1/2}$ of 3 weeks or longer were the hepatopancreas (45 days), egg masses (39 days), stomach (28 days), gills (24 days), and intestines (23 days).[60] Those tissues in which the $t_{1/2}$ was 1 week or less were the green gland, heart, tail muscle, claw muscle, male gonads, and plasma.[60] Over 95% of hepatopancreas extract showed that the PCB level was unchanged.[60] The very slow metabolism and excretion of PCB in the lobster are probably related to the very low microsomal mixed-function oxidase activities of lobster hepatopancreas.[60]

Adult minnows, *Phoxinus phoxinus*, were fed food containing Clophen® A50 in the following concentrations: control 0.09, low 25, medium 270, and high 2500 μg PCB per gram dry weight for 40 days.[71] The next 5 days were the "accumulation period", and the remaining 255 days of the experiment served as a "clearance period".[71] On day 45, the efficiency in uptake of PCB from the food varied between 22 and 36%.[71] Levels of total PCB corresponded to 1-, 5-, 100-, and 500-fold, respectively, that of the background levels of PCB in minnows from the catching area.[71] At day 300, the low, medium, and high levels showed decreases to 74, 38, and 35% of the level at day 45.[71] Stimulated growth in the minnows was noted; PCB might have had an effect on some hormone system, such as an increase in thyroid or pituitary activity.[71]

Fathead minnows (*Pimephales promelas*) were exposed to Aroclor® 1248 and 1260 to determine acute and chronic toxicity as well as bioconcentration of the PCB mixtures in the fish.[91] The bioconcentration factor for PCBs was independent of the PCB level in the water; in adult females at 25°C, it was approximately 1.2×10^5 for PCB 1248 and 2.7×10^5 for PCB 1260.[91] Females accumulated about twice as much PCBs as the males because of the greater amount of lipid in the female tissues. Exposed fish placed in untreated water eliminated less than 18% of the body burden after 60 days.[91] Fish exposed to PCB 1248 had eliminated only 18% of the original PCB burden after 28 days.[91] Fish exposed to PCB 1260 lost only 1% after 42 days.[91]

The adsorption, accumulation, and elimination of PCB (Clophen® A50) in goldfish have also been studied. When fish remained for 13 days in water containing 0.1 ppm Clophen® A50, a PCB concentration of about 70 ppm accumulated in the fish.[55] The fish were then transferred to freshwater to determine elimination.[55] The PCB content in the fish was halved during the first 3 weeks of the experiment, but the half-life for PCB increased during the experimental period.[55] Concentrations of PCB in fat tissue of the fish were calculated to be more than 10,000 times greater than PCB content in water.[55] Research conducted in 1982 showed guppies exhibiting half-life of ^{14}C-PCB (Aroclor® 1254) to be 3.3 days.[79] This same study also showed cichlids (*Cichlasoma facetum*) to have a half-life of ^{14}C-PCB of 5.1 days (Table 2).[79]

The rate and extent of bioaccumulation of HCB in killifish (*Fundulus similis*) continuously exposed to low concentrations of HCB have been determined along with the rate and extent of depuration in these animals.[78] After an 11-day exposure, HCB-exposed fish were removed from the dosing tank and placed into the depuration tank.[78] The HCB concentration decreased exponentially for 2 days; during this time, tissue concentration fell from about 70 to 1.8 mg/g; after 1 week tissue concentration in the depurated fish had leveled off at 0.8 mg/g.[78] The half-life for loss of HCB from the whole body was calculated to be 9.3 hr.[78]

The average bioaccumulation factor for HCB in *F. similis* was calculated to be 375.[78] The concentration in tissue fell exponentially with a half-life of 9 to 12 hr to within control levels.[78]

Data collected on the uptake, retention, and elimination of Aroclor® 1254 by larval striped bass (*Morone saxatilis*) showed that maximal transfer of ^{14}C-Aroclor® 1254 from water to

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fish was noted after 48 hr, at which time, the fish had accumulated 60% of the PCB available in the system.[76] PCB uptake between 24 and 48 hr was slow and became nearly asymptotic between 34 and 48 hr, suggesting that an equilibrium had developed.[76] No further uptake was noted between 48 and 120 hr.[76] After 48 hr of release, whole body concentrations of PCB were about 18% less than the concentration accumulated during uptake.[76] The 48-hr release concentrations were significantly less than the concentration levels after 24 hr of uptake, and significantly less after 24 hr of release.[76]

Spot fish (*Leiostomus xanthurus*) were exposed to Aroclor® 1254 (1 ppb) for 56 days and then placed in flowing water free of PCB for 84 days.[76] Residue analyses of pooled samples of brain, gills, liver, muscle, and remaining tissues were conducted.[76] Residues in all tissues were summed to compute levels in the whole fish.[76] The maximum concentration in the whole fish was 3.7×10^6 times that in the water. Liver concentrated the greatest amount.[76] After 84 days of flushing, the relative amount (parts per million) in the whole fish dropped 73% and the absolute amount in micrograms decreased 61%.[76]

Acute and chronic toxicity uptake and retention of Aroclor® 1016 to pinfish (*Lagodon rhomboides*) has been determined.[73] The PCB was not toxic to pinfish at 100 μg PCB per liter for 96 hr, but significant mortality occurred when pinfish were exposed to 32 μg PCB (Aroclor® 1016) per liter for 42 days.[73] Pinfish exposed to 1 μg PCB per liter for 56 days accumulated the PCB with maximum concentrations attained in whole fish by 21 to 28 days.[73] Maximum whole-body residue (wet weight) was 17,000 times the nominal concentration in test water.[73] After 56 days exposure to 1 μg PCB per liter, the pinfish were placed into PCB-free water for 56-day depuration. At this time, concentrations in the fish had decreased by 51%.[73]

Rainbow trout (*Salmo gairdneri*) were exposed to 1.6 and 9.0 μg 2,2',4,4'-tetrachlorobiphenyl per liter for 5 days, and then transferred to fresh water.[74] The bioconcentration factor at steady state was 9550 ± 1610 in trout muscle with a clearance half-life of 28 days.[74]

Residues of PCBs in lake trout ranging from 1 to 12 years old have been analyzed.[103] The residues were found to increase progressively with age reaching an average of 15.7 ppm PCBs (measured as Aroclor® 1254) in 12-year-old fish.[103] In 1978, lake trout of increasing age (6 to 12 years) were again sampled to study the persistence of these compounds in the fish.[103] Fresh weight concentrations in flesh of 12-year-old fish were about 13 ppm of PCBs, which showed that the concentrations had remained the same.[103]

The distribution and persistence of 2,5,2',5'-(^{14}C) tetrachlorobiphenyl in rainbow trout (*Salmo gairdneri*) for 140 days have been studied.[104] The trout were exposed to ^{14}C-TCB for 26 hr and then transferred to PCB-free water.[104] The distribution of ^{14}C was determined immediately following the exposure and at 1, 2, 7, 15, 28, and 56 days later.[104] Carcass muscle, skin, lower gastrointestinal tract, and fat contained most of the ^{14}C and a total of 88% of the radioactivity was detected in these tissues.[104] During the first 14 days, ^{14}C in adipose tissue, carcass, and eyes increased, possibly at the expense of other tissues.[104] The disappearance of ^{14}C from most tissues studied appeared to be a biphasic process.[104] Approximately 30% of the compound was eliminated after 2 weeks, but only a 6% loss was observed in the following 126 days.[104] The initial rapid loss of the ^{14}C-TCB had a half-life of 2.66 years.[104] The bile and blood initially contained relatively high concentrations of ^{14}C, but disappearance from these sites was rapid and nearly complete.[104]

In 1976, Hansen et al.[105] observed a slight net decrease (218 to 182 μg) in body burden of PCB in 56 days when channel catfish (*Ictalurus punctatus*) were no longer given Aroclor® 1242-contaminated feed after 84 days of exposure.[105] However, when fish were fed PCB for 140 days, there was no change in body burden after 56 days of PCB-free feed.[105]

The distribution and excretion of a (^{14}C)-pentachlorobiphenyl in the lobster and the dogfish shark have also been determined.[106] These authors reported that the specific radioactivity (dpm/mg tissue) was higher in dogfish shark liver than in any other tissue following the

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parenteral administration of PCB.[101] The delayed biliary excretion of radioactivity and the excretion of only very small quantities of biliary metabolites suggested that dogfish sharks biotransformed the dosed compound very slowly.[100] Unaltered PCB was not excreted in the bile and the biliary radioactivity was not converted to organic soluble material by β-glucuronidase hydrolysis indicating that glucuronide conjugates of phenols were not major biliary metabolites.[103]

The disappearance of radioactivity from blood followed a multiple exponential decay relationship with time.[102] The $T_{1/2}$ of ^{14}C-PCB was estimated for the three components of the curve. The α, β, and γ components of the radioactivity disappearance curve had $t_{1/2}$ values of 4, 27, and 730 min, respectively.[102]

Many marine species have some hepatic microsomal mixed-function oxidase activity, but this activity is generally considerably lower than that found in mammalian liver.[102] Reduced rates of oxidative xenobiotic metabolism in marine vertebrates and invertebrates would partially account for the persistence of lipophilic compounds, such as PCBs, in these species.[102]

In 1977, Mizutani et al.[95] studied all possible isomers of symmetrically substituted tetrachlorobiphenyls (TCBs) in connection with their structures. Twenty female mice were fed (*ad libitum*) a diet containing 10 ppm of each isomer for 20 days at which time treated diets were replaced with a control diet.[95] Biological half-lives were calculated from slopes and intercepts of regression lines. The liver was removed and analyzed separately from the remainder of carcass and showed the following:

2,3,2',3'-TCB	Carcass 0.9 days	
2,4,2',4'-TCB	Carcass 9.2 days	Liver 7.8 days
2,5,2',5'-TCB	Carcass 3.4 days	Liver 2.6 days
2,6,2',6'-TCB	No residues detected at end of 20 days' exposure	
3,4,3',4'-TCB	Carcass 0.9 days	
3,5,3',5'-TCB	Carcass 2.1 days	Liver 2.2 days

There were clear differences in the BHL of the isomers, but no apparent relationship between substitution pattern and BHL could be observed.[95] Lower storage ratio values for 2,3,2',3'-, 3,4,3',4'-, and 2,6,2',6'-TCB imply that these orientations of chlorine substituents are favored for ready metabolic degradation.[95] The actual magnitude of accumulation during the course of a prolonged daily exposure depends upon both the BHL and storage ratio of each compound.[95] For example, the BHL of 3,5,3',5'-TCB was at most two times that of 2,3,2',3'-TCB, whereas the former exhibited more than a 40-fold accumulation at the end of exposure.[95] This discrepancy can be accounted for by the large difference in storage ratio between these two isomers.[95] No apparent relationship between substitution patterns and BHC was observed.[95]

Later in 1980, Mizutani et al.[104] examined the biological half-lives of all symmetrically substituted HCB and decachlorobiphenyl isomers in mice and reported that the half-lives for HCB isomers were much greater than those for tetrachlorobiphenyl isomers previously studied.[95] However, 2,3,6,2',3',6'-HCB was rapidly metabolized and showed a very short half-life.[104] Mizutanis' 1977 study[95] did point out that the biological half-lives of some tetrachlorobiphenyls seem to be determined by the rate of releasing from body fat mass rather than by the rate of metabolism. Therefore, another likely explanation for the rapid decline of 2,3,6,2',3',6'-HCB may be offered by a certain physicochemical nature which permits easy releasing of this isomer from the fatty compartment.[104]

^{14}C-labeled 2,2',4,4',6-pentachlorobiphenyl was fed to rats for 8 days in their chow and organ and tissue samples taken at necropsy. The authors reported that the highest concentrations were in (1) abdominal fat, (2) subcutaneous fat, (3) lymph nodes, (4) ovaries, (5) adrenal, (6) sciatic nerve, and (7) liver.[98] The elimination of 2,2',4,4',6-PCB followed a

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biexponential rate expression with an α-phase half-life of 0.90 ± 0.1 and 0.95 ± 0.1 days for females and males, respectively.[92] The β-phase half-lives were 4.2 ± 0.1 and 3.8 ± 0.1 days for females and males, respectively. In 7 days, the males excreted 46.6% and the females 38.3% of the administered dose.[92] The majority of the excreted radioactivity was found in the feces: 83% in males and 81% in females.[92] Hydroxylated metabolic products were also found.[92]

Pigeons which were fed for 28 days with wheat dressed with 500 ppm Aroclor® 1242 showed muscle and fat with a steady logarithmic decay with half-lives of 50 and 125 days, respectively.[91] The brain exhibited a logarithmic decay with a half-life of 50 days.[91] After an initial drop in levels, the liver residues also follow a logarithmic decay with a half-life of 140 days.[91] More highly chlorinated biphenyls are the most potent inducers of hepatic microsomal systems.[91] Pigeons and quail metabolize PCBs at a rate generally dependent on the amount of chlorine in the molecule.[91] These authors also reported that hepatic microsomal enzymes are sensitive to inducing by chlorinated hydrocarbons.[91]

Male and female rhesus monkeys receiving various levels (1.5 or 3.0 g Aroclor® 1248 per kilogram in a single oral dose) of PCBs demonstrated 90% absorption of PCBs from the gastrointestinal tract and distribution in organs of high lipid content.[93] Hydroxylated metabolites were formed in the liver and excreted through the biliary and urinary routes.[93] Lower chlorinated components were more rapidly metabolized and excreted, while concentrations of the highly chlorinated biphenyls persisted in the adipose tissue in excess of 2½ years.[93] The detection of the urinary metabolite (trans-3,4-dihydro-3,4-dihydroxytetrachlorobiphenyl suggests that the mechanism of metabolism is through an arene oxide intermediate.[93] In vivo and in vitro studies demonstrated binding of PCBs with macromolecules.[93]

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Chapter 6

MODIFICATION OF PCBs BY BACTERIA AND OTHER MICROORGANISMS

Kensuke Furukawa

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I. INTRODUCTION

Metabolic breakdown of PCBs by microorganisms is considered to be one of the major routes of environmental degradation for these widespread pollutants.[1] Environmental monitoring has demonstrated that the PCBs found in environmental samples are the more highly chlorinated biphenyls, i.e., those containing five or more chlorines per biphenyl molecule.[1,2] This is circumstantial evidence that the less-chlorinated biphenyls degrade more rapidly than the more highly chlorinated ones. The occurrence and distribution of PCB-degrading microorganisms and their capability of biodegrading PCBs have been investigated in the last decade. Since the report by Ahmed and Focht[3] in 1973 that two species of *Achromobacter* are capable of growing on biphenyl and 4-chlorobiphenyl, a number of studies have indicated that pure cultures of microorganisms and naturally occurring microbial populations are capable of degrading PCBs.[3-16] There is evidence that a variety of factors affect the microbial degradation of PCBs in the environment. The degree of chlorination in the various components of PCBs might be the most significant factor. Water solubility and volatility of PCBs, pH, temperature, oxygen, nutrient supplies in the environment, emulsification of PCBs and adsorption by various materials such as soil, sediment, sludge, and biological materials all combine to varying extents, to affect microbial degradation of PCBs in ecosystems. The present review describes interactions between PCBs and microorganisms (mainly bacteria), biodegradability, and metabolic transformations of various PCBs.

II. PCBs AND MICROORGANISMS

The ubiquitous occurrence of bacteria which can utilize biphenyl as a sole carbon and energy has been reported. Ohmori et al.[17] isolated 39 bacterial strains capable of utilizing biphenyl from soil. Lunt and Evans[18] described a Gram negative soil bacterium that oxidized biphenyl to phenylpyruvic acid. A strain of *Pseudomonas putida* metabolized biphenyl to benzoic acid via 2,3-dihydro-2,3-dihydroxybiphenyl, 2,3-dihydroxybiphenyl, and 2-hydroxy-6-oxo-6-phenylhexa-2,4-dienoic acid.[17,19] Gibson et al.[19] position further evidence for initial oxidative attack at the 2,3-position on the biphenyl molecule. In 1973 degradation of mono- and dichlorobiphenyl to chlorobenzoic acids was shown by two species of *Achromobacter*.[4] Later, conversion of PCBs to chlorobenzoic acids was shown for certain pure strains and by naturally occurring microorganisms.[5,10]

Furukawa et al.[20] described the interaction of PCBs and bacteria, demonstrating that an *Alcaligenes* sp. and an *Acinetobacter* sp. rapidly adsorbed a large amount of ^{14}C-labeled 2,5,2'-trichlorobiphenyl onto the cell surface and then the compound was gradually metabolized. The metabolic products containing radioactive carbon, such as a ring-cleaved compound and 2,5-dichlorobenzoic acid were gradually released from the cells and moved into the aqueous layer.

PCB-degrading or PCB-resistant bacteria recovered from estuarine and marine samples represent seven bacterial genera: *Pseudomonas*, *Vibrio*, *Aeromonas*, *Micrococcus*, *Acinetobacter*, *Bacillus*, and *Streptomyces*.[21] Twenty isolates of PCB-degrading bacteria from the Hudson River represented five genera: *Acetobacter*, *Acinetobacter*, *Alcaligenes*, *Klebsiella*, and *Pseudomonas*.[22] Sylvestre[23] described a method for the isolation of 4-chlorobiphenyl-degrading bacteria from soil samples and activated sludge samples. The method is based on the observation that when a PCB solution is spread over an agar plate previously inoculated with an environmental sample, the film of PCB deposited on the surface of the plate becomes clarified around some of the colonies. Fourteen strains obtained by this method were all strictly aerobic, motile, Gram negative rods, and able to grow on 4-chlorobiphenyl as the sole carbon source.

PCB-sensitive bacteria are also known. The lipophilic nature of these chlorinated hydro-

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carbons enhances their toxic properties by promoting the efficient binding of such substances to cells. Of 85 different estuarine bacteria, growth of 26 strains was inhibited to a varying extent by PCBs.[16] In contrast to resistant bacteria, sensitive strains were mainly amylolytic and proteolytic. Of 17 bacterial cultures tested, growth of two open-ocean isolates, including one *Pseudomonas*, was consistently inhibited by Aroclor® 1254 at a concentration as low as 10 μg/ℓ.[16] The *Pseudomonas* also had reduced carotenoid levels and an altered filamentous morphology. The effect of Aroclor® 1254 was immediate and reversible for at least 18 hr after initial exposure. The PCBs had no effect on respiration rates and protein synthesis at the growth-inhibiting concentration of the Aroclor®. On the other hand, rates per cell of adenine uptake and adenine incorporation into DNA and total nucleic acids by the cells treated with PCBs were significantly lower than in control cells. Lower chlorinated PCBs were highly mutagenic to *Salmonella typhimurium*, whereas higher chlorinated ones were only weakly mutagenic.[20]

In contrast to bacteria, there is relatively little information about interaction between PCBs and fungi or yeasts. The following effects were observed between *Aspergillus flavus* and PCBs:[21] no metabolic change of PCBs, accumulation of various Aroclors in the mycelium, no significant effect on nucleic acid content, an obvious delay of sporulation, and a decrease of colony diameter. The magnitude of these effects was highest for Aroclor® 1232 and lowest for Aroclor® 1260. Tejedor et al.[22] observed that growth and oxygen uptake of *Saccharomyces cerevisiae* were suppressed by PCBs and that these effects increased as the chlorine content of the PCBs decreased. There is evidence that fungi, yeast, and a cyanobacterium oxidized biphenyl primarily at the 4-position to form 4-hydroxybiphenyl and 4,4'-dihydroxybiphenyl.[3,20-22] These results are similar to those reported for the mammalian metabolism of biphenyl and chlorinated biphenyls.[23-29]

III. BIODEGRADABILITY OF PCBs

Chlorine is known to alter the resonant properties of aromatic substrates and alter the electron density of specific sites. These alterations may result in a deactivation of the primary oxidation of chlorinated biphenyls by microorganisms. Furthermore, the positions occupied by substituted chlorines have stereochemical effects on the affinity between enzymes and their substrate molecules. Many workers have reported that both pure chlorobiphenyls and commercial PCBs are degraded by certain pure strains or by mixed cultures. Mixed cultures, dominated by *Alcaligenes odrans*, *A. denitrificans*, and an unidentified bacterium, degraded Aroclor® 1242.[19] The gas chromatography profile of Aroclor® was greatly altered after 5 days incubation. Tucker et al.[7] demonstrated that commercial PCB mixtures which contain predominantly mono- and dichlorobiphenyls readily undergo primary biodegradation by activated sludge microorganisms, and that as the levels of tri-, tetra-, and pentachlorobiphenyls increase, the degradation rates decrease accordingly. Similar results were obtained for the degradation of Kanechlor® (KC) 200, 300, 400, and 500, which are primarily di-, tri-, tetra-, and pentachlorobiphenyls, respectively.[30] Baxter et al.[9] showed that addition of biphenyl to the substrate enhanced the biodegradation of certain PCBs, indicating that co-metabolism could be involved in the degradation process of highly chlorinated biphenyls. Liu[16] also observed the enhanced biodegradation of Aroclor® 1254 by the addition of Aroclor® 1221 to the growth medium. Further investigation demonstrated that the rate of PCB biodegradation is greatly enhanced by growing *Pseudomonas* cells in a PCB-lignin-sulfonate emulsion. The use of lignin-sulfonate in the growth medium allow the cells to overcome the substrate-limiting factor of surface area. A kinetic study, using resting cells, indicated that Aroclor® 1221 was degraded much faster (980 μg/mg cell dry weight/hr) than Aroclor® 1254 (43 μg/mg cell dry weight/hr).

Furukawa et al.[31] studied the effect of chlorine substitution on biodegradability of PCBs.

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Table 1
EFFECT OF CHLORINE SUBSTITUTION ON BIODEGRADABILITY OF VARIOUS ISOMERIC PCBs BY BACTERIA

Isomer group	PCB chlorine position	Degradation rate[a] (nmol/ml/hr)	
		Alcaligenes sp. Y42[b]	*Acinetobacter* sp. P6[b]
A	2-	>50	>50
	3-	>50	>50
	4-	>50	>50
B	2,3-	>50	46.4
	2,4-	>50	>50
	2,5-	>50	>50
	2,6-	0	4.1
	3,4-	>50	>50
	3,5-	>50	>50
C	2,2'-	8.3	14.0
	2,4'-	48.2	49.1
	3,3'-	—	18.5
	4,4'-	18.2	25.2
D	2,3,4-	35.1	32.0
	2,3,6-	0	0
	2,4,5-	46.0	32.4
	2,4,6-	3.1	46.0
E	2,5,2'-	1.6	5.1
	2,5,3'-	42.1	41.3
	2,5,4'-	21.8	30.4
	2,4,4'-	41.5	40.2
	3,4,2'-	15.6	38.6
F	2,3,4,5-	25.8	19.1
	2,3,5,6-	0	0
G	2,5,2',3'-	8.7	7.3
	2,4,2',4'-	0	0
	2,4,3',4'-	0	0
	2,5,2',5'-	0	3.5
	2,6,2',6'-	0	0
	3,4,3',4'-	0	0
H	2,4,5,2',5'-	0.6	0

[a] A 500 nmol amount of each compound dissolved in ethanol (10 mM each) was suspended in 10 ml of phosphate buffer (pH 7.5). Degradation rate was calculated after 1 hr of incubation for mono-, di-, and trichlorobiphenyls and after 2 hr of incubation for tetra- and pentachlorobiphenyls.

[b] The cell number was 2×10^8 cells/ml in *Alcaligenes* Y42 and 4.4×10^8 cells/ml in *Acinetobacter* P6.

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Chlorinated biphenyls can theoretically consist of as many as 210 different components. Of the possible isomers, 103 are considered most probable. For this study 33 pure isomers of PCBs and single strains of the bacteria, *Alcaligenes* sp. and *Acinetobacter* sp. were employed. The former was grown on biphenyl, and the latter was grown on 4-chlorobiphenyl as a sole carbon source. Both strains could co-metabolize a variety of PCB components to varying extents. The results presented in Table 1 indicate the following general information on the

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Table 2
METABOLIC PRODUCTS OF VARIOUS PCB ISOMERS TRANSFORMED BY *ACINETOBACTER* sp.

Group	Major product	PCB isomers
i	Chlorobenzoic acids (Cl no. ≤ 4)	2-, 3-, 4-, 2,3-, 2,4-, 2,5-, 3,4-, 3,5-, 2,2'-, 3,3'-, 4,4'-, 2,3,4-, 2,4,5-, 2,5,2'-, 3,4,2'-, 2,3,4,5-, 2,4,2',4'-, 3,4,3',4'-
ii	Meta-cleavage compounds	2,4'-, 2,4,4'-, 2,5,4'-
iii	Dihydroxy compounds	2,6-, 2,3,6-, 2,4,2',5'-, 2,4,5,2',5'-
iv	Trihydroxy compounds	2,4,6-
v	Unknown compounds	2,3,2',3'-, 2,3,2',5'-, 2,4,5,2',3'-
vi	No metabolism	2,3,5,6-, 2,6,2',6'-

relationship between chemical structure and microbial breakdown of PCBs. (1) Degradation rate markedly decreases as chlorine substitution increases. (2) PCBs containing two chlorines in the *ortho*-position of a single ring (i.e., 2,6-) and each ring (i.e., 2,2'-) show a striking resistance to degradation, as illustrated in the studies with 2,6-, 2,2'-, 2,3,6-, 2,5,2'-, 2,3,5,6-, and 2,6,2',6'-chlorobiphenyls. 2,4,6-Trichlorobiphenyl is the exception in this series of PCBs. This compound is rapidly metabolized through an alternative pathway by *Acinetobacter* sp., while the same compound is slowly metabolized by *Alcaligenes* sp. through the major pathway to 2,4,6-trichlorobenzoic acid. (3) PCBs containing all chlorines on a single ring are generally degraded faster than those containing the same number on both rings. (4) PCBs having two chlorine atoms at the 2,3-position of one ring such as 2,3,2',3'-, 2,3,2',5'-, and 2,4,5,2',3'-chlorobiphenyls are much more susceptible to microbial attack, compared with other tetra- and pentachlorobiphenyls. Members of this series of PCBs are metabolized through an alternative pathway with accumulation of unknown compounds. (5) Preferential ring cleavage of the biphenyl molecules occurs with a nonchlorinated or lesser chlorinated ring.

It is also known that chlorine substitution greatly affects metabolism and excretion of PCBs by mammalian and avian species. A number of less-chlorinated PCBs (i.e., mono-, di, tri-, and tetrachlorobiphenyls) are readily metabolized and excreted by mammals and birds.[42-46] However, the rate of metabolism drops sharply as the number of chlorines per biphenyl molecule increases.[46,47]

IV. MICROBIAL METABOLISM OF PCBs

In bacterial metabolism studies with 36 pure PCB isomers (chlorine numbers 1 to 5), Furukawa et al.[48,49] demonstrated that 33 compounds were oxidized by an *Acinetobacter* sp. (Table 2). From the analysis of metabolic intermediates, it was found that a number of the PCB components listed in Table 2 are converted to the corresponding chlorobenzoic acids by an oxidative route as illustrated in Figure 1. Like other aromatic hydrocarbons,[50,51] PCBs are considered to be metabolized via a deoxygenase catalyzed reaction such as Gibson et al.[19] had demonstrated with biphenyl. A species of *Beijerinckia* metabolized biphenyl to *cis*-2,3-dihydroxy-1-phenylhexa-4,6-diene (*cis*-2,3-dihydro-2,3-dihydroxybiphenyl). This suggests that a cyclic peroxide is an initial oxidation product in the metabolism of biphenyl. Two atoms of molecular oxygen might be incorporated at the 2,3-position of a nonchlorinated

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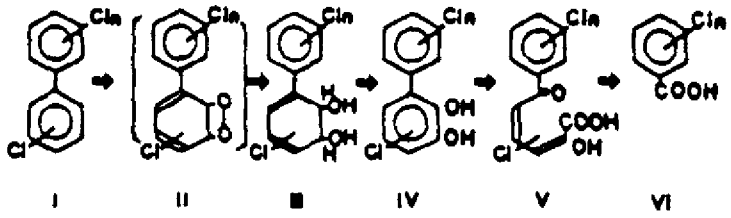

FIGURE 1 Major metabolic pathway of PCBs by bacteria

or less chlorinated ring of PCBs to form *cis*-dihydrodiol compounds (III in Figure 1). The dihydrodiols are then dehydrogenated to yield 2,3-dihydroxy compounds (IV). The *meta*-cleavage occurs between C1 and C2 of the 2,3-dihydroxy compounds to yield chlorinated derivative of 2-hydroxy-6-oxo-6-phenylhexa-2,4-dienoate (V). For many PCB isomers the *meta*-cleavage compounds thus formed are usually hydrolyzed readily to chlorobenzoic acids (VI). Of 36 PCBs, 18 isomers were converted to the corresponding chlorobenzoic acids by this major pathway in *Acinetobacter* sp. This major pathway to chlorobenzoates is also common in other pure cultures such as *Alcaligenes* sp.,[9,46] *Achromobacter* sp.,[4] and *Pseudomonas putida*[17,18] and naturally occurring microbial cultures[19] with both isomeric chlorobiphenyls and commercial PCB mixtures.[46] However, alternative pathways to the chlorobenzoate pathway are demonstrated with other PCB isomers as shown in Table 2. For example, 2,4'-, 2,4,4'-, and 2,5,4'-chlorobiphenyls which all have chlorine substituents at the 2,4'-position are readily converted to the *meta*-cleavage compounds through the major pathway illustrated in Figure 1. These *meta*-cleavage compounds are rather stable and accumulate in the reaction mixture during prolonged incubation. Dihydroxy compounds accumulate in the reaction mixture from the 2,6-, 2,3,6-, 2,4,2',5'-, 2,5,2',5'-, and 2,4,5,2'-chlorobiphenyls. 2,4,6-Trichlorobiphenyl is quickly metabolized to a trihydroxy compound via a dihydroxy compound. Thus, the trihydroxy compound predominantly accumulates during the late stages of incubation. PCBs which possess chlorine atoms at the 2,3-position such as 2,3,2',3'-, 2,3,2',5'-, and 2,4,5,2',3'-chlorobiphenyls seem to be metabolized by an alternative route. Large amounts of unidentified product always accumulate along with the appropriate chlorobenzoic acid from this series of PCBs. These unidentified compounds are also produced from the commercial PCB mixtures, Kanechlor® 400.[46] Thus, the metabolic alterations of PCBs by bacteria are greatly dependent on chlorine substitution.

There is evidence that fungi, yeast, and a cyanobacterium (*Oscillatoria* sp.) metabolize biphenyl and low chlorinated biphenyls to monohydroxy and dihydroxy compounds as is also demonstrated by mammalian systems.[22-29] Fungal species of several genera are able to grow on biphenyl and form 2- and 4-hydroxybiphenyls and 4,4'-dihydroxybiphenyl.[29] Dodge et al.[29] demonstrated that *Cunninghamella elegans* grown in dextrose broth transformed biphenyl to produce 2-, 3-, and 4-hydroxybiphenyls as well as 4,4'-, 2,4'-, and 2,5-dihydroxybiphenyls. Wainhöfer et al.[3] observed that *Rhizopus japonicus* converted 4-chlorobiphenyl to 4-chloro-4-hydroxybiphenyl. There is a report that *Candida tropicalis* oxidizes biphenyl to 4-hydroxybiphenyl.[31] A cyanobacterium, *Oscillatoria* sp. also oxidizes biphenyl to 4-hydroxybiphenyl.[32] However, ring cleavage which is common in bacteria has not been reported in fungi and yeasts.

V. BIODEGRADATION OF COMMERCIAL PCBs AND THEIR METABOLIC PRODUCTS

Based upon observation with pure isomers of PCBs, Furukawa et al.[46] using *Acinetobacter*

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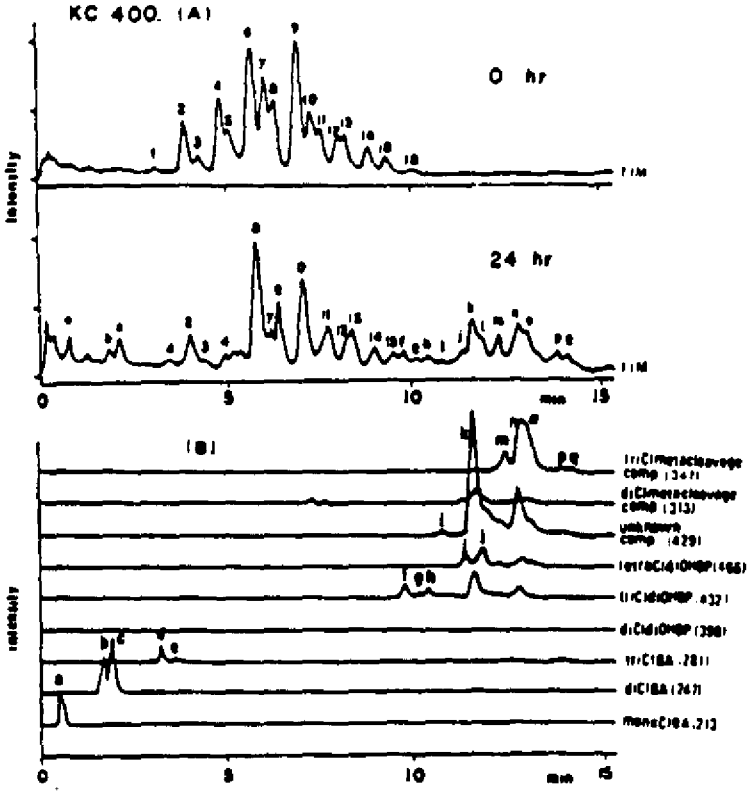

FIGURE 2. Biodegradation of Kaneclor® 400 by *Acinetobacter* sp. and metabolic products. (A) Total ion monitor on GC/MS of KC 400 at 0 hr and after 24 hr incubation with organisms. PCB components are numbered and the metabolites are expressed by letters. (B) Mass chromatogram of metabolites was monitored by mass chromatography. Abbreviations: BA, benzoic acid; BP, biphenyl.

sp. examined the biodegradability and metabolic products of commercial PCB mixtures (Kaneclors®). KC 200 (primarily dichlorobiphenyls) and KC 300 (primarily trichlorobiphenyls) were readily degraded by *Acinetobacter* sp. so that total ion monitor (TIM) readings on gas chromatography/mass spectrometry (GC/MS) were greatly changed after 4 hr incubation. The peaks of PCB components disappeared rapidly or decreased in size and were replaced by the peaks of metabolic products. Monochlorobenzoic acids accumulated predominantly in the reaction mixture from KC 200. Several metabolic products such as mono- and dichlorobenzoic acids, dichloro- and trichlorodihydroxy compounds, the *meta*-cleavage products with two and three chlorines were observed from KC 300. KC 400 (primarily tetrachlorobiphenyls) is also susceptible to biodegradation. The TIM profile was greatly changed after 24 hr incubation by the decrease of various PCB components (di-, tri-, and tetrachloro isomers) and by the concomitant formation of metabolites as presented in Figure 2. A variety of metabolites of mono-, di-, and trichlorobenzoic acids, trichloro- and tetrach-

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loro dihydroxybiphenyls, the *meta*-cleavage products (chlorine no. 2 to 4), and in addition certain unknown compounds with two chlorines in the molecules were detected. The unknown compounds can be derived from 2,3,2',3'- or 2,3,2',5'-tetrachlorobiphenyls or both (see Table 2). On the other hand, KC 500 components (primarily pentachlorobiphenyls) were refractory and hardly metabolized. Only dihydroxy compounds of pentachlorobiphenyls were detected. Thus, a variety of metabolic products can be formed and accumulate from commercial PCB mixtures by bacteria.

VI. TOTAL DEGRADATION OF PCBs AND PLASMIDS

A number of bacterial strains are known to convert various PCB components into the corresponding chlorobenzoates, so that this oxidative route appears to be a common pathway for PCB degradation by bacteria. In the natural environment a mixed culture acting on the many components of PCBs might be expected to give a more complete degradation than would a single organism. Chlorobenzoates formed from many PCB components by bacteria can be decomposed by other bacteria. Horvath and Alexander[43] demonstrated that 20 isolates representing 9 bacterial genera co-metabolized one or more of 22 substituted benzoates. Some of the chlorinated benzoates can be acted upon by pure cultures of such genera as *Arthrobacter*,[44] *Brevibacterium*,[31] *Alcaligines*,[30] and *Pseudomonas*[27,30] and the metabolic processes have been studied in detail. The transformation and fate of chlorobenzoates under natural conditions has been studied by some workers. Dewey et al.[45] observed biological degradation of 2,3,6-trichlorobenzoic acid in soil. GiGeronimo et al.[46] reported that microorganisms in sewage decomposed *o*-, *m*-, and *p*-chlorobenzoic acids and also 3,4-dichlorobenzoic acid.

It is well known that plasmids can endow bacterial species with the ability to catabolize various hydrocarbons and other organic compounds.[47-53] A plasmid encoding conversion of 4-chlorobiphenyl to 4-chlorobenzoic acid has been demonstrated in *Klebsiella* sp.[54] Furukawa and Chakrabarty[55] also suggested that the plasmid pKF1 specified the catabolism of biphenyl and chlorinated biphenyls into benzoate and chlorobenzoates in *Acinetobacter* sp. and *Arthrobacter* sp. On the other hand, Harriman et al.[56] and Chatterjee and Chakrabarty[57] have demonstrated that introduction of TOL and other plasmids (pAC25) specifying chlorobenzoate degradation and subsequent selection in a chemostat with 4-chloro- or 3,5-dichlorobenzoic acid leads to the development of strains that can rapidly utilize 4-chloro- and 3,5-dichlorobenzoic acids. The 4-chlorobenzoate-degrading *Pseudomonas putida* harbors a plasmid pAC27, while the 3,5-dichlorobenzoate-degrading strain harbors a plasmid pAC31.[58] Both of the strains carry a portion of the TOL on the chromosome, and the plasmids pAC27 and pAC31 specify the corresponding chlorocatechol-degradative pathway i.e., 4-chlorocatechol and 3,5-dichlorocatechol. A combined culture of the PCB-dissimilating *Acinetobacter* sp. or *Arthrobacter* sp. (harboring the putative 53 Mdal plasmid specifying conversion of chlorobiphenyls to chlorobenzoic acids) and genetically constructed mono- or dichlorobenzoate-utilizing pseudomonads (harboring plasmids encoding complete utilization of mono- or dichlorobenzoates) allowed more than 98% utilization of mono- or dichlorobiphenyls with the liberation of equivalent amounts of chloride ions (Figure 3).[58] A combination of PCB-degrading strains and chlorobenzoate-degrading strains leading to total degradation of mono- and dichlorobiphenyls is an example of the role of various degradative plasmids in the total mineralization of some chlorinated synthetic compounds.

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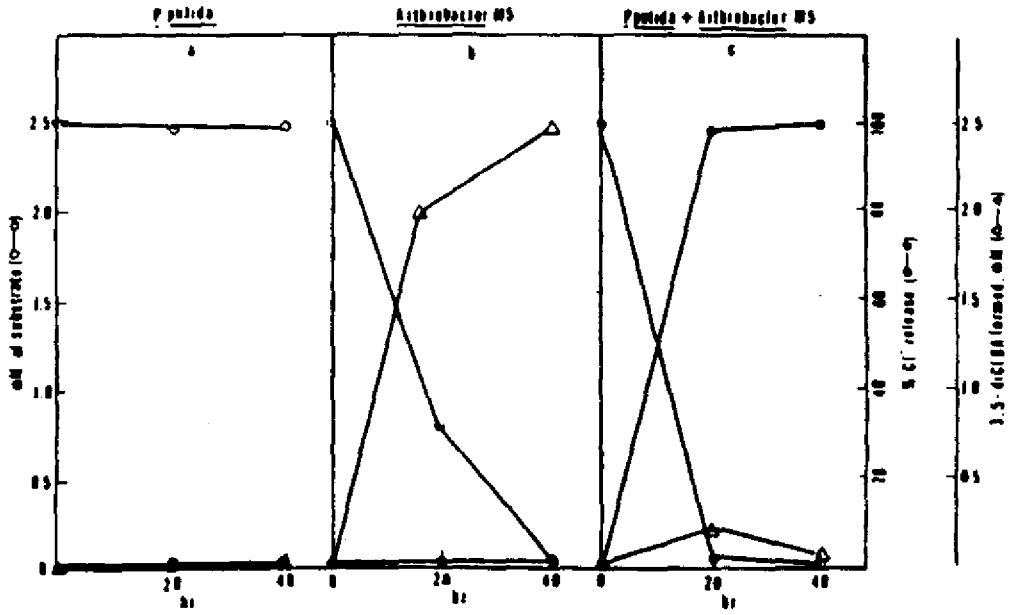

FIGURE 3 Total degradation of 3,5-dichlorophenyl by a mixed culture of *Arthrobacter* sp. M5 (harboring plasmid pKF1 specifying degradation of chlorophenyls to chlorobenzoic acids) and *Pseudomonas putida* (harboring plasmid pAC31 specifying utilization of 3,5-dichlorobenzoic acid). Substrate (3,5-dichlorophenyl) consumption (○), product (3,5-dichlorobenzoic acid) formation (△), and chloride release (□) by resting cells of 3,5-dichlorobenzoate-degrading *P. putida* (a), PCB-degrading *Arthrobacter* M5 (b), and a mixed culture of *P. putida* and *Arthrobacter* M5 (c). An extensive chloride release and little 3,5-dichlorobenzoic accumulation were observed in the mixed culture. However, a large amount of 3,5-dichlorobenzoic accumulation, and no chloride release was observed in pure culture of *Arthrobacter* M5. *P. putida* strain had no effect on the degradation of the 3,5-dichlorophenyl. (From Furukawa, K. and Chakrabarty, A. M., *Appl. Environ. Microbiol.*, 44, 619, 1982. With permission.)

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Chapter 7

EFFECT OF PCBs ON REPRODUCTION IN MAMMALS

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I. INTRODUCTION

Chemically induced reproductive abnormalities have been documented among mammalian wildlife species throughout the industrialized world. Some of these effects have been attributed to the polychlorinated biphenyls (PCBs) which have accumulated in the environment due to their widespread industrial use and chemical stability. Most mammals are exposed to these chlorinated hydrocarbons via an aquatic or terrestrial food chain. The lipophilic nature of the PCBs result in their accumulation in adipose tissue. Although a constant exchange between fatty deposits and plasma probably occurs, increased mobilization of PCBs takes place during pregnancy, lactation, stress, and in a time of starvation. This persistence has stimulated many acute and chronic toxicity studies over the past decade to delineate the potentially harmful effects of exposure on reproductive function.

Reproduction can be modified at several developmental stages, the consequences of which may be expressed immediately or at a later time in life. Subacute or chronic exposure to PCBs pre- or postnatally, pre- or postpubertally, or as adults has been found to be detrimental to reproductive function. In experimental studies, the demonstrable consequences of exposure are dependent upon the type and level of PCB utilized, route of administration, the test animal used, and the physiologic endpoint. The definitive characterization of the toxicologic properties of PCBs is difficult due to the fact that the commercial products are mixtures of isomers; the problem is further complicated by the frequent presence of other toxic contaminants.

It is the purpose of this chapter to provide a current assessment of the effects of PCBs on reproduction in mammals. The chapter will be organized by reference to maturational reproductive stages. The possible site(s) and/or mechanism(s) of action will be discussed although these aspects remain largely speculative due to the tenuous nature of present evidence.

II. DIFFERENTIATION AND DEVELOPMENT

In recent years there has been a growing concern about the exposure of pregnant women to chemicals and possible teratogenic effects on the developing fetus. Prenatal exposure to PCBs may place reproductive development at risk, the effects of which may not be immediately apparent but could be expressed later as functional or behavioral abnormalities. Unfortunately, few studies have been conducted concerning the effects of prenatal exposure to the chlorinated biphenyls on subsequent postnatal reproductive function. That PCBs cross the placenta, albeit slowly, has been demonstrated in mice,[1] rats,[2] rabbits,[3] and monkeys.[4]

A. Morphology

No teratogenic effects of PCBs have been demonstrated on reproductive structures. There are reports, however, of cleft palate, cleft lip, brachydactyly, and syndactyly induced in mouse pups[5] and pigs[6] by prenatal exposure to PCBs. Chow et al.[7] reported histological changes in the central nervous system of mice exposed in utero to pure tetra-CB from days 6 to 10 of gestation. These authors found cylindrical CNS peninsulas in the spinal and cranial nerve roots, suggesting that the PCB may have affected the early organization of interneuronal junctions or development of synapses. This change in nervous tissue organization is associated with a malady known as "spinning syndrome" which developed in mice prenatally exposed to PCB.[7,8] Although not an established detriment to reproduction, these results illustrate the potential for untoward effects on intrauterine differentiation.

Adult reproductive abnormalities may be attributed to subtle developmental alterations caused by exposure to PCB in utero. While not teratisms by strict definition there are morphological and functional differences which, either directly or indirectly, resulted from

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exposure to PCBs during development. Gellart and Wilson[9] administered 30 mg/kg/day Aroclors® 1221, 1242, or 1260 to pregnant Sprague-Dawley rats from days 14 to 20 of gestation. Sacrifice at 6 months of age revealed a significant increase in the absolute weight of the testes of males exposed to Aroclor® 1260. Fertility was not affected.

B. Reproductive Function

The reproductive neurotoxicity of PCBs was corroborated by Gellart[10] when he injected neonatal rats with Aroclor® 1221 during the postnatal "critical period" of CNS development. In rats during the first 5 days following birth the hypothalamo-pituitary axis is "programmed" for cyclic or acyclic release of gonadotropins. It has been experimentally demonstrated that this period of development is sensitive to hormonal perturbations which alter sex-specific hormone patterns.[11] Specifically, neonatal injection of estrogens or androgens into certain rodents causes functional and behavioral sterility. Thus, female rat pups injected with Aroclor® 1221 (10 mg/day) on the second and third days following birth presented an acyclic gonadotropin pattern resulting in the anovulatory-persistent vaginal estrus syndrome. The reported estrogenic activity of the lower chlorinated biphenyls (<48% chlorination)[12] probably accounts for the altered neuroendocrine differentiation in rats, especially as the administered dose was very high (2000 mg/kg/day). A direct toxic effect on the CNS could not account for these results as Aroclors® 1242, 1254, and 1260 were ineffective.

Because reproduction is a highly integrated function with behavioral and metabolic as well as hormonal components, exposure to an environmental compound could alter behavior or extragenital systems producing infertility even in the absence of toxic effects on the reproductive endocrine system or genital tract morphology. As an example, liver function can be reprogrammed or "imprinted" by exogenous gonadal steroids.[13-15] Female rat pups so treated demonstrate, at puberty, enzymatic rates for reductive and oxidative steroid reactions characteristic of the male liver. Consequently, changes in hepatic metabolism of steroids induced by any compound during development could modify reproductive function and/or behavior. An hepatic "imprinting" effect has, in fact, been reported for some pure PCB congeners. Lucier and McDaniel[16] administered PCBs orally on days 8 to 18 of gestation to pregnant rats at the following doses: 4-chlorobiphenyl, 30 mg/kg/day; 3,4,3',4'-tetrachlorobiphenyl, 3 mg/kg/day; 2,4,5,2',4',5'-hexachlorobiphenyl, 30 mg/kg/day. Generally speaking, the congeners were specific enzyme inducers although there was some overlap in activities. Tetrachlorbiphenyl was the most potent transplacentally as it "feminized" the postpubertal male liver by repressing the normally high activity of UDP glucuronyltransferase. Because of the reported estrogenic activity of the lower chlorinated biphenyls, one might speculate that the PCB interacts with the cytosol receptor. It could be translocated to the nucleus where it acts on genetic material resulting in either immediate modulation of enzyme levels or latent gene expression at puberty.

C. Summary

Although the labile nature of the developing conceptus is well known, relatively few studies concerning prenatal exposure to the ubiquitous PCBs on the subsequent postnatal fertility of the offspring have been conducted. The limited evidence indicates that the PCBs do not affect organogenesis of the genital tract. Of concern are those studies which reveal morphological and functional neurotoxic effects and the latent effect on hepatic steroid metabolizing enzymes. Both consequences of PCB exposure could compromise the capacity of an organism to reproduce itself. However, the relationship of these observations in laboratory rodents to human fetal development has yet to be established.

III. MALE REPRODUCTION

The primary role of the reproductive system lies in the continuation of the species. The

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male reproductive system is organized to develop and transmit the male germ cells to the female ultimately resulting in the formation of a third animal of the same species. However, from fertilization through sexual maturity, the male reproductive system is susceptible to chemically induced modifications which could prevent a successful mating.

A. Sex Organs

As previously discussed, PCBs have no known teratogenic effects on the development of the male reproductive tract in mammals. Likewise, a direct effect of postnatal administration of PCBs on adult male reproductive organs has not been clearly demonstrated. Oral administration of Aroclor® 1254 at levels ranging from 62.5 to 4000 ppm/day for 2 weeks to adult male albino mice was reported to have no effect on sex organ weights.[17] Similarly, Orberg and Lundberg[18] reported that 0.25 g/day of Clophen® A-60 for 28 days had no significant effect on sex organ weights of intact adult male albino mice. Castrated males were administered the same dosage of Clophen® A-60 for 28 days but in addition were given 70 μg testosterone propionate for 10 days beginning on the 19th day of PCB administration. When compared with castrated male controls receiving testosterone propionate only, the seminal vesicle weights were significantly lower in the PCB plus androgen treated males. The authors suggested that the lack of an effect of PCB in intact males contrasted with an effect in castrates was due to two factors: enhanced androgen metabolism and the absence of the testes-hypophysis feedback mechanism to compensate for altered hormone removal. Merson and Kirkpatrick[19] on the other hand, reported a significant decrease in seminal vesicle weights of mature male white-footed mice (*Peromyscus*) fed 200 ppm/day Aroclor® 1254 for 4 weeks. There was no weight change at 100 ppm for the 4-week period. In a related study it required 400 ppm/day of Aroclor® 1254 to cause a reduction in seminal vesicle weight when a 2-week feeding period was utilized.[20] There was no change in organ weights of the *Peromyscus* males when 100 and 200 ppm/day was fed for 2 weeks.

Kanechlor® 500 was included in the diet of adult male Sprague-Dawley rats for 4 weeks at a dose level of 100 ppm/day.[21] No effect on reproductive organs were observed. Likewise, testes, prostate, and seminal vesicle weights were not changed in cottontail rabbits fed 25 ppm Aroclor® 1254 for 20 days.[22] In contrast, when male rats were given Aroclor® 1254 at 500 mg/kg by oral gavage as a single treatment and sacrificed 12 days later[23] or 500 ppm of Aroclors® 1254 and 1260 in the feed for 8 months,[24] there was a significant increase in testes weight. In spite of the inconsistency of the existing reports, organ weight analysis could be a useful parameter for assessing the effects of PCBs. This would require more uniform and rigid experimental conditions. Perhaps such studies could be combined with histological examination of the affected tissue to determine whether the effect of the PCB was direct or indirect.

B. Spermatogenesis

Testicular toxicity should be reflected by some impairment of spermatogenesis. Sanders et al.[20] reported that Aroclor® 1254 in the diet of adult albino male mice at a level of 200 ppm/day significantly reduced the number of sperm per milligram of testis but did not affect gonadal weights. They found no effect at 50 ppm after the 15-day treatment period. A similar effect of Aroclor® 1254 was observed in adult white-footed male mice by Sanders and Kirkpatrick.[20] A treatment regimen of 400 ppm/day for 2 weeks reduced the number of sperm per testis. There was no effect at 100 or 200 ppm.

The frequency of mitotic figures in adult rat spermatogonia decreased following oral gavage of an acute dose of 5000 mg/kg Aroclor® 1242.[25] Acutely administered levels of 2500 or 1250 mg/kg had no effect on spermatogonia. Interestingly, when 500 mg/kg were administered per os for 4 days there was a significant decrease in spermatogonia. This suggests duration of exposure is an important parameter to consider when assessing toxicity of PCBs.

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Mink are reportedly very susceptible to the toxic effects of PCBs. When adult males were fed 20 ppm daily of Aroclor® 1016 or 5, 10, 20, or 40 ppm Aroclor® 1242, mortality was 100% within 6 months at treatment levels of 20 ppm or greater.[27] Spermatogenesis, however, was not affected during the exposure period. Platonow and Karstad[28] earlier described the lack of an effect of Aroclor® 1254 on male mink, noting that they continue to produce normal numbers of viable spermatozoa throughout exposure.

Male beagles fed Aroclor® 1254 (100 ppm) for 2 years showed aspermatogenesis and diffuse hyperplasia of the interstitial cells of the testes.[29] One of four rhesus monkeys developed a low sperm count between 12 and 18 months while on a diet containing 5 ppm Aroclor® 1248.[30] The sperm count returned to normal 1 year after PCB was removed from the diet.

C. Fertility

Fertility evaluation in males following prenatal, postnatal, or adult exposure could be a more sensitive assessment of a functional impairment than changes in organ weights or altered spermatogenesis. Subtle changes in testicular weights, sperm numbers, and libido may not be apparent when examined as a single endpoint, but when indirectly assessed as an estimate of fertility might result in a measurable effect on fecundity. Orberg[31] administered daily oral doses of 50 μg of pure trichlorobiphenyl or hexochlorobiphenyl to mice from days 5 to 19 of gestation or from birth through day 12 of lactation. Male pups subjected to each treatment were subsequently mated with nonexposed females which resulted in no differences in numbers of pups sired when compared with controls. Kihlstrom et al.[32] injected postpartum NMRI strain mice with 50 mg/kg Clophen® A-60. Treatment began on the day of delivery and then weekly for three successive weeks during lactation. After reaching sexual maturity, the male mice which received the PCBs via their mother's milk were mated with control females. The frequency of implantations and the resorption rate was not different from control matings. Interestingly, when both treated males and females were mated, the number of implantations decreased significantly; whereas similar to males, when treated females were mated to control males, there was no effect on implantation.

Pregnant Sprague-Dawley rats were gavaged daily from days 14 to 20 with 30 mg/kg of either Aroclor® 1221, 1242, or 1260.[9] At sexual maturity the prenatally exposed male offspring were placed with nontreated breeder females for 2 weeks. Based on the number of young produced, there was no apparent detrimental effect of the Aroclors®. Daily exposure of mature rats to 70 ppm Aroclor® 1254 for 9 weeks in the drinking water did not affect fertility.[33]

Four mature male rhesus monkeys were fed a diet containing 5 ppm Aroclor® 1248 for approximately 1 year with no loss of breeding capacity.[30] However, as previously mentioned, one of these males had a decreased sperm count following 12 to 18 months exposure.

D. Summary

Definitive conclusions regarding the hazard of PCB exposure to male reproductive function cannot be reached because of the different experimental conditions utilized by various authors. In general, however, chronic exposure to levels of PCB at or near concentrations humans may encounter in the environment has minimal or no effect in animal models. While certain aspects of male reproductive function were altered by high levels of PCBs, no changes in male fertility have been reported.

IV. FEMALE REPRODUCTION

The cyclic nature of female reproductive function presents a series of physiological events subject to the influence of drugs and chemicals. Complex interactions within the hypoth-

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alamo-hypophyseal-gonadal axis increase the vulnerability of the pre- and postpubertal female to direct chemical influences upon fertility.

A. Sex Organs

Morphological consequences of PCB exposure on the female reproductive tract have been infrequently examined. Sanders and Kirkpatrick[26] fed 25 or 100 ppm of Aroclor® 1254 to adult female white-footed mice for 3 weeks. No notable effects of PCB ingestion were seen on ovarian or uterine weights. Female rat pups were injected (s.c.) with 1 or 10 mg of Aroclor® 1221 or 1 mg of Aroclors® 1242, 1254, or 1260 on the second and third days following birth.[10] Sacrifice at 7 to 8 months of age revealed no changes in ovarian or uterine weights but an increase in the absolute weight of the pituitary gland was found in rats receiving 10 mg of Aroclor® 1221 as a neonate. This difference, however, might not have been significant if relative rather than absolute gland weights had been calculated.

Gellart[10] investigated the uterotrophic activity of Aroclors® 1221, 1242, 1254, and 1260 in immature female (22 day) Sprague-Dawley rats. A single s.c. injection was administered followed by sacrifice at 24 hr. A significant increase in uterine weight was observed only in those animals treated with Aroclor® 1221 at a dose level of 1 g/kg. In a similar study Ecobichon and MacKenzie[33] injected 8-mg doses of several pure PCBs and commercial Aroclors® i.p. into immature female rats. The animals were sacrificed 18 hr after treatment and uterine weights, fluid, and glycogen contents were determined. In general, the lower-chlorinated PCBs increased uterine weights while the higher-chlorinated biphenyls increased uterine fluid and glycogen. The increase in uterine weight as well as increased fluid retention and glycogen content are indicators of estrogen action and support Bitman and Cecil's[12] report of estrogenic activity of the lower chlorinated Aroclors® (< 48% chlorination). In contrast, uteri from New Zealand white rabbits were significantly smaller following exposure to 300 mg Aroclor® 1254 administered weekly by oral intubation for 14 weeks.[26] Aroclors® 1221 and 1242 failed to affect the size of the uterus.

B. Puberty

The factors controlling the onset of puberty remain largely unresolved. Nevertheless, the normal maturation of the hypothalamo-hypophyseal-gonadal axis is requisite for cyclic reproductive function in female mammals. Thus, any chemically induced lesion during this critical developmental phase might modify the onset of puberty and subsequent reproductive success.

Unfortunately, there is a paucity of information on PCB exposure during this labile developmental period. Gellart[10] injected neonatal rats with 10 mg of Aroclor® 1221 on days 2 and 3 following birth. He reported the average age of vaginal opening in treated rats as less than 28 days compared to 42 days for untreated controls. This "precocious" puberty indicates an altered neuroendocrine function possibly induced by the reported estrogenic activity of high levels of Aroclor® 1221.[12] No other studies were identified which examined the effects of PCBs on pubertal development.

C. Estrous or Menstrual Cycles

The discrete neurological, neuroendocrine, and endocrine components that are manifest at puberty persist as the integrated control of estrous and menstrual cycles. These components exert some measure of control over each other and are additionally influenced by elements in the internal and external environments. Concern for harmful external factors such as the ubiquitous PCBs has stimulated many studies to evaluate their effects on estrous cycles.

Albino mice (NMRI strain) were given a single intraperitoneal (i.p.) injection of Clophen® A-60 at 20 mg/kg/24 hr after exhibiting a cornified vaginal smear.[37] The length of the estrous cycle was significantly increased for three cycles after which cycle length returned to normal. Orberg and Kihlstrom[26] administered 0.025 mg/kg Clophen® A-60 orally for 75 days to

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adult NMRI strain mice. The average length of the estrous cycle was 8.7 days as compared to 6.6 days for controls. Conversely, cycle lengths were not altered in mature white-footed mice fed 100 ppm Aroclor® 1254 for 3 weeks[38] or in adult rats fed 75 or 150 ppm Aroclor® 1242 daily for 36 weeks.[39]

Microscopic examination of ovaries from rats exposed to 150 ppm Aroclor® 1242 for 36 weeks revealed a reduction in the number of follicles.[39] Whether this was a direct or indirect effect on the follicles was not established but the reduction correlated well with the total inhibition of mating also reported.

The sequence of endocrine events occurring during the estrous and menstrual cycles is essentially the same. Consequently, it is not surprising that chronic exposure to low levels of PCBs has profound effects upon cyclic phenomenon in rhesus monkeys. Menstrual cycles were affected by 2.5 and 5.0 ppm Aroclor® 1248 fed in the diet daily for 7 months.[40] There were increases in the duration of menses and in menstrual bleeding, especially in the 5.0 ppm treatment group. Menses were 5 to 7 days longer in animals on the PCB diet than in the controls. Occasional amenorrhea was also noted in the treated animals.

Mueller et al.[41] administered 4 mg/kg daily of Clophen® A-30 via gastric intubation to mature cycling female rhesus monkeys. Treatment was initiated after confirmation of ovulation by laparoscopy and continued through the following cycle. The PCB treatment blocked ovulation in two of four monkeys in the ensuing cycle while inducing a double ovulation in one of four females. The site of action of Clophen® A-30 could not be established but in both anovulatory animals there was an indication of inadequate estrogen secretion. This suggests an ovarian site of action which is supported by the histopathological findings of Jonsson et al.[39] of decreased ovarian follicles in PCB treated rats.

D. Fertility

The failure to produce young is an excellent toxicity marker for chronic exposure to PCBs although single or multiple sites of action cannot be distinguished. Overall reproductive performance was completely blocked in rats receiving Aroclor® 1242 at 150 ppm/day for 36 weeks.[39] Treated females were exposed to normal males but no matings occurred, even though vaginal cytology indicated normal estrous cycles. It is difficult to reconcile apparent normal conditions during the cycle with the reported decrease in numbers of follicles and an absence of mating behavior.

Bleavins et al.[37] fed mature female mink from 5 to 40 ppm Aroclor® 1242 daily for 9 months. Complete reproductive failure occurred in animals fed diets containing 5 to 20 ppm. Subsequent mortality was 100% in mink on 20 or 40 ppm and 67% with the diet containing 10 ppm. Aroclor® 1016 reduced reproductive success 50% at 20 ppm but caused no deaths. These authors reported ferrets to be less sensitive than mink to the effects of PCBs as 20 ppm Aroclor® 1016 had no reproductive effects and 20 ppm Aroclor® 1242 was the minimal level required to block reproductive function. No mortality occurred in ferrets.

E. Summary

It is apparent that PCBs cause perturbations in female reproductive function. Depending upon the animal model utilized, PCBs variously affect the reproductive tract, neuroendocrine function (puberty, estrous, and menstrual cycles, ovulation) and general reproductive performance. The relationship of these observations in experimental animals to the human female is not yet established. The data must, however, be considered to provide an ominous warning of the potential danger in chronic low-level exposure.

V. PREGNANCY, PARTURITION, AND LACTATION

The complexity of pregnancy and maternal behavior from fertilization through weaning offers many physiological and behavioral intricacies vulnerable to alteration by extrinsic

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factors. Consequently, the pregnancy, the fetus, and the young are potentially at risk; the increasing concern about exposure of pregnant women to drugs or chemicals and the possible overt effects on the offspring is thus understandable. New life begins at fertilization with the union of the two germ cells. Under normal conditions, the zygote passes into the uterus where implantation and the formation of the placenta occur. The developing embryo is there nurtured until term and parturition. Studies of PCB-induced lesions in this process have not identified a site of action in the preimplantation events of pregnancy. The endpoint most accessible during early pregnancy for toxicity studies is the number of implantation sites. Otherwise, the number of live young born is utilized to assess toxic intervention during pregnancy.

A. Fecundity

Orberg and Kihlstrom[38] fed Clophen® A-60 daily for 10 weeks at a treatment level of 0.025 mg which reduced the number of implantations in mated NMRI mice. Orberg[42] subsequently administered 0.05 or 0.5 mg/kg pure tri(2,5,4') or hexachlorobiphenyl (2,4,5,2',4',5') via oral intubation to NMRI mice from days 1 to 6 of pregnancy. Females given 0.5 mg of either chlorinated compound had a significantly lower frequency of implanted ova than control females. In an earlier study, Torok[43] dosed pregnant NMRI strain mice per os from days 1 to 3 of gestation with 375 or 750 mg/kg daily of dichlorobiphenyl (2,2'). Both treatment levels resulted in decreased litter size due to high embryo mortality and decreased implantations in the high dose group. Conversely, Kanechlor® 500 in the diet of pregnant mice had no effect on the number of fetuses or offspring at 10 or 100 ppm/day throughout pregnancy.[44]

Linder et al.[34] conducted two-generation studies in Sherman rats with Aroclors® 1254 and 1260. Aroclor® 1254 was fed at dietary levels of 1, 5, 20, and 100 ppm daily while Aroclor® 1260 was fed at levels of 5, 20, and 100 ppm. Chronic exposure was initiated at 3 to 4 weeks of age in the F_0 generation and at weaning in the F_1 animals. Treatment was continuous through mating, gestation, and lactation. The F_0 rats fed 100 ppm of Aroclor® 1254 for either 62 or 188 days before each mating produced smaller litters than controls in both pregnancies (F_{1A} and F_{1B}). Small litters (F_{1B}) also resulted from 20 ppm in the 188-day parental treatment group. F_{1B} rats bred after exposure for 129 or 274 days (conception to mating) to 20 or 100 ppm had smaller litters than age-matched controls. No effect on reproduction resulted from treatment with Aroclor® 1260 except at 500 ppm for 67 days which reduced litter size. Baker et al.[33] reported fetal resorptions in rats treated with 70 ppm Aroclor® 1254 in their drinking water for 9 weeks. The females were placed with the males 1 week after the initiation of treatment.

In rats, chronic low-level exposure to PCBs is potentially more hazardous to a successful pregnancy than is subacute exposure except at extremely high levels. It required 500 ppm Aroclor® 1254 throughout gestation to reduce litter size in Osborn-Mendle rats.[45] In studies in which Aroclor® 1254 was fed to pregnant Sherman[1,34] or Wistar[46] rats between days 6 and 15 of gestation, there were no effects on litter size or number of live births. Dose levels ranged from 6.25 to 100 mg/kg daily. Survival to weaning was reduced by the 100 mg/kg treatment level.

Rabbits and guinea pigs are apparently more susceptible to the effects of PCBs than are rats and mice. Treatment of pregnant rabbits with PCB 1254 at levels of 12.5 mg/kg or greater from days 7 to 28 caused abortions, resorptions, stillbirths, and maternal deaths.[46] Less than 12.5 mg/kg for the duration of pregnancy had no effect on live litter size.[3] Guinea pigs were given a technical grade PCB (Clophen® A-50) or a pure hexachlorobiphenyl (2,4,5,2',4',5') via oral intubation.[47] The treatment level of Clophen® A-50 was 2.2 mg/day (total of 100 mg) from days 16 to 60 of gestation. The hexachlorobiphenyl was similarly administered to two groups, one receiving a total of 25 mg and the other 100 mg from days

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16 to 60 of pregnancy. The Clophen® A-50 caused a significantly higher incidence of fetal death whereas 6-CB had no effect at either dose level.

Reports by mink ranchers of severe reproductive failures and high kit mortality in their colonies which were given diets containing fish and animal byproducts focused suspicion on the possibility of PCB contamination. This prompted several investigative studies to substantiate the magnitude of this problem. Aulerich et al.[48] fed adult mink diets containing 30% Lake Michigan coho salmon or the control diet of ocean perch plus PCBs for 6 months or greater. Tissue levels of PCBs in most Great Lakes fish are known to be relatively high (approximately 10 to 15 ppm). The PCBs were combined and added to the control diet at 30 ppm, 10 ppm each of Aroclors® 1242, 1248, and 1254. Total reproductive failure occurred in females fed 30% coho salmon as their source of PCBs. Of 65 females, 59 failed to whelp and from the six term pregnancies only ten kits were born; two were alive but died with 24 hr. No term pregnancies were observed in those females which received control diet supplemented with the PCB mixture. All mink receiving the PCB supplemented diet died between the beginning of the breeding season and the end of the whelping period. In a later study, Aulerich and Ringer[47] mixed different amounts of Aroclor® 1254 ranging from 1 to 15 ppm with the diet for 5 or 11 months of exposure. The degree of 1254 toxicity was directly proportional to the total intake of the compound. Females fed Aroclor® 1254 at 5 ppm for 2 months before breeding averaged 0.8 kit per litter which compares with 0.3 kits per litter from females fed 1254 at 2 ppm for 7 months prior to breeding. They also compared the chronic reproductive toxicity of 2 ppm daily of Aroclors® 1016, 1221, 1242, or 1254 fed for approximately 11 months. Only 1254 had any detrimental effect on reproductive function at the low chronic exposure level.

Jensen et al.[39] fed 3.3 or 11 ppm PCB to adult female mink for 66 days. The number of implantation sites were not different between treated and control females but the number of kits delivered was significantly reduced in the low dose treatment group while no kits were born in the high dose group. Platonow and Karstad[38] fed 1 and 10 mg/kg of Aroclor® 1254 to cows 10 days before slaughter. Following slaughter, the muscles and organs were ground and combined into rations for adult mink containing 0.64 and 3.57 ppm of total PCB. No live kits were produced by females receiving the greatest level of PCB and only 1 of 12 females whelped in the low level diet group.

Forty-six bred bitches were given Aroclor® 1254 in the diet at concentrations of 0.25, 1.0, or 5.0 mg/kg daily from the day of breeding.[6] Significant decreases in the number of term pregnancies, the number of live pups per litter at birth, and the number of pups alive after 2 weeks resulted from dietary exposure to the 5.0 mg/kg dose. There was no effect of the lower dose levels on reproductive performance.

Domestic livestock have been exposed to PCBs from such sources as paints and sealants on silo walls being accidentally incorporated into rations. In addition to the potential danger of human exposure by consumption of animal products, the economic impact of impaired reproductive performance on livestock breeding programs would be damaging. Hansen et al.[31] fed 20 ppm Aroclor® 1242 to sows of proven fertility. Treated sows were exposed from one cycle prior to mating until the young were weaned. Control sows farrowed significantly more pigs per litter than treated sows (11.4 ± 4.1 vs. 6.4 ± 4.4). In addition, treated sows weaned fewer pigs per litter and at much lighter weaning weights. Similarly, Earl et al.[6] reported dose-related decreases in the number of pregnancies and the number of pigs farrowed per litter in miniature sows receiving 1.0, 10.0, or 30.0 mg/kg Aroclor® 1254. Exposure was from 21 days prior to breeding through weaning. Studies of PCB effects on reproductive performance in cattle, sheep, or goats have not been conducted, at least to this author's knowledge, which leaves a rather large void regarding PCB influences on livestock production.

Nonhuman primates have been found to be susceptible to PCBs when treated with levels

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at or near that to which humans may be environmentally exposed. Adult female rhesus monkeys were fed a diet containing 2.5 or 5.0 ppm of Aroclor® 1248.[4,30,46] After 7 months of exposure to the PCB, attempts were made to breed the females to nontreated males. All females on the 2.5 ppm diet and six of eight animals on the 5.0 ppm diet conceived. Only one live term infant was born in the high dose group due to resorptions, abortions, and one stillbirth. Five of eight females delivered live infants at the low exposure level. At birth, the surviving infants were smaller than controls but otherwise appeared normal. During the nursing interval, the surviving infant from the mother receiving 5.0 ppm and two infants from mothers receiving 2.5 ppm died of PCB intoxication. At weaning all surviving infants showed rapid improvement in toxic PCB symptoms.

Following removal from the experimental diet for 1 year, the female rhesus monkeys were rebred.[30,13] Although conception rate, pregnancies, and live births were normal, the infants were smaller and unthrifty. Two infants born to mothers which had received 5.0 ppm Aroclor® 1248 in the diet subsequently died.

Allen et al.[30] examined the effect of lower exposure levels of Aroclor® 1248 in rhesus monkeys. Dietary levels of 0.5 and 1.0 ppm were administered three times per week for 7 months. The females were bred to control males with normal term pregnancies resulting. The infants, however, were smaller and gained weight less rapidly than controls.

The insidiousness of chlorinated biphenyl contamination was illustrated in a report by Altman et al.[13] Harems of breeding age rhesus monkeys at the Perrine Primate Center in Miami showed similar reproductive problems as reported in experimental studies. There was a high incidence of abortions and stillbirths. The offspring were small and unthrifty with a poor survival rate. The cause was determined to be PCBs in the concrete sealant on cage floors. The floors were resurfaced and the problem disappeared with time. Interestingly, a breeding colony of squirrel monkeys was housed on these concrete floors but showed no PCB intoxication symptoms.

A pilot study conducted with three pregnant cynomolgus monkeys (*Macaca fascicularis*) also illustrated the detrimental effect of PCB 1254 on pregnancy.[34] Two females receiving 100 μg/kg daily beginning on day 60 of pregnancy delivered stillborn infants. One female similarly dosed with 400 μg/kg delivered a term infant which died at 139 days postpartum of acute confluent bronchopneumonia. Although numbers were small, these results support the findings in rhesus monkeys.

It is difficult to speculate at which stage of pregnancy PCBs exert their effect. Limited numbers of studies in which chronic administration of PCBs were initiated before mating and continued until the young were weaned indicated that ovulation and fertilization were essentially unaffected. The adverse effects of the chlorinated hydrocarbons appear to be blocked or delayed implantation or fetal loss through resorption, abortion, or stillbirth. Whether these consequences result from transplacental effects on the fetus or to nonspecific physiological changes in the maternal organism is difficult to differentiate. Several studies have demonstrated that PCBs are minimally transferred across the placenta in many mammalian species.[40,44,59,70] Transplacental passage ranges from less than 1% of the maternal dose[56,59,63] to as high as 6%[37] being dependent upon the stage of pregnancy when the fetal PCB level was determined. Although the placenta is an effective barrier to PCB transfer, the PCB used, percentage of chlorination, and duration of dosing are variables in determining fetal uptake.

B. Lactation

Although PCBs do not directly affect lactation, the most significant source of PCB exposure for offspring is mother's milk.[33,50,61,66] The lipophilic nature of PCBs is exemplified by the magnitude of their accumulation in animal fat depots. Thus, milk, rich in fats provides a vehicle by which the fat soluble PCBs can be transferred from mother to infant. Vodicnik

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and Lech[37] injected virgin mice with pure 6-CB (2,4,5,2',4',5') 2 weeks before mating. Sacrificing pre- and postpartum animals, they confirmed that transfer of the chlorinated biphenyl to offspring through the milk is much greater than transplacentally, at least for the hexochlorobiphenyl. The lactating mice eliminated almost all of their body burden of 6-CB in one lactation period. Bailey et al.[66] treated lactating rhesus monkeys with Clophen® A-30 at 16 mg/kg daily for 30 days. Concentrations of PCBs were higher in the milk than in the serum of dosed mothers. Serum levels of PCBs in the infants were two to three times higher than in their mothers. Sacrifice of a mother-infant pair revealed a 2- to 93-fold greater tissue concentration in the majority of infant tissues. Whether this situation applies to humans awaits determination of PCB pharmacodynamic similarities between humans and laboratory animals. Measurable concentrations of PCBs have been reported in the milk of "non"-exposed women.[67,68]

C. Summary

It is now well established that PCBs can be found in wildlife in many areas of the environment. Definitive studies, however, on the relationship between PCBs and reproductive function in terrestrial mammals is nonexistent. There have been efforts to correlate high tissue residues of the chlorinated biphenyls with breeding deficiencies in marine mammals. High tissue levels of PCBs were associated with low reproduction in Baltic seals[69] and Harbour seals[70] and in premature births of California sea lions.[71] A later study of the premature parturient females and offspring suggested an interrelationship between PCBs and disease agents, i.e., the San Miguel Sea Lion Virus and *Leptospira pomona* were recovered from specimens obtained from prematurely delivering cows and pups.[72] Reproductive failure has also been reported to be associated with PCB-induced pathological changes in the uteri of Baltic gray, common, and ringed seals.[73]

The suspected correlation between high tissue levels of PCBs and poor reproductive performance in selected marine mammal populations is a foreboding signal for all mammals, both terrestrial and aquatic, especially endangered species. More definitive observations of the effect of PCBs on pregnancy and fetal or neonatal survival in experimental animals substantiate the vulnerability of this period of life. Perhaps more ominous is the possibility that in utero exposure may alter subsequent adult reproductive success, an effect not apparent at birth. Potentially, the survival of whole species or populations is at risk.

VI. MECHANISM OF ACTION

Despite numerous studies concerning the deleterious effects of PCBs on mammalian reproduction, the mechanism and/or site of action remains virtually unknown. The complex interrelationships of female cyclic reproductive function offer many sites for a PCB-induced lesion in contrast to the male; nevertheless, it takes only one alteration in either sex to produce infertility. Reproductive success can be modified directly via a toxic PCB action on the differentiated reproductive tract or indirectly via a lesion in the neuro-gonadal axis. Other possible sites of action which might induce reproductive dysfunction are the nonreproductive endocrine organs, hepatic function, and behavior. One or any combination of these organs/structures might be affected by exposure to PCBs thus altering some aspect of reproduction either directly or indirectly.

A. Liver

One of the more apparent toxic manifestations of PCBs is on the liver. That the liver is a primary target organ is supported by studies which revealed a relatively high accumulation of orally administered PCBs in the livers of mammals, fetal through adult.[1,4,33,66,74] Grossly observable changes include hepatomegaly, and depending on dosage, a fatty infiltration of the liver. Cellular changes reported include an increase in smooth endoplasmic reticulum,

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an increase in cytoplasm around the nucleus, cytoplasmic inclusion, and atypical mitochondria.[34,36,75,76]

Commercial preparations and purified PCBs are potent inducers of hepatic mixed function oxidases and cytochrome P-450. This has been adequately demonstrated in several mammalian species including mice,[42,77,78] rats,[80-82] rabbits,[3,36] mink,[83] and ferrets.[83] In addition Chen and Dubois[79] reported that in rats enzyme induction was greater in males than females and in young rather than adults fed Aroclors® 1221, 1254, and 1260. Enzyme induction has also been reported in fetal and neonatal mice and rats exposed to PCBs either transplacentally or through breast milk.[10,77,78,84,85]

Generally, discontinuation of exposure to PCBs results in a slow decay of enzyme activity. There are reports, however, that suggest that the PCB induction may be permanent. Lucier and McDaniel[10] and Dieringer et al.[86] reported that transplacental and lactational exposure of neonatal rats to 4-CB or 6-CB feminized the male liver. UDP glucuronyltransferase activity, a nonsteroid metabolizing enzyme was repressed in neonatally exposed adult males to levels similar to that found in females. Thus, in rats, the chlorinated biphenyls not only alter hepatic metabolism by transient enzyme induction but possibly by an irreversible "imprinting" of postpubertal hepatic enzymes.

Liver homogenate preparations have been the experimental approach utilized most often to demonstrate changes in enzyme activity following exposure to PCBs. Although these studies show increased activity in specific steroid hydroxylating enzymes and suggest a mechanism by which PCBs could alter reproductive function, the in vitro system cannot account for compensatory homeostatic mechanisms which operate to offset induced hepatic steroid metabolism.

Studies designed to assess in vivo steroid metabolism following PCB exposure are scarce. Derr[86] injected rats i.p. with either 10 or 40 mg Aroclor® 1254 every third day for 2 weeks followed by i.v. administration of progesterone 4-[^{14}C] on day 16. The ratio of parent progesterone to metabolites decreased at both PCB treatment levels resulting in a 2.6-fold increase in progesterone metabolism. Other studies not designed to directly examine steroid metabolism have reported changes in steroid patterns following PCB exposure. Whether these alterations resulted from PCB-induced liver enzymes or direct gonadal effects were not determined. Platonow et al.[87] reported a significant decrease in urinary estrogens and dehydroepiandrosterone (DHA) in boars administered 100 mg of Aroclor® 1254, either as a single dose of 10 mg/day for 10 days. Rhesus monkeys were dosed with 4 mg/kg daily of Clophen® A-30 via gastric intubation and serum hormone levels monitored from ovulation through the following ovulation.[41] Three of four PCB-treated monkeys had subnormal serum estrogen levels.

Jonsson et al.[39] fed rats 150 ppm of Aroclor® 1242 for 36 weeks which completely eliminated mating and significantly decreased plasma progesterone. There were no significant changes in 17-α-hydroxyprogesterone although mean levels in PCB-treated animals were lower than controls. An antisteroid effect of Aroclor® 1254 was reported by Spencer.[88] He noted that administration of the PCB during decidualized pseudopregnancy reduced uterine glycogen. As glycogen is a sensitive indicator of estrogenic activity, he interpreted the results as evidence for the antisteroidal role of the PCB although no site of action was indicated.

B. Gonads

In spite of indisputable changes in mixed function oxidases, a direct effect of the chlorinated biphenyls on the gonads and/or steroidogenesis is a distinct possibility. A direct action on steroid production would preclude any homeostatic compensatory mechanism thus accounting for depressed circulating steroids. Several studies support a gonadal site of action. Jonsson et al.[39] in addition to reporting reduced progesterone levels, also noted histologically distinct ovarian stromal changes after prolonged exposure of rats to Aroclor® 1242. In

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harmony with these findings Brandt[7] injected (i.v.) either a mixture of two 6-CB-[^{14}C] isomers or 8-CB-[^{14}C] to pregnant female and adult male mice. The animals were sacrificed at various intervals from 4 min to 32 days after treatment for autoradiographic localization of the carbon label. In pregnant females there was a marked and selective uptake of 6-CB-[^{14}C] in the corpora lutea with a much lower concentration in the follicular walls and ovarian interstitium. Testicular uptake was low but persistent during the first few days following treatment. The radiocarbon-CB appeared to localize in the interstitial tissue and androgen-producing cells of the testes. The octachlorobiphenyl-[^{14}C] also accumulated in the corpora lutea but after a few hours was much higher in the ovarian stroma than the luteal tissues. This author found a similar pattern of accumulation of 6-CB in the hamster.[89] In contrast, the lower [^{14}C] chlorinated biphenyls as well as radiolabeled Aroclor® 1242 and 1254 did not accumulate in the gonads. Apparently, the degree of chlorination and the stereostructure determine the affinity of the molecule for different tissues. Nevertheless, the accumulation of some PCBs in gonadal tissues provides support for a direct effect on these steroid producing organs.

An intriguing effect of PCB on steroidogenesis was reported by Fuller et al.[90] Heavily luteinized ovaries were induced by PMS-HCG priming of immature albino rats. Following formation of corpora lutea 20 mg/kg of Clophen® A-30 was administered i.p. for 2 days, then the ovaries were removed, sliced, and incubated for 2 hr in the presence of luteinizing hormone (LH). Addition of LH to vials containing PCB-exposed luteal tissue resulted in approximately a 100% increase in progesterone synthesis above basal levels, whereas there was only a 31% increase with LH in nonexposed luteal tissue. This is suggestive of a synergistic relationship between PCB and LH or a priming effect due to estrogenic activity of the Clophen® A-30. These results appear contradictory to the PCB-induced decrease in steroid levels reported earlier; however, the closed in vitro system and the induced corpora lutea are different from the complex physiological interactions of an in vivo model.

C. Estrogenic Activity

The estrogenic activity of Clophen® A-30 suggested above is supported by reports of weak estrogenic activity of the lower chlorinated Aroclors®. Gellert[16] found 1 g/kg of Aroclor® 1221 to be uterotrophic in immature rats, whereas Bitman and Cecil[13] reported Aroclors® 1221, 1232, 1242, and 1248 to be minimally estrogenic based on the 18-hr glycogen response of the immature rat uterus to a single 8-mg injection. Ecobichon and MacKenzie[35] measured uterine weight, percent water, and percent glycogen in immature rats following an 8-mg injection (i.p.) of various PCBs. They tested a series of Aroclors® from 1016 to 1260 and isomerically pure chlorobiphenyls (mono-, di-, tetra-, and hexa-CB). In general, at least two of the uterine parameters were increased by each of the commercial preparations except Aroclor® 1016. Of the pure chlorobiphenyls, only those labeled in the 2 position affected the uterus, all parameters being increased with 2-chlorobiphenyl, but only tissue weight by the 2,2' isomer. Little estrogenic activity was noted with increased chlorination except for an increase in uterine weight with the 2,4,6,2',4',6'-hexachlorobiphenyl.

Estrogen-sensitive cells in target organs contain cytoplasmic receptors. Binding to a specific receptor is one of the initial events required for estrogen action. Thus, interaction of any of the PCBs with the receptor would be supportive evidence for the reported estrogenic activity. Nelson[91] correlated the inhibition of [^{3}H]-estradiol binding in vitro to the rat uterine receptor by Aroclors® 1221 and 1254 to the uterotropic effect of these PCBs in vivo. The Aroclors were injected i.p. at a level of 50 mg/kg to assess their uterotropic activity. A significant increase in uterine weight was induced by Aroclor® 1221 with a slight but nonsignificant increase following Aroclor® 1254. This correlated well with the in vitro results showing greater than 40% inhibition of binding for Aroclor® 1221 and less than 30% binding

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for Aroclor® 1254. The greater affinity of Aroclor® 1221 for the estrogen receptor does not establish its estrogenicity but does give credibility to reports of weak estrogenic activity of the lower chlorinated biphenyls. Whether this effect is environmentally consequential has not been determined.

D. Central Nervous System

During development the central nervous system (CNS) passes through a "critical steroid-sensitive period" in which organization of the neural mechanisms regulating hormonal and behavioral patterns in the adult are determined. This period occurs following birth in some rodents[91,92] and during fetal development in higher mammals.[91-93] Evidence indicates that behavioral disruption is not primarily attributed to demonstrated alterations in patterns of steroidogenesis in gonads of treated animals but rather to an alteration of the nervous tissues regulating sexual behavior.

As described earlier, Aroclor® 1221, similar to neonatally administered steroids, significantly advanced the age of vaginal opening and induced subsequent sterility in rats treated with 10 mg on days 2 and 3 following birth.[10] Sexual behavior was not studied in these animals but as the results parallel studies using estrogens a modification of reproductive behavior by the PCB is likely.

There are limited data which support a PCB-induced disorganization of nervous elements underlying the expression of normal behavior. Any effect of an animal's behavior patterns could potentially result in infertility. The mechanism by which the PCBs affect the nervous system is obscure. Chow et al.[7] reported that mice exposed to 3,4,3',4'-tetrachlorobiphenyl in utero developed morphological changes in the spinal and cranial nerve roots. These histopethologic alterations were associated with the motor dysfunction "spinning syndrome" in the 4-CB exposed mice. Earlier, Offner et al.[94] had shown Aroclor® 1254 to cause degeneration of neurons in rat brain cell cultures within 25 days of exposure.

Sexual behavior has not been studied following perinatal exposure to PCBs in any mammalian species to this author's knowledge. Nevertheless, other behavioral activities have been compared between perinatally PCB-exposed and control animals which illustrate neurotoxic lesions and potential adverse effects on reproduction. Pregnant rats fed 20 or 100 mg/kg Kanechlor® 500 produced offspring with retarded learning as measured by the number of errors in water-filled multiple T-maze testing at 13 weeks of age.[95] Open field behavior was not affected by this treatment. Bowman et al.[96] reported locomotor hyperactivity and deficiency in learning discrimination problems in three infant rhesus monkeys whose mothers had been fed 2.5 ppm Aroclor® 1248 throughout pregnancy and lactation. The PCB-exposed group showed fourfold the locomotor activity of controls at both 6 and 12 months of age. These same monkeys were retested at 44 months of age for locomotor activity.[97] Unexpectedly, there was a marked hypoactivity in previously hyperactive monkeys. The change in locomotor activity appeared 4 years after exposure and reportedly 3 years after fat biopsies revealed a clearance of PCB residues from the mesenteric fat. Although the number of animals was small, those results underscore the potential neurotoxic hazard to the developing organism.

E. Thyroid

Another extragenital effect of PCBs which could possibly alter reproductive function is the reported depression of circulating thyroxine. Thyroid hormones have been implicated in reproductive function for a long time, both clinically and experimentally. The reproductive system in the immature animals is more readily affected than the adult. Rats made hypothyroid by feeding thiouracil during pregnancy and lactation produced smaller litters at weaning with retarded sexual development of the young. In many species reproduction occurs despite hypothyroidism but fecundity is usually subnormal. In the human female one finds a tendency

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for abortion. Thyroidectomized rabbits become pregnant but exhibit a high incidence of abortions and stillbirths. Similar results have been reported for guinea pigs, sows, and ewes. Delayed puberty, infertility, and estrus and menstrual cycle irregularities are all symptoms of hypothyroidism. In males, hypothyroidism is associated with a reduction in libido and atrophy of seminiferous tubules and Leydig cells. The excess secretion of TSH induced by thyroid deficiency may reduce hypophyseal secretion of gonadotropin, thus reducing testis function. It should be noted that many of the signs of hypothyroidism are the same as those commonly seen after chronic exposure to PCB.

The literature concerning the relationship of the thyroid to reproduction is extensive[100] although a direct relationship has never been established. Nevertheless, ultrastructural lesions of thyroid follicle walls and decreased serum thyroxine were reported in offspring of rats fed 500 ppm Aroclor® 1254 from conception through lactation. This was associated with a reduction in litter size and body weights at weaning.[45,101] The concentration of PCBs in the thyroid of an infant-mother pair of rhesus monkeys was reported by Bailey et al.[66] as 93 to 1 at sacrifice. The mother was treated with 16 mg/kg of Clophen® A-30 for 30 days during lactation. Mink exposed to 5 ppm Aroclor® 1254 were hypothyroid during estrus and the breeding season.[102] They failed to bear young. Pigs farrowed by sows treated with 20 ppm of Aroclor® 1242 for the duration of pregnancy had atrophied thyroids when sacrificed at weaning (8 weeks).[31] The site of action of PCB has not been determined but could result from a direct effect of PCB on the secretion of thyroid hormone or possibly on hypothalamic control of TSH. Although information regarding the effect of PCBs on thyroid function is limited, the evidence justifies continued investigation.

F. Adrenal

Certain disorders of the adrenal gland, especially in females, may lead to oligo-ovulation, anovulation, amenorrhea, precocious or delayed puberty, and infertility. This effect on reproductive function may be directly or indirectly correlated with abnormal adrenal function. This is due to the fact that the gonads and adrenals have overlapping functions. Gonadal abnormalities may affect the normal pituitary-adrenal axis and adrenal malfunction may disturb the normal pituitary-gonadal relationship. Therefore, it is reasonable to assume that a PCB effect on the adrenal could disrupt reproductive development and/or function.

Brandt[7] described a rapid accumulation of [^{14}C]-6-CB and [^{14}C]-8-CB in the adrenal cortexes of male, pregnant female, and fetal mice. Prolonged retention (> 30 days) in the adrenal was observed with both labeled congeners after a single oral or i.v. dose. Sanders and Kirkpatrick[30,34] showed decreased plasma corticoid levels in white-footed mice treated with Aroclor® 1254. Inao[103] reported atrophy of the adrenals and a decreased corticosteroid production in rats after feeding Kanaclor® 400. A significant decrease in plasma corticosteroids was also noted by Bruckner et al.[104] in male rats following i.p. injections of Aroclor® 1242. Conversely, Sanders et al.[17,32] reported an increase in corticords in adult white mice treated with Aroclor® 1254. Wasserman et al.[100] also observed a hyperfunction of the zona fasciculata associated with elevated plasma levels of corticosterone in rats treated with "PCB's 1221".

An explanation for the conflicting reports of hypo- and hyperadrenal function is not apparent. The differences in experimental conditions and stress factors make comparisons futile.

G. Summary

When all potential mechanisms by which mammalian reproductive function can be modified by PCBs are considered, it becomes apparent that no single site of action predominates. First, it is difficult, if not impossible, to distinguish PCB-induced changes in hepatic steroid-metabolizing enzymes from a direct effect on endocrine function. While direct chemical

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damage to the secretory organ may result in decreased hormonal output, the weakly estrogenic PCBs could effect endocrine function by virtue of their competitive affinity for hormone receptors within the organ. Altered receptor availability and/or increased hormone metabolism change trophic hormone thresholds, thus disrupting feedback patterns between the endocrine organs and hypothalamus. This might directly or indirectly modify some phase of the reproductive process. Determination of the definitive site(s) of action remains an elusive goal.

VII. CONCLUSIONS

This chapter has attempted to summarize and integrate as many relevant studies as possible into hypotheses of how environmental PCBs affect mammalian reproductive function. The many studies of varying species, sex, dosages, route and duration of administration and PCB preparations, as well as conflicting findings between studies have made such a goal almost unattainable. Nevertheless, several generalizations relating to their potential deleterious actions on reproduction in wildlife, livestock, and human populations through chronic exposure were derived from the experimental literature.

A point which becomes immediately apparent is that sensitivity to chronic (sublethal) exposure to PCBs increases with higher mammalian orders. In addition, developing organisms are more sensitive than adults and females more sensitive than males to the chlorinated biphenyls. The liver is undoubtedly a major target organ but conclusive evidence that establishes a direct relationship between PCB-induced steroid metabolizing enzymes and reproductive dysfunction is lacking. A demonstration of the accumulation of PCBs in the gonads in conjunction with reports of altered steroid patterns in various mammalian models was suggestive of a direct action of PCBs on endocrine function. Evidence of estrogenic activity for the lower chlorinated biphenyls supports a direct effect on reproductive function. Nevertheless, whether the effect of the PCBs is directly on the reproductive system or acts indirectly through hepatic enzyme induction or on nonreproductive endocrine organs, the outcome is a diminished capacity of the organism to reproduce itself. Finally, the persistence of PCBs in our environment threatens the very existence of the most vulnerable species of wildlife.

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Table 1
SUMMARY OF PCB EFFECTS ON DIFFERENTIATION AND DEVELOPMENT

PCB	Dose	Treatment (days)	Major effect	Species	Ref.
Kanechlor® 500	100 mg/kg	13—21 P	Low weight female fetuses	Mouse	97
2,4',5-Trichlorobiphenyl, 2,4,5,2',4',5'-hexachlorobiphenyl	50 µg	5—22 P	Increased postnatal growth		42
2,2'-Dichlorobiphenyl	750 mg/kg	1—3 P	Reduced fetal weights		56
	500 mg/kg	1—6 P			
3,4,3',4'-Tetrachlorobiphenyl	32 mg/kg	10—16 P	Motor dysfunction in offspring		7, 8
Aroclor® 1221	1 g/kg	2	Persistent vaginal estrus syndrome	Rat (neonatal)	10
3,4,3',4'-Tetrachlorobiphenyl	3 mg/kg	5, 11, 13, 15, 18 P	Permanent postpubertal male liver	Rat	16, 84
Aroclor® 1248	2.5 ppm	1—165 P	Low birth weights	Rhesus monkey	52
	5.0 ppm				

Note: P = pregnancy

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Table 2
SUMMARY OF PCB EFFECTS ON MALE REPRODUCTION

PCB	Dose	Treatment (days)	Major effect	Species	Ref.
Aroclor® 1254	62.5 ppm	14	No effect	Mouse	17
	4000 ppm	14	No effect		
Aroclor® 1254	10 ppm	15	Reduction in sperm per testicular area		25
	200 ppm				
3-CB	50 μg/day	5 P—W	Offspring showed no effects on reproduction at adults		31
6-CB					
Aroclor® 1254	200 ppm	28	Decreased seminal vesicle weight	White-footed mouse	19
	400 ppm	14	Decrease in seminal vesicle weight; decrease in number of sperm per testis		20
Aroclor® 1242	5000 mg/kg	1	Decreased number of developing spermatogonia	Rat	36
	500 mg/kg	4			
Aroclor® 1254	500 mg/kg	1	Increased testis weight		21
Aroclor® 1260	50 mg/kg	14—30 P	Increased testis weight		9
Kanechlor® 500	100 ppm	28	No effect		21
Aroclor® 1254	70 ppm	63	No effect		33
Aroclor® 1254	25 ppm	28	No effect	Cynomolgus rabbit	22
Aroclor® 1016	20 ppm	270	No effect	Mink/ferrets	27
Aroclor® 1242	5—60 ppm	270	No effect		
Aroclor® 1254	100 mg/kg	1	Decreased testis DNA and testis estrogens	Boar	37
	10 mg/kg	10			
Aroclor® 1248	5 ppm	300	Decreased sperm count in 1 of 4 exposed males	Rhesus monkey	30

Note: P = pregnancy; W = weaning

Table 3
SUMMARY OF PCB EFFECTS ON FEMALE REPRODUCTION

PCB	Dose	Treatment (days)	Major effect	Species	Ref.
Clophen® A-60	20 mg/kg	1	Increased length of estrous cycles	Mouse	37
Clophen® A-60	0.025 mg/day	75	Increased length of estrous cycles		38
3-CB	50 μg/day	5 P-W	Offspring showed no effects on reproduction at adults		31
6-CB					
Aroclor® 1254	25 ppm	21	No effect	White-footed mouse	34
	100 ppm				
Aroclor® 1221	1000 mg/kg	1	Uteroscopic	Intolerant rat	10
	10 mg/day	2	PVE syndrome	Neonatal rat	
Aroclor® 1254	70 ppm	63	No effect	Rat	33
Aroclor® 1016	20 ppm	180	50% Reproductive failure	Mink/ferrets	27
Aroclor® 1242	5 ppm	180	Reproductive failure		
	10 ppm	180	Greater than 66% mortality		
Aroclor® 1242	20 ppm	180	Reproductive failure		
Aroclor® 1254	300 mg/event	100	Decreased uterine weight	Prepubertal rabbit	36
Aroclor® 1242	2.5 ppm	210	Altered menstrual cycles, amenorrhea, increased urinary ketosteroids	Rhesus monkey	40
	5.0 ppm				
Clophen® A-50	4 mg/kg	36	Decreased estrogen levels, blocked ovulation in 50% of animals		41

Note: P = pregnancy; W = weaning

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Table 4
SUMMARY OF PCB EFFECTS ON PREGNANCY AND PARTURITION

PCB	Dose	Treatment (days)	Major effect	Species	Ref.
3-CB	0.5 mg/day	1—6 P	Decreased frequency implantation	Mouse	42
2-CB	375 mg/kg 750 mg/kg	1—3 P	Increased duration of pregnancy, decreased litter size, decreased term pregnancies		43
Aroclor® 1254	25 ppm	6—15 P	Reduced uterine glycogen and ovarian protein during pseudopregnancy (decidualized) and pregnancy	Rat	88
Aroclor® 1254	300 ppm	6—15 P	Reduced fetal survival rate		—
Aroclor® 1254	100 ppm	W—67 or 186	Reduced survival to weaning		
	500 ppm	W—67 or 186	Fewer litters, smaller litter size, 100% pup mortality		
Aroclor® 1254	20 ppm	W—62 or 188 2 Generations	Fewer pups per liter F_1 and F_2		24
	100 ppm	2 Generations	Increased mortality in F_1; decreased mating performance of F_1 adults		
Aroclor® 1260	500 ppm	M—67 and 186	Reduced live litter size and survival to weaning to F_{1a} and F_{1b}		
Aroclor® 1254	100 mg/kg	7—18 P	Decreased pup survival to weaning		
Aroclor® 1254	10—15 mg/kg	7—15 P	No effect		2
Aroclor® 1254	6.25—100 mg/kg	6—15 P	No effect		46
Aroclor® 1254	50 ppm	1—21 P	No effect reproduction; reduced serum T_3 and T_4		45
	500 ppm	1—21 P	Decreased litter size; reduced serum T_3 and T_4		
Aroclor® 1254	70 ppm	63	Fetal resorption		33
Aroclor® 1221	10 mg/kg	1—28 P	No effect reproduction	Rabbit	3
Aroclor® 1254	10 mg/kg	1—28 P	No effect		
Aroclor® 1254	12.5 mg/kg 25.0 mg/kg	1—28 P	Resorptions, abortions, fetal deaths, maternal deaths		46
Clophen® A-50	2.2 mg	16—60 P	Increased fetal deaths	Guinea pig	47
4-CB	2.2 mg	16—60 P	No effect reproduction		
Aroclor® 1221 Aroclor® 1248 Aroclor® 1254	30 ppm (combined)	180	Failed to reproduce	Mink	48
Aroclor® 1016	2 ppm	300 ±	No effect		49
Aroclor® 1221	2 ppm	300 ±	No effect		
Aroclor® 1242	2 ppm	300 ±	No effect		
Aroclor® 1254	2 ppm	300 ±	Reduced litter size		
Aroclor® 1254 (Contaminated beef)	0.64 ppm 3.57 ppm	160	Total reproductive failure		28
Aroclor® 1254	0.5 ppm	270	No effect reproduction		102
	2.0 ppm	270	Failed to bear young		
	5.0 ppm	270	Failed to bear young		

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Table 4 (continued)
SUMMARY OF PCB EFFECTS ON PREGNANCY AND PARTURITION

PCB	Dose	Treatment (days)	Major effect	Species	Ref.
Aroclor® 1254	1.0 mg/kg	0—60 P	No effect	Dog	6
	5.0 mg/kg	0—60 P	Reduced pregnancies, live pups; increased resorptions		
Aroclor® 1242	20 ppm	182 ±	Farrowed and weaned fewer pigs	Sow	51
Aroclor® 1254	1 mg/kg	182 ±	Farrowed and weaned fewer pigs		6
Aroclor® 1248	2.5 ppm	180	Conception normal but 3 of 8 resorbed	Rhesus monkey	40
	5.0 ppm	180	6 of 8 pregnant; 3 aborted, 1 resorbed, 1 stillbirth		

Note: P = pregnancy; W = weaning; M = mating.

Table 5
SUMMARY OF POSSIBLE PCB SITES OF ACTION

PCB	Dose	Treatment (days)	Major effect	Species	Ref.
Aroclor® 1254	500 mg	1 (day 19 P)	Induced hepatic enzymes in offspring	Mouse	77
Aroclor® 1254	62.5 ppm	14	Increased corticosterone		17
	4000 ppm	14	Increased corticosteroids and adrenal weights		
6-CB	100 mg/kg	1	Induced hepatic enzymes		78
Aroclor® 1254	400 ppm	14	Decreased corticosterone	White-footed	20
Aroclor® 1254	400 ppm	21	Decreased plasma corticoids	Mouse	14
	100 ppm				
Aroclor® 1254	10 mg/day	5 (over 2 weeks)	Increased metabolism of progesterone	Rat	96
Aroclor® 1242	75 ppm	252	Decreased plasma progesterone		39
	150 ppm	252	Decreased plasma progesterone Decreased ovarian follicles		
Aroclor® 1254	50 ppm	7	Induced hepatic enzymes		81
	10 ppm	14	Induced hepatic enzymes		80
	100 μM	27	Degeneration of cultured brain cells		96
Clophen® A-30	30 mg/kg	1	Enhanced LH induced ovarian progesterone synthesis in vitro		90
Aroclor® 1221	250 ppm	70	Increased plasma corticosterone		103
Aroclor® 1254	70 ppm	63	Induced hepatic enzymes		33
1-CB	30 mg/kg	5, 11, 13, 15, 18 P	No effect on liver		16
4-CB	3 mg/kg	5, 11, 13, 15, 18 P	Induced hepatic enzymes; fenestrated adult male liver		84
6-CB	30 mg/kg	5, 11, 13, 15, 18 P	Induced hepatic enzymes; fenestrated adult male liver		82
Aroclor® 1254	25 mg/kg	6	Induced hepatic enzymes		
Aroclor® 1254	50 ppm	1—21	Reduced serum T_3 and T_4		45
	500 ppm		Reduced serum T_3 and T_4		
Aroclor® 1221	100 ppm	—	Greater than 40% inhibition of E_2 binding to uterine receptor in vitro	Rat (immature)	91
Aroclor® 1254	100 ppm	—	Less than 30% inhibition of E_2 binding to uterine receptor in vitro		

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Table 5 (continued)
SUMMARY OF POSSIBLE PCB SITES OF ACTION

PCB	Dose	Treatment (days)	Major effect	Species	Ref.
Aroclor® 1221	50 mg/kg	1	Uteroruptive		
Aroclor® 1254	50 mg/kg	1	No effect		
Aroclor® 1221	8 mg/kg	1	Increased uterine glycogen		42
Aroclor® 1232	8 mg/kg	1	Increased uterine glycogen		
Aroclor® 1242	8 mg/kg	1	Increased uterine glycogen		
Aroclor® 1248	8 mg/kg	1	Increased uterine glycogen		
Aroclor® 1254	8 mg/kg	1	No effect		
Aroclor® 1260	8 mg/kg	1	No effect		
Aroclor® 1262	8 mg/kg	1	No effect		
Aroclor® 1268	8 mg/kg	1	No effect		
Aroclor® 1016	20 ppm	28	Weak induction of liver enzymes	Mink/ferret	83
Aroclor® 1242	20 ppm	28	Induced liver enzymes		
Aroclor® 1254	0.5 ppm	270	Increased T_3		102
	2 ppm	270	Hypothyroid		
	5 ppm	270	Hypothyroid		
Aroclor® 1254	10 mg/kg	1	No effect	Boar	87
	1 mg/kg	10	No effect		
	100 mg/kg	1	Decreased uterus DNA and estrogens		
	10 mg/kg	10	Decreased uterus DNA and estrogens		
Aroclor® 1248	2.5 ppm	1 P—W	Hyperactive at 6 and 12 months of age; learning deficiencies	Rhesus monkey	98
Aroclor® 1248	2.5 ppm	1 P—W	Hyperactive at 44 months of age		99
Clophen® A-30	4 mg/kg	36	Decreased serum estrogen		41

Note: P = pregnancy; W = weaning.

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Chapter 8

USE OF ORGANISMS TO QUANTIFY PCBs IN MARINE AND ESTUARINE ENVIRONMENTS

David J. H. Phillips

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I. ABSTRACT

PCBs have entered the environment from a variety of industrial sources. Their low water solubility, highly lipophilic character, and considerable resistance to degradation are the cause of their bioaccumulation and persistence in biota. The quantification of PCBs in the aquatic environment is possible through the analysis of water, sediments, or biota. Of these methods, the analysis of so-called bioindicator or sentinel organisms has certain practical and theoretical advantages, and is increasingly becoming the method of choice in surveys of PCBs.

Several problems must, however, be overcome if the results of such surveys are to give an accurate picture of the general contamination of aquatic ecosystems by PCBs. The first of these concerns is the accurate analytical quantification of PCB residues in aquatic biota. Difficulties arise because of a lack of standardized methods for the analysis and quantification of PCBs, coupled with the selective accumulation of some PCB compounds by organisms. Improvements in analytical techniques are required to permit separation of PCBs into individual components, each of which should be considered a unique contaminant in its own right.

The other problems inherent in the use of bioindicator organisms to monitor PCBs mainly concern biological variables. The lipid content of the organisms studied is of very considerable importance in determining the levels of PCBs accumulated within the tissues. The effects of this parameter on the results may be reduced or eliminated by basing analytical data on both wet and lipid weights. Season of collection or organism condition is also important; temporal variability may occur due to fluctuations in pollutant availability, biological changes (commonly involving the sexual cycle, but including in some instances factors such as growth and migration of organisms), and alterations in water quality (particularly salinity and temperature). Size, age, or weight of the individuals sampled may also influence the PCB concentrations present, particularly in finfish. Finally, both organism's sex and its vertical position in the water column may affect its content of PCBs.

Each of these variables must be taken into account by the researcher undertaking bioindicator surveys for PCBs. The influence of each variable can be reduced, or sometimes eliminated, by certain methods, many of which involve care at the stage of organism sampling. If such precautions are taken, the use of bioindicators to monitor PCBs in aquatic environments is a powerful tool, giving rise to accurate and rapid estimates of the time-integrated bioavailability of these compounds in the various study locations.

II. INTRODUCTION

Polychlorinated biphenyls (PCBs) were first produced commercially in 1929 in the U.S. Between 1929 and 1977, about 600,000 t of PCBs were manufactured in the U.S. alone; a similar additional amount was manufactured in Japan, the U.S.S.R., and parts of Europe.[1-3] These compounds were used for a variety of purposes, e.g., as dielectric fluids in transformers and capacitors, as heat transfer or hydraulic fluids, and as lubricating oils and plasticizers. Many of the uses can be classified as dissipative, i.e., recovery of the PCBs has not occurred, and the PCBs have therefore entered the general environment.

Estimates of the total amount of PCBs in the global environment vary, but it is probable that about 15% of the PCBs manufactured to date has already escaped to enter a "mobile environmental reservoir"; much of the remainder is not in closed systems and may reach the general environment at some future time.[3-6,7]

The possible persistance of effects of PCBs in the environment received remarkably little attention for more than 35 years after their introduction into commercial usage. However, in the mid-1960s, chemists began to take note of unidentified peaks on chromatograms of

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extracts of biota containing DDT and its metabolites.[8,9] These compounds were identified by Sören Jensen in 1966 as PCBs, and he showed that they were present in biological tissues prior to the introduction of DDT.[8,10]

PCBs enter the general environment by a variety of routes.[6] One of the most important routes is transportation in air, which permits the efficient and rapid dissipation of PCBs from point sources to distant areas.[11-14] In addition to the airborne route, marine environments receive PCBs from urban run-off,[12-16] from wastewater discharges,[17-19] from rivers,[20] and directly from dumped sewage sludge[21] and antifouling paints.[22,23]

Once in the aquatic environment, PCBs exhibit a bioaccumulative tendency similar to that of DDT and its metabolites. This similarity is based upon the very low water solubilities of the two groups of compounds, coupled with their extreme lipophilic nature and great resistance to degradation. As a result of the diffuse airborne and waterborne inputs and the great persistence and stability of PCBs, these compounds may be found in biota throughout the world, including both poles,[24-26] the deep sea,[27] the offshore oceans,[11,28-30] and inland lakes and coastal waters.

The recent realization of the ubiquity, persistence, and possible toxic effects of PCBs in aquatic environments has given rise to partial bans or restrictions on the use of these compounds.[3,5,30,31,32] However, such restrictions will not solve the current or even the future problems, as considerable amounts of PCBs have already been introduced to the general environment and will continue to leach to the final marine sediment sink from all sources. In addition, the restrictions to date apply in general only to the developed nations of the Northern Hemisphere, and PCB usage in the tropics and subtropics continues unabated; indeed, in some areas usage is increasing to keep pace with industrial development.

As a result, it is clear that PCBs will continue to be present in aquatic environments for many decades. Their effects in the ecosphere depend, of course, on dosage; if sufficient data on the amounts of PCBs present in an aquatic ecosystem can be accumulated, decisions can be made on how to deal with the problems engendered by their presence. It is therefore clear that one of the primary needs is the development of an accurate and efficient method of quantifying PCBs in aquatic environments. This chapter addresses the methods available for this purpose, with particular emphasis on the use of aquatic organisms (so-called "biological indicators").

III. PCB QUANTIFICATION IN THE AQUATIC ENVIRONMENT

As Phillips[33-35] has pointed out, conservative pollutants such as trace metals, organochlorine pesticides, or PCBs, may be quantified in the aquatic environment by the analysis of natural waters, sediments, or aquatic organisms. None of these methods is ideal, each possessing certain disadvantages. The three methods are discussed briefly below.

A. Analysis of Water

Considerable amounts of data have been amassed concerning PCB concentrations in marine and fresh waters. Table 1 shows typical concentrations reported for open oceans, coastal waters, and estuaries or rivers. It is evident that PCB levels vary over a considerable range in any one type of water. However, as might be expected, open ocean concentrations of PCBs[36-40] are generally less than those found in coastal waters[41-46] and rivers exposed to industrial sources of PCBs are commonly even more highly contaminated.[47-50]

The PCBs present in aquatic environments partition between the soluble phase and the particulate phase of the water mass. Pavlou and Dexter[51] considered this partitioning in detail, and concluded that at the normal suspended loads encountered in coastal waters (0.5 to 10 g/m³), less than 20% of the PCB would be adsorbed to inorganic particulate matter. However, this situation will alter under conditions where either particularly large amounts

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Table 1
RANGES IN PCB CONCENTRATIONS (ng/ℓ) REPORTED FOR OPEN OCEANS, COASTAL WATERS, AND ESTUARIES OR RIVERS

Area	Location	Range in PCBs (ng/ℓ)	Ref.
Open oceans	North Atlantic	<1—150	36
		0.4—41	37
	Sargasso Sea	0.9—3.6	38
	North-South Atlantic	0.3—8.0	39
	Mediterranean Sea	0.2—8.6	40
Coastal waters	Southern California	2.3—36	41
	Northwest Mediterranean	1.5—38	42
	Atlantic coast, U.S.	10—700	43
	Baltic coasts	0.3—139	44
		0.1—28	45
	Dutch coast	0.7—8	46
Estuaries/rivers	Wisconsin rivers, U.S.	<10—380	47
	Rhine-Meuse system, Holland	10—200	48
	Tiber Estuary, Italy	9—1000[a]	48
	Brisbane River, Australia	ND—50	49
	Hudson River, U.S.	<100—2.6 × 10⁶	50

Note: ND, not detectable (no limits quoted).

[a] As decachlorinated biphenyl equivalents.

Table 2
CONCENTRATIONS OF PCBs (RANGES, ng/ℓ) IN THE DISSOLVED AND SUSPENDED FRACTIONS OF WATER SAMPLES FROM RIVERS DRAINING TO THE RHINE-MEUSE ESTUARY[48]

		PCB conc. in water (ng/ℓ)	
Rivers	Date	Dissolved	Suspended
Lek	June 1974	14—80	40—300
Maas	August 1976	13—20	25—40
Waal/Oude Maas	June 1974	40—100	50—230
	August 1976	120—200	130—160
Nieuwe Maas	June 1974	80—200	50—100
	December 1974	10—75	20—400
	August 1976	60—180	80
Estuary mixing zone	June 1974	50—120	80—200
	December 1974	20—100	40—100
	August 1976	40—65	25—40

Note: Filtration was by Whatman GF/C filters.

of suspended material are present, or PCB concentrations are unusually high. Thus, in turbid rivers, concentrations of PCBs in the particulate fraction may exceed those in solution; an example of such a situation is shown in Table 2. In highly contaminated areas, PCBs may exceed their water solubilities by adsorbing to particulates.[50-52]

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Although these general comments may be made concerning the partitioning of PCBs between solution and particulates, it must be emphasized that present knowledge of such matters is far from adequate. Thus, it is uncertain as to precisely how PCBs partition between all the various components of natural waters, which may include inorganic particulates of various sizes and geochemical types, plankton (zooplankton, phytoplankton, and microplankton), and colloids, humates, and several other ill-defined classes of particulates. Indeed, the data gathered to date frequently suffer from a lack of attention to the methods of filtration employed[33] and results are in any event applicable only in terms of the filtration methods employed. While there is little doubt that PCBs exhibit high affinities for phytoplankton and zooplankton,[34-40] their affinities for inorganic particulates, microplankton, and colloids or humates are much less well understood, and the transfer of PCBs from one phase or compartment to another has received little attention.[41]

In addition to partitioning between the soluble and various particulate components of natural waters, PCBs also partition between surface films and subsurface waters. Thus, for example, Raybaud[42] found higher concentrations of DDT, DDE, lindane, and PCBs in surface films of sea water near the mouth of the River Huveaune, off Marseilles. The surface film exhibited PCB levels of 46 to 121 μg/ℓ, whereas water from 40 cm below the surface contained only 0.1 to 0.21 μg/ℓ PCBs. Other authors have confirmed the disparity between PCB concentrations in the surface microlayer and subsurface waters,[12,31,43-47] and many other contaminants are also known to be present at higher concentrations in surface films. This factor is important not only in terms of the sampling of water for PCBs but also because of the possibility of volatilization or codistillation of PCBs from the surface microlayer to the atmosphere.[43]

The researcher wishing to quantify PCBs by analysis of water is thus faced with several practical problems. Decisions must be made not only on how and at what depth the sample should be taken, but also on what method of filtration will be used. There is no standard methodology, and it is obvious from the examples given above on the effects of particulate load and surface films that the results of any survey depend heavily on the particular methodology employed. As a result, as Bernhard[48] states, "... in the present state of knowledge, probably only data from the same author can be used for the detection of differences between samples taken at different sites and/or different dates."

Other problems also exist in attempting to quantify PCBs in aquatic ecosystems by the analysis of water. The concentrations of PCBs present are low compared to many other types of pollutants such as trace metals, commonly varying between nanograms per liter and micrograms per liter levels. The contamination of samples by extraneous sources of PCBs or by the collection equipment employed is therefore a real and ever-present problem.[22] In addition, large volumes of water must be collected and (usually) filtered before preconcentration, presenting practical problems.

Even if such problems of contamination and sample handling can be overcome, the temporal fluctuation of PCB concentrations in water may be extreme. This is particularly the case in estuarine sites close to PCB sources but occurs also in coastal waters more remote from inputs. In the Tiber Estuary, Puccetti and Leoni[49] found variations of greater than an order of magnitude between samples taken sequentially at the same site, and similar variations have been noted by others.[49] These fluctuations in ambient PCB levels in water may be caused by hydrological factors, the episodic nature of inputs from industrial sources, scavenging by plankton, or several other factors. The effects of such fluctuations are severe in terms of the efforts required to elucidate real mean levels of PCBs in a study area. Some method of time-averaging samples must be found, or several samples must be collected at each site, to confirm representative concentrations. If bulking of samples (which is difficult owing to the large volumes required and possibilities of contamination) is not possible, and time-integrating water samplers are not available, the investigator has no real choice but to

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Table 3
RANGES IN CONCENTRATION OF PCBs (ng/g DRY WEIGHT) REPORTED FOR SEDIMENTS IN AREAS RANGING FROM RELATIVELY UNCONTAMINATED TO HIGHLY CONTAMINATED

Area	PCB conc.	Ref.
Mediterranean Sea	0.8—9.0	42
Gulf of Mexico	0.2—35.0	50
Chesapeake Bay	4—400	43
Lake Superior	5—390	12
Tiber Estuary	28—770	48
Rhine-Meuse Estuary	50—1,000	46
New York Bight	0.5—2,200	69
Palos Verdes Peninsula	30—7,900	70
Hudson River	tr—6,700	50
Escambia Bay	190—61,000	50

Note: tr, trace.

collect and analyze several samples at each location studied. This effectively diminishes the number of locations which may be sampled.

In summary, the analysis of natural waters as a method for quantifying PCBs in the aquatic environment is fraught with problems. Practical problems involve the need for multiple sampling at each location studied to overcome temporal fluctuations in ambient PCB levels, and the requirement for large-volume samples. Researchers must ensure the sample is taken while avoiding extraneous sources of PCBs and contamination by the sampling equipment. Once the samples are collected, they must be filtered, and this introduces an arbitrary element into the results, as any method chosen will relegate PCBs in certain particulates to the "suspended" phase while retaining PCBs on finer material in the "soluble" phase. Finally, as there is no definite relationship of bioavailability of PCBs to the operationally defined soluble and particulate phases, uncertainty remains as to the possible effects of any PCB concentrations found in waters on the indigenous aquatic biota.

B. Analysis of Sediments

The use of sediment analysis as a method of quantifying PCBs in aquatic environments has definite advantages over the analysis of water considered above, although certain practical and theoretical problems remain.

The concentrations of PCBs found in sediments are generally much higher than those in water. Typical concentrations of PCBs in sediments are shown in Table 3, values being selected to represent a range from sediments in relatively uncontaminated ocean areas,[42,50] through moderately contaminated coastal or lake sediments,[12,43] to highly polluted estuarine sediments.[46,48,50,69,70]

Because PCB levels in sediments are greater than those in water, the practical problems at sampling are diminished somewhat. Samples of reasonably small volume are generally sufficient and analysis is simpler as organic extraction procedures replace the preconcentration techniques used for water analysis. The problem of contamination, while ever-present, is substantially reduced compared to water analysis, because of the greater amounts of PCBs in the sample.

In addition, PCB levels in sediments represent a time-averaged indication of contamination at the sampling site, rather than a space/time-defined sample as in the case of water analysis.

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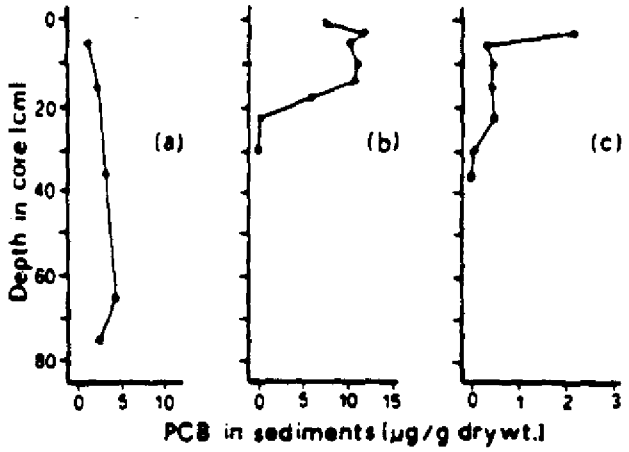

FIGURE 1. Variation in PCB concentrations in sediments with depth. (a) New York Harbor;[48] (b) Hudson River, 83 mi upstream of Manhattan Island;[20] (c) the Christiaensen Basin (note altered horizontal scale).[48]

This time integration facility is important, as it maximizes information output per sample, and permits detailed large-scale surveys to be completed with the minimum of effort, each study location being sampled (usually) only once.

Despite these advantages, the use of sediment analysis to quantify PCBs in aquatic environments is open to certain criticisms, both in terms of practical difficulties and interpretive uncertainties. The practical problems essentially involve the requirement for sediments to be representative of the bottom in the locations sampled, and to be undisturbed. Most sampling involves the use of remote coring or grab devices, deployed from a vessel some considerable distance above the sediments. Under these conditions it is generally impossible to satisfy the skeptic that undisturbed samples which are representative of the general bottom have been taken.

The requirement for sediment samples to be representative of the location studied is self-evident and need not be elaborated, except perhaps to point out that in coastal areas sediments are commonly very heterogeneous over short distances. The requirement for undisturbed samples merits further discussion, however. It will be clear that all sediments must exhibit vertical heterogeneity of PCBs, as these compounds have only been in use for about half a century. The depth to which PCBs penetrate will thus be equivalent (approximately, ignoring reworking of sediments) to about 50 years' worth of sedimentation, assuming that significant deposition of PCBs to sediments has occurred throughout their commercial usage. This depth will vary markedly from place to place, depending on the rate of sedimentation. In open oceans, sedimentation is very slow and only a thin layer of the surface sediments will be contaminated. Elder et al.[43] found PCBs only in the top 1 cm of open-sea Mediterranean sediments, and considered that most or all of the contaminants were present in the top 1 mm. By contrast, the sedimentation rate in turbid estuaries may be very rapid, approaching or even exceeding 1 cm/year. Thus, West and Hatcher[48] found significant quantities of PCBs at 36 to 39 cm depth of sediment in the Christiaensen Basin of the New York Bight. Similarly, Bopp et al.[20] noted PCBs at greater than 40 cm sediment depth in areas of known rapid sedimentation in the Hudson River system, which drains to the New York Bight. These data are shown in Figure 1. It is evident on scrutiny of the profiles shown that different results will be obtained from different types of sampling methods

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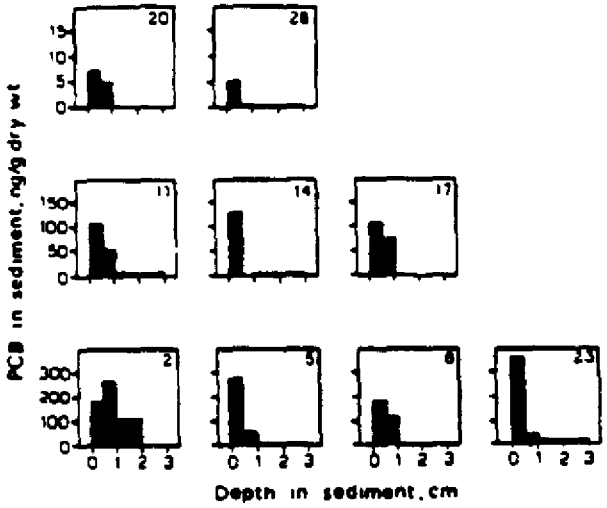

FIGURE 2. Vertical distributions of PCBs in sediments from various areas in Lake Superior. Numbers at upper right of each figure denote stations sampled. Note different vertical scale of each horizontal row.[12]

if the methods vary in ability to retain the surface sediment. This view is echoed by Eisenreich et al.,[12] who noted that their values of PCB concentrations in Lake Superior sediments exceeded those of other authors by factors of 2 to 10. They ascribed this difference to their use of an oceanographic box corer, which they felt to be superior to grab samplers or gravity/piston corers because the recently deposited sediment was retained. The conclusion appears valid, as their data show that PCB concentrations in Lake Superior sediments are generally much higher in the surface 0.5 cm, although the vertical distribution of PCBs varied between stations (Figure 2). It is thus evident that researchers must be cautious in comparing data for PCBs in samples of sediments taken with different types of sampling equipment. Much benefit would accrue from the standardization of methods, and it would appear that the use of box corers would be preferable.

In addition to this practical problem, a major theoretically based problem exists in using sediment samples to quantify PCBs in aquatic environments. This involves the effect of the rate of sedimentation on the PCB levels present in sediments, and this problem has been almost completely ignored to date. In general, authors treat results of PCBs in sediments as a direct function of the degree of contamination of areas by these compounds, i.e., as a function of the magnitude of a PCB source in the various areas. Thus if one area contains sediments with PCB levels fivefold greater than those of a second area, the conclusion is generally that the area exhibiting higher PCB levels is exposed to a PCB source of greater magnitude. This interpretation may, however, be completely erroneous, as the PCB levels in sediments depend on at least two major factors; one of these is the magnitude of the PCB source, and the other is the rate of particle sedimentation. Thus, the concentrations of PCBs present in sediments depend not on the PCB loading from a source, but on the relationship of this loading to the amounts of suspended material upon which the PCBs sediment. In areas of rapid sedimentation (e.g., turbid estuaries draining urbanized deforested catchments), PCBs will be "diluted" by the large available particulate load, and concentrations of PCBs in the sediments downstream may be expected to be relatively low. By contrast, PCBs may be present at higher concentrations in sediments of estuaries with low turbidity, as the solids

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load available for PCB adsorption is much smaller. This argument is of course somewhat simplistic, as the particulate load in an estuary may influence partitioning of the PCBs between the soluble and particulate-adsorbed phases; however, it is clear that the PCB levels in sediments cannot be simply correlated to the magnitude of local PCB sources.

Certain other factors also influence the PCB concentrations found in aquatic sediments. The first and most important of these is sediment grain size. Several authors have reported that the finer fractions of sediments contain higher concentrations of PCBs, and this relationship (which is similar to that for trace metals) is considered to be due to the greater surface area available for PCB adsorption onto smaller particles.[71,72] In some instances, the effects of grain size can be extreme; for example, Duinker and Hillebrand[46] reported values for PCBs in Waal and Lek sediments of the Rhine-Meuse system as <40 ng/g dry weight for the coarse fraction and 2400 to 3400 ng/g dry weight for the fine fraction. If no account is taken of the effects of particle size on the results of surveys of PCBs in sediments, the survey results may be interpreted erroneously, with areas of fine sediments being considered to be closer to PCB sources. Few authors have addressed this problem to date, and no standard methodology exists to take account of the influence of particle size on results of PCBs in sediments. Authors studying trace metals in sediments (which also vary in concentration with particle size) often sieve whole sediments to collect a defined fraction which is then analyzed. Some authors go further than this and propose the need for measurement of surface areas of sediments.[73] By contrast, researchers studying PCBs in sediments generally extract whole sediments for analysis, thus leaving the results open to the interference of the effects of particle size.

In addition to particle size, the organic matter content of sediments may influence the PCB levels present. If a survey area varies in organic content of sediments, this effect may interfere with the distribution of PCBs and lead to erroneous conclusions concerning the magnitude and location of PCB sources.

Finally, it must be mentioned that sediments act as a long-term sink for PCBs. Thus, PCBs sedimented on organic or inorganic particulate material may become buried by further material and hence be lost to the general PCB cycle. In this event, the contamination is of little significance (except perhaps as an indicator of historical PCB inputs at that location), and may bear little relation to present-day levels of contamination of the biota in the water column. This argument was used by Phillips[33-35] to support the use of bioindicators rather than sediments for monitoring trace elements; in this case, metals deposited even to surface sediments are generally of relatively low bioavailability (except perhaps to benthic deposit feeders). In the case of PCBs, however, the argument is rather less valid, as it is known that PCBs in surface sediments are bioavailable both to benthic organisms and to those in the overlying water column.[63,74-81] Indeed, in areas where inputs of DDT or PCB have diminished following controls, sediments are thought to act as a major source of these compounds. This is the case for DDT off the California coast[82-86] and for PCBs in the Great Lakes.[87] PCBs desorbed from sediments re-enter the global cycle and may not only be accumulated by local biota, but may also be released to the atmosphere via the surface microlayer.[87]

In summary, there can be little doubt that the analysis of sediments can be a most useful tool in attempts to quantify PCBs in aquatic ecosystems. Several advantages exist in the use of sediments rather than water; these include greater ease of sample handling and existence of time-averaged PCB levels. However, certain problems remain concerning the use of sediments and this author cannot improve on the words of Pavlou and Dexter[81] who considered that "... sediments can provide a rapid assessment of the mean residue levels and dynamics of persistent organic chemicals such as PCBs ... However, it should be emphasized that such data must be interpreted with care. Even though the levels in sediments represent temporal mean concentrations, they depend also on other factors, such as the sedimentation

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rate in the area, the depth of sediment taken for analysis, the particle size, and the natural organic matter content."

C. Analysis of Organisms

The use of aquatic biota as quantitative indicators of pollutant levels in aquatic environments is a relatively recent development. Early studies of this type were carried out in the 1960s and involved quantification of radionuclide abundance in marine ecosystems. In the last decade, the technique has been adapted to the study of stable trace elements, organochlorine pesticides and PCBs, and most recently, hydrocarbons.

The basic theory behind the use of so-called indicator (or sentinel) organisms is that these species accumulate pollutants in their tissues in amounts proportionate to those in their ambient environment. The analysis of pollutant levels within their tissues thus provides a time-averaged indication of pollutant bioavailability at each study location.[33-35]

The ideal indicator organism has the following attributes:[36-40]

1. It accumulates the pollutant studied in proportion to the average levels present in the ambient waters
2. It should be sedentary in order to be representative of the area in which it is collected
3. It should be abundant throughout the study area and should tolerate brackish water
4. It should tolerate the presence of high levels of the pollutant studied without being toxicologically affected
5. It should be of reasonable size, giving adequate tissue for analysis
6. It should be easy to sample and should preferably be hardy enough to survive in the laboratory
7. It should be relatively long-lived, to permit time-integration of pollutant levels over several months or years

The use of such an indicator organism to quantify pollutants in aquatic environments has several advantages over the analysis of water or sediments. Pollutant concentrations accumulated by the organism will generally be several orders of magnitude greater than those in the surrounding water; hence analysis is simpler. The time-integration of ambient levels of pollutants exhibited by the organism permits the elucidation of average levels of contamination, without the need for multiple sampling at each study location. In addition, the analysis of pollutant levels in the indicator organism eliminates the problems of estimating bioavailability of pollutants, such as is required with water or sediment analysis; pollutants present in the organism are by definition bioavailable, although it must be remembered that "bioavailability" may vary with species.

However, the use of biological indicators to quantify pollutants in aquatic environments is subject to various problems. Thus, certain extraneous variables exist, the effects of which will interfere with the simple correlation between pollutant levels in the organism and those in the ambient waters. In the case of PCB monitoring, the most important of these factors is the lipid content of the organism used. Other factors of possible importance include the kinetics of PCB isomers in the organism, the condition, age or sex of the organism, the mobility of the organism, and so on. Researchers wishing to employ indicator organisms to monitor PCBs in the aquatic environment should be cognizant of these problems, and should design surveys carefully so as to minimize the influence of extraneous variables on the results of the surveys. If used correctly, biological indicators will give rise to extremely accurate and detailed profiles of PCB abundance in various locations. This may act as a basis for the elucidation of PCB sources or for the monitoring of temporal trends in PCB abundance. However, if biological indicator surveys are not planned correctly, the conclusions therefrom may be completely erroneous.

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Table 4
THE MOLECULAR COMPOSITION OF AROCLORS® 1242 AND 1254[1]

Component	Abundance (% by weight) Aroclor® 1242	Aroclor® 1254
$C_{12}H_{10}$	<0.1	<0.1
$C_{12}H_9Cl$	1.0	<0.1
$Cl_2H_8Cl_2$	16.0	0.5
$Cl_3H_7Cl_3$	49.0	1.0
$C_{12}H_6Cl_4$	25.0	21.0
$C_{12}H_5Cl_5$	8.0	48.0
$C_{12}H_4Cl_6$	1.0	23.0
$C_{12}H_3Cl_7$	<0.1	6.0
$C_{12}H_2Cl_8$	ND	ND

Note: ND, not detected.

The remainder of this chapter addresses the major problems inherent in the use of indicator organisms to quantify PCBs in aquatic environments, and proposes solutions to these problems.

IV. ISOMER QUANTIFICATION, BEHAVIOR, AND KINETICS IN ORGANISMS

The theoretical number of PCB isomers which may exist is 210.[9] Commercial preparations of PCBs, manufactured by chlorinating biphenyl with anhydrous chlorine using iron filings or ferric chloride as a catalyst contain mixtures of compounds of different degrees of chlorination and different positions of chlorine substitution. Table 4 shows the composition of Aroclors® 1242 and 1254 as an example.[1] Other commercial PCB preparations, such as Phenochlors® and Clophens®, are also sold as several products, each a mixture of compounds differing in their overall degree of chlorination.

This heterogeneity of commercial PCBs gives rise to a number of problems in terms of quantification of these compounds. These problems should be clearly understood by researchers wishing to use biological indicators to quantify PCBs in the aquatic environment. The first of these problems is that the usual method of analysis employed for PCB studies (electron capture gas chromatography) may not sufficiently separate the individual compounds present in a sample. Thus, peaks commonly contain more than one isomer and the separation of individual components is taxing. This problem is now being at least partially overcome by the use of glass capillary columns, which are preferable to the earlier packed columns; however, the analytical techniques merit particular attention and future improvements are anticipated.[1,9]

The second (and equally important) problem concerning PCB analysis is that PCBs in environmental samples are generally quantified by comparing peak heights in the sample to those in commercial standards. No standardized method exists, however, which gives rise to inconsistencies. Authors generally choose a standard which is closest in relative peak heights to the sample chromatogram. Unfortunately, the two rarely match completely, and quantification therefore depends on which peaks are chosen. Some authors prefer to choose a peak corresponding to the major peak in the standard, others use four or more different peaks, and still others may use total peak area or publish two sets of results based on two different standards.

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It is clear from this that the analytical quantification of PCBs is no straightforward matter. However, a third problem exists where organisms are used as indicators of PCBs, and this involves the unusual capacity of aquatic biota to selectively accumulate PCB isomers. Thus, whereas in studies of PCBs in water or sediments, PCB profiles generally vary only marginally from commercially available standards,[46,90,91] the PCBs found in aquatic biota may vary dramatically from those in the standards.

The selective accumulation by organisms of different PCB isomers or PCB components varying in chlorination is a function of the physicochemical properties of PCB components in the environment in addition to the biological effects of accumulation. Both aspects merit attention here, as they both influence the PCB profiles found in studies of indicator organisms.

PCB components of different chlorination exhibit differences in their solubility in water.[6,92,93] As a general rule, the higher the chlorination of a biphenyl component, the lower its water solubility. Thus, for example, Haque and Schmedding[92] reported solubilities of 1 μg/ℓ for 2,4,5,2',5'-pentachlorobiphenyl, 27 μg/ℓ for 2,2',5,5'-tetrachlorobiphenyl, and 638 μg/ℓ for 2,4'-dichlorobiphenyl. This difference is also exhibited by the PCB mixtures, as these also vary in overall chlorination: for example, Mackay and Wolkoff[93] reported solubilities of 2.7 μg/ℓ for Aroclor® 1260, 12 μg/ℓ for Aroclor® 1254, 54 μg/ℓ for Aroclor® 1248, and 240 μg/ℓ for Aroclor® 1242. Although different authors report different absolute solubilities of PCBs due to the different methodologies employed to study this parameter, all results show this general inverse relationship between water solubility and degree of chlorination. It should be noted in addition that different isomers with the same degree of chlorination also vary in solubility; Table 5 shows extensive data from Lee et al.[94] which confirm these variations.

These differences in water solubility of the various PCB components are particularly important, as they influence the partition coefficients of the components. A molecule with high chlorination (and thus low water solubility) will favor the inorganic particulate phase rather than remaining in solution. By contrast, a biphenyl of low chlorination with high water solubility may remain in solution, and a much smaller proportion of the total load of the component will be adsorbed to inorganic particulates.[95,96] This is important when considering the dispersion of PCBs from a point source; components of lower chlorination may be expected to exhibit greater dispersion than those of higher chlorination, as they will remain in solution longer. This tendency was revealed particularly well in the studies of Richardson and Waid,[97] who found that the PCBs in sediments of Port Phillip Bay varied according to distance from the shore-based sources. Inshore sediments in Corio Bay, close to the major inputs of PCBs, exhibited a large proportion of highly chlorinated components, resembling Aroclors® 1254 and 1260. By contrast, offshore sediments contained mainly lower-chlorinated biphenyls, chromatograms resembling Aroclor® 1242. Essentially similar results, although on a larger geographical scale, were noted by Tanabe et al.,[98] in studies of PCBs in the Antarctic, Western Pacific, and Indian Oceans. Greater amounts of the higher-chlorinated biphenyls were found in coastal areas than in the open oceans. In this case, the higher vapor pressures of the lower-chlorinated PCBs, and hence a more efficient aerial transport, undoubtedly also played a part. A similar effect may be responsible for the unusually high proportion of PCBs represented by components of lower chlorination in the central part of the Southern Bight of the North Sea, offshore from the Rhine-Meuse Estuary, as mentioned by Duinker and co-workers.[46,99] It is notable here in addition, that individual isomers with the same degree of chlorination may also exhibit differences in partitioning between solution and particulate material,[71] although these differences are generally minor compared to those between components of different chlorination.

The differences between PCB components in terms of water solubility not only lead to variations in partitioning between solution and inorganic particulates as described above, but also give rise to differences in bioaccumulative tendencies. Several authors have shown

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Table 5
COMPARATIVE WATER SOLUBILITIES OF ISOMERS OF AROCLOR® 1242; NOTE THE DIFFERENCES BETWEEN THE RAPIDITY OF DISSOLUTION OF DIFFERENT ISOMERS[a]

Peak no.	No. of chlorines	Identification	Concentrations (ng/ℓ) attained in days shown					
			7	20	30	50	149	251
1	1	2					124.60	121.59
2	1	3						
3	1	4					7.50	15.07
4	2				1.00	1.69	5.83	9.06
5	2	2,2'			3.18	1.78	20.80	21.18
6	2					2.93	23.24	23.76
7	2	2,3'			9.10	14.07	19.63	30.60
8	2	2,4'	2.90	27.30	71.18	78.45	141.85	138.93
9	3	2,6,2'			1.40	1.72	8.13	8.33
10	3	2,5,2'	5.52	22.97	41.05	41.20	62.48	61.35
11	3							2.15
12	3	2,4,2'						0.48
13	3					1.71	10.00	4.50
14	2	3,4'						2.98
15	2 & 3	4,4';2,3,2'		8.83	16.25	17.19	26.15	35.30
16	3	2,4,3'					29.83	27.47
17	3	2,4,4'	2.55		4.08	4.63	9.83	9.87
18	3	2,5,4'	20.22	35.15	43.28	48.20	63.76	65.07
19	3	3,4,2'					2.59	2.59
20	4	2,5,2',5'	1.58	6.67	8.94	8.76	24.05	22.25
21	4					0.64	2.66	3.59
22	4				0.55	0.49	3.78	4.09
23	3 & 4	2,4,2',5'				2.11	17.95	19.51
24	4	2,4,2',4'	11.12	7.39	8.93	9.12	19.85	15.88
25	4						4.15	3.37
26	4						1.11	1.09
27	4	2,3,2',5'				0.69	12.75	12.83
28	4					0.74	4.10	3.13
29	4					1.42		2.50
30	4	2,3,3',4'					9.15	7.21
31	3					0.59	4.53	4.70
32	4						4.10	2.70
33	4						5.43	2.63
34	3						0.73	0.82
35	4	2,4,3',4'	1.96			2.18	13.83	11.62
36	5							
37	5						2.53	0.99
38	5							
39	5		0.71				4.79	2.47
40	5							2.84
Total			46.56	108.31	208.94	240.53	671.86	702.67

that the tendency of a pollutant to become concentrated in aquatic biota (the "bioconcentration potential") is correlated to the partition coefficient of that pollutant in the *n*-octanol/water system.[101-108] Thus, in general, contaminants of low water solubility, and high affinity for organic phases, will be of high bioconcentration potential. It should be realized that this rule is only generally true, and the precise tendency of a pollutant to bioaccumulate is governed not only by its lipophilicity but also by other factors, such as the biodegradability

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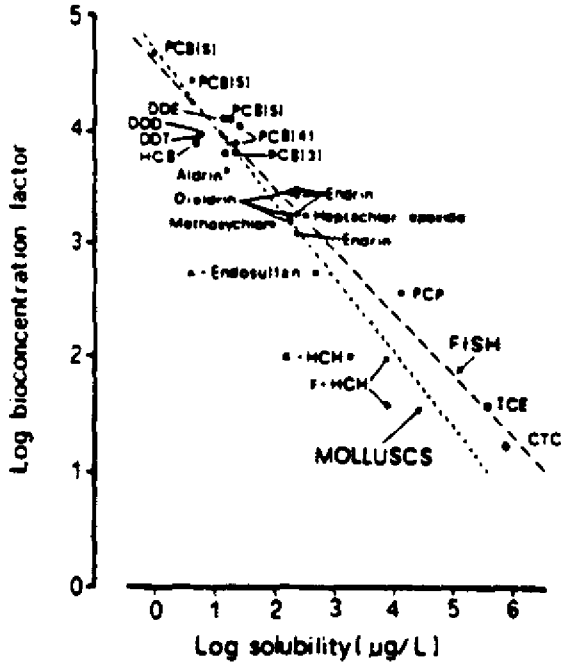

FIGURE 3. Correlation between water solubility of organic pollutants and their bioconcentration factors in molluscs (filled circles) or fish (open circles). Numbers in brackets for PCBs indicate degree of chlorination. TCE is tetrachloroethylene; CTC is carbon tetrachloride.[101,107-113]

or persistence of the contaminant in the organism. However, the use of *n*-octanol/water partition coefficients is certainly valid in a broad sense as an index of the bioconcentration potential of contaminants.

Data on the correlation between the bioconcentration of pollutants and either their water solubility or their partition coefficients in the *n*-octanol/water system have been recently summarized in the form of figures by Ernst;[107] Figures 3 and 4 show these summaries, which are a synthesis of results from several authors.[101,108-113] It should be noted that both figures are plotted on log-log scales; hence, while the correlations appear particularly good, they would be less impressive on linear plots. Nevertheless, the general relationships are sound, and the relatively minor variability imposed thereon is due to differences in biodegradability of contaminants and other factors, such as uncertainties in accurately assessing water solubilities of the various compounds. It is also notable that the PCB components shown are among the most highly bioconcentrated contaminants, correlating to their very low water solubilities and high *n*-octanol/water partition coefficients. The differences between the different PCB components follow the rules described above, biphenyls of greatest chlorination exhibiting lowest water solubilities and highest partition coefficients.

The discussion above deals with the physicochemical aspects of bioaccumulation only, and could be summarized by the statement that biphenyls of higher chlorination should accumulate to greater extents in biota than lower chlorinated types. However, the organisms themselves may also exhibit selective accumulation of isomers, and this need not be consistent with the above picture. This selective bioaccumulation of PCB components will be discussed

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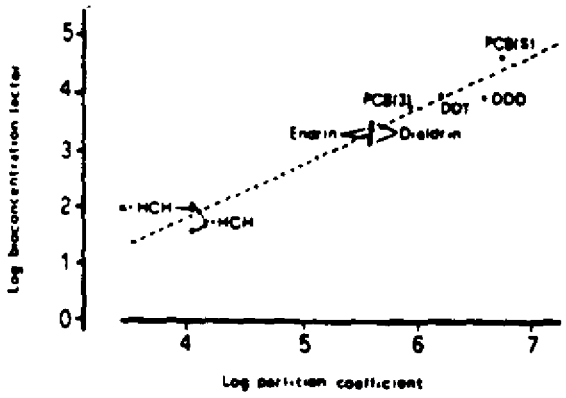

FIGURE 4. Correlation between partition coefficients (in n-octanol/water system) of organic pollutants and their bioconcentration factors in bivalve molluscs. Numbers in parentheses for PCBs indicate degree of chlorination.[illegible]

here in relation to the food web, citing data for primary producers first and progressing up trophic levels through other families to top predators.

Very little information is available on selective accumulation of PCB components by phytoplankton, despite considerable studies of the uptake of either commercial PCBs or individual PCB components by phytoplankton[illegible] and of the effects of PCBs on phytoplankters.[illegible] Most authors consider the uptake of PCBs by phytoplankton to be a passive adsorption process initially, followed by absorption into the cell. There is little evidence for selectivity in the accumulation of different PCB components over and above that which may be explained on the basis of the physicochemical properties of the isomers themselves. Thus, for example, Wang et al.[113] found that higher chlorinated PCB components were taken up to a greater extent by *Chlorella fusca* than were lower chlorinated components. This tendency correlated to the water solubility of each compound, i.e., to its lipophilicity, and no overt biological selectivity was noted.

Information on selective uptake of PCB components by macroalgae is also very sparse. Macroalgae have been used very little as bioindicators of PCBs[124] compared to their frequent use for studies of trace metals. However, Amico et al.[125] published comparative studies of PCBs in 12 species of seaweed and the mussel *Mytilus edulis* from the east coast of Sicily. They noted that the algae exhibited much larger proportions of lower chlorinated PCB components than the mussels, and speculated that this may be based on a lack of metabolic breakdown of the lower chlorinated components in the algae, or on differential uptake of components by the different organisms. Without information on the PCBs in the surrounding waters, these two alternative hypotheses cannot be tested. It might also be noted here that the salt marsh cordgrass *Spartina alterniflora* was shown to exhibit selective uptake of PCBs by Mroszek et al.[126] Analysis of both stems and roots of *S. alterniflora* revealed an enrichment of lower chlorinated biphenyls and a paucity of higher chlorinated components in the plant, compared to Aroclor® 1254 used for dosing.

The selective uptake of PCB components by zooplankton was studied by Clayton et al.[74] in Puget Sound. Table 6 shows data for concentration factors (PCBs in lipids/PCBs in water) for chlorobiphenyls of different chlorine content for various sites, and the corresponding dominant plankton group at each site. It is evident that at most sites, the PCB components of higher chlorination were accumulated in greater amounts; this tends to reflect the con-

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Table 6
PARTITIONING VALUES OR CONCENTRATION FACTORS (PCBs in lipids/PCBs in water) FOR ZOOPLANKTON IN DIFFERENT REGIONS OF PUGET SOUND[a]

Region	Conc. factor × 10^6			Dominant fauna
	N = 4[b]	N = 5	N = 6	
Elliott Bay	1.06(±0.81)[c]	1.42(±1.06)	2.17(±1.66)	Euphausiids
Main Basin	1.07(±0.61)	1.90(±1.25)	3.18(±2.32)	Copepods
Whidbey Basin (Port Gardner)	0.80(±0.38)	1.09(±0.53)	1.47(±0.75)	Copepods/ euphausiids
Hood Canal	0.98(±0.56)	0.74(±0.52)	0.43(±0.32)	Ctenophores
Sinclair Inlet	1.12(±0.68)	3.61(±2.02)	6.90(±3.45)	Ctenophores
Admiralty Inlet and Straits of Juan de Fuca	0.34(±0.21)	0.28(±0.18)	0.29(±0.18)	Copepods

[a] N = number of chlorines/biphenyl.
[b] Values in parentheses are standard deviations.

clusions from the physicochemical considerations above. However, at Hood Canal and Admiralty Inlet, the opposite pattern was found, tetrachlorobiphenyls being accumulated to greater extents than penta- or hexachlorobiphenyls. These patterns presumably rely on species differences in preferred components, and may possibly be correlated to the particular lipids present in the dominant species. It is relevant, in addition, that Wyman and O'Connors[77] reported that copepods of the genus *Acartia* accumulate much of their PCBs from phytoplankton food; the possibility that the selective accumulation of PCB components in secondary and higher levels of the food web depends on selectivity at the primary level must not be overlooked.

Selective accumulation of PCB components has also been reported in the scud *Gammarus pseudolimnaeus* by Sanders and Chandler,[127] who measured ^{36}Cl-labeled PCB uptake from water in continuous flow laboratory studies. Table 7 shows the results of this study, which revealed progressively greater uptake of lower chlorinated biphenyls. "Enrichment factors", representing the relative peak areas of the sample peak and the equivalent peak in Aroclor® 1254, decrease from 2.00 in the trichlorobiphenyl, to 1.71 to 1.90 for tetrachlorobiphenyls, 1.00 to 1.31 for pentachlorobiphenyls, 0.49 to 0.85 for hexachlorobiphenyls, and 0.25 to 0.50 for heptachlorobiphenyls. The ranges given for biphenyls containing four to seven chlorine atoms reflect differences between individual isomers. The authors noted that no lower-chlorinated components than those in the dosed Aroclor® 1254 were present in scud, confirming the absence of reductive dechlorination, and suggesting that this is truly selective uptake. However, it is possible that the observed results were brought about at least partially by the higher water solubilities of the components of lower chlorination leading to greater bioavailability, particularly as the exposure level was quite high at 1.6 μg/ℓ; as discussed previously, the biphenyls of higher clorination exhibit low water solubilities and their dissolution may not have occurred.

McLeese et al.[78] also reported interesting data on the selective accumulation of PCB components, in this case in a sea water/sand system using the polychaete *Nereis virens* and the shrimp *Crangon septemspinosa*. Unfortunately only five peaks of Aroclor® 1254 were quantified in samples, which tends to limit the usefulness of the data. However, as shown in Figure 5, spiked sand exhibited rather greater amounts of lower chlorinated components than the parent Aroclor® 1254 mixture, and *Nereis virens* tended to preferentially accumulate biphenyls of lower chlorination to accentuate this effect. However, the biphenyls present in

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Table 7
ENRICHMENT FACTORS FOR COMPONENTS OF AROCLOR® 1254 IN SCUD (*GAMMARUS PSEUDOLIMNAEUS*)[127]

Peak no.	Relative retention time	Number of chlorines	Enrichment factor, sample 1	Enrichment factor, sample 2	Average 1 + 2	Aroclor® 1254 RPA
1	0.27	3	2.00	2.00	2.00	0.03
2	0.37	4	1.69	1.72	1.71	0.29
3	0.45	4	1.81	1.81	1.81	0.11
4	0.50	4	1.80	2.00	1.90	0.05
5	0.63	5	1.31	1.31	1.31	0.54
6	0.77	5	1.00	1.00	1.00	1.00
7	0.98	5	1.13	1.16	1.15	0.38
8	1.07	5	1.07	1.16	1.12	0.57
9	1.19	6	0.33	0.66	0.49	0.03
10	1.31	6	0.84	0.85	0.85	0.65
11	1.59	6	0.55	0.55	0.55	0.33
12	1.67	6	0.80	0.80	0.80	0.30
13	1.79	7	0.43	0.57	0.50	0.07
14	2.04	6	0.56	0.60	0.58	0.45
15	2.61	6	0.50	0.62	0.56	0.08
16	3.22	7	0.25	0.50	0.37	0.06
17	3.68	7	0.25	0.25	0.25	0.04

Note: The enrichment factor in each case represents the relative peak area (RPA) of the sample peak and the equivalent peak in Aroclor® 1254.

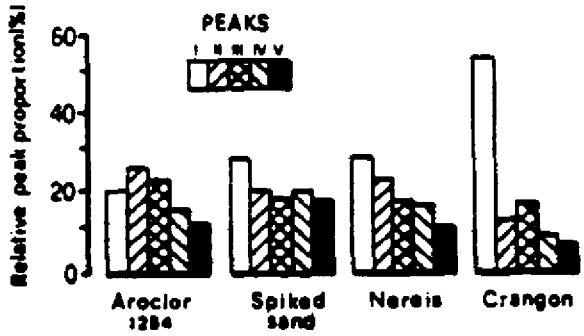

FIGURE 5. Proportion of quantified Aroclor® 1254 peaks as a percentage of total amount for Aroclor® 1254 standard, spiked sand, and *Nereis virens* or *Crangon septemspinosa* exposed to the spiked sand for 32 days.[76]

Crangon septemspinosa after an exposure of 32 days showed an even greater preferential accumulation of components of lower chlorination. In these cases, it is possible that uptake of components occurred after solubilization from the spiked sand. In the case of the polychaete, this may have occurred via interstitial water, with additional uptake occurring directly from the sand. The shrimp, however, would be dependent wholly on solubilized PCB components. This hypothesis explains the selective uptake found, as biphenyls of lower

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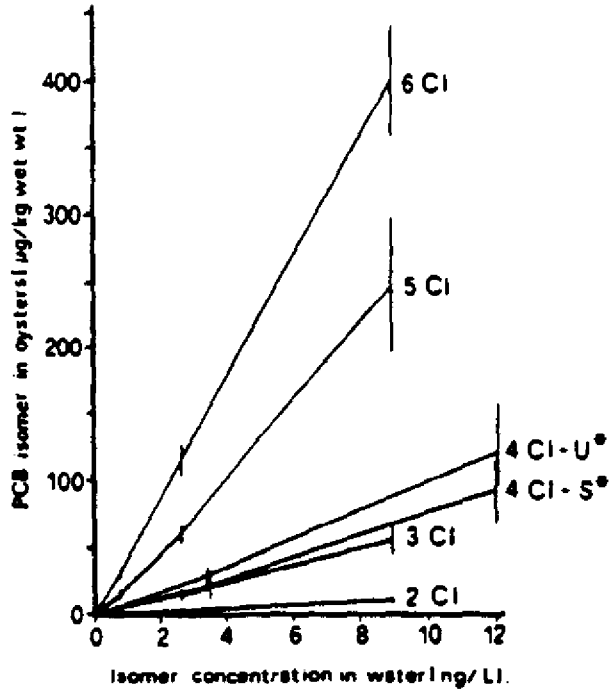

FIGURE 6. Relationship between PCB concentrations attained in oysters (*Crassostrea virginica*) and PCB exposure concentration in ambient sea water. Oyster values are means from days 28 and 63 of uptake; vertical lines are standard deviations. Isomers used were 2,3-dichlorobiphenyl (2 Cl); 3,4,2'-trichlorobiphenyl (3 Cl); 2,5,2',5'-tetrachlorobiphenyl (4 Cl-S*); 2,3,2',5'-tetrachlorobiphenyl (4 Cl-U*); 2,3,4,2',5'-pentachlorobiphenyl (5 Cl); and 2,4,5,2',4',5'-hexachlorobiphenyl (6 Cl).[48]

chlorination are known to be easier to solubilize from particulates (see above). It may be noted here also that, despite similar studies of PCB uptake by polychaetes from sediment and water, other authors[74,75] did not report selective uptake of PCB components; this probably reflects the fact that many authors do not attach particular significance to alterations in relative peak heights of chromatograms of PCBs.

The selective accumulation of PCB components by bivalve molluscs has been the subject of several studies, although it is notable that no consensus may be derived from these. Vreeland[48] investigated the uptake of six individual biphenyls by the oyster *Crassostrea virginica*. She found uptake to be proportional to PCB dose, and to vary dramatically with the degree of chlorination of the isomer (Figure 6). Equilibrium concentration factors based on wet weights were quoted as follows: dichlorobiphenyl, 1200; trichlorobiphenyl, 6200; symmetrical tetrachlorobiphenyl, 7400; unsymmetrical tetrachlorobiphenyl, 11,000; pentachlorobiphenyl, 27,000; and hexachlorobiphenyl, 48,000.

By complete contrast, Courtney and Denton[28] found that clams (*Mercenaria mercenaria*) fed Aroclor® 1254 adsorbed to the surface of alumina preferentially retained biphenyls of lower chlorination in the muscular foot and visceral mass (Table 8). This correlated to the excretion of disproportionately large amounts of higher chlorinated isomers in the feces. Clams from Southampton water also exhibited preferential enrichment of lower chlorinated

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Table 8

VARIATION IN PEAK HEIGHT OF TISSUE AND FECES RESIDUES OF PCBs COMPARED TO AROCLOR® 1254[100]

Aroclor® peaks	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Chlorine atoms/mol	3	4	4	4	5	4 & 5	5	5	5	6	5	5 & 6	6	6	6	7	7
Standard	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Visceral mass																	
21-day exposure	315	190	227	237	135	184	113	122	117	74	97	69	55	49	55	37	30
1-month postexposure	322	181	210	414	133	185	113	122	117	77	96	70	55	50	56	38	32
3-month postexposure	274	152	162	257	120	173	114	121	120	74	100	75	60	32	56	30	34
6-month postexposure	361	151	162	312	123	177	113	118	120	77	101	79	53	47	56	44	41
Feces																	
2-week postexposure	48	26	27	61	44	35	65	60	60	129	97	141	152	177	182	217	229
3-month postexposure	227	56	74	151	83	84	86	99	103	108	95	105	87	126	129	192	252
6-month postexposure	224	60	81	200	67	79	66	82	91	123	84	110	108	170	161	290	275

Note: Clams (*Mercenaria mercenaria*) were exposed to 12.3 µg/l Aroclor® 1254 (adsorbed to the surface of sediment) for 21 days; residues were determined immediately after exposure and at various postexposure intervals.

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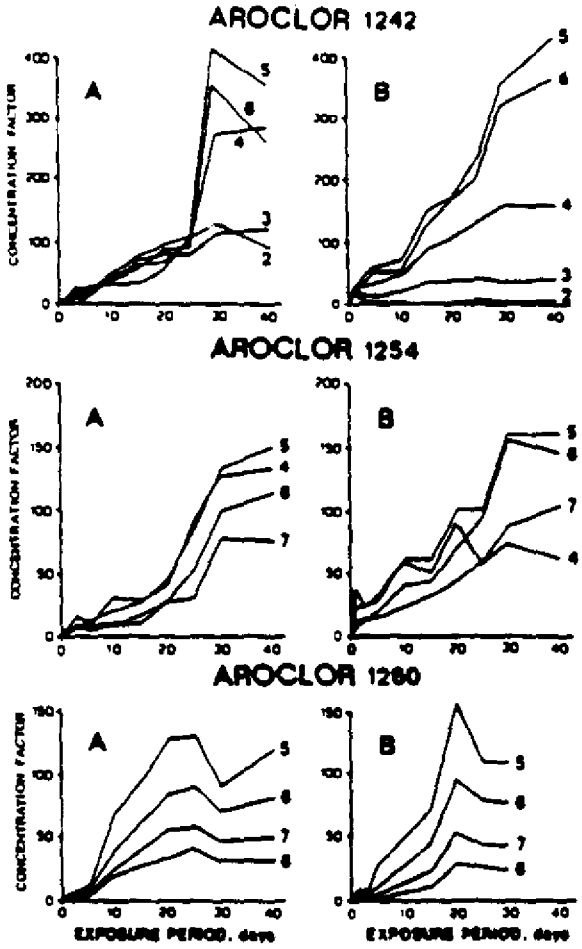

FIGURE 7. Concentration factors describing the uptake of PCBs varying in degree of chlorination from Aroclors® 1242, 1254, and 1260 by (A) *Macoma balthica* and (B) *Cerastoderma edule*. The numbers 2 to 8 represent the degree of PCB chlorination. Uptake was from aluminum oxide particles in water.[134]

biphenyls compared to Aroclor® 1254.[129] A more recent report from Deubert et al.[130] has confirmed the preferential uptake of lower chlorinated biphenyls by *M. mercenaria*.

These differences between species were compounded by the investigations of Langston,[131,133] who found yet another pattern of selective accumulation of PCB components in the bivalves *Cerastoderma edule* and *Macoma balthica*. Both these species were found to preferentially accumulate pentachlorobiphenyls; isomers with less chlorine atoms were accumulated in decreasing amounts from 4 to 2 atoms, and those with more chlorine atoms in decreasing amounts from 6 to 8 atoms (Figure 7). Such a pattern of accumulation has also been noted for PCB uptake in the oyster *Crassostrea virginica* by Nimmo et al.[132] Langston[133] also noted, however, that differences existed in the persistence of chlorobiphenyls

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containing the same number of chlorine atoms in different substitution positions. As a general rule, isomers with the most *ortho*-substituted chlorine atoms were the least persistent. Species differences were also found: *C. edule* excreted dichlorobiphenyls much faster than did *M. balthica*.

It is clear from the above discussion that at least with respect to bivalves, considerable interspecific differences exist with respect to PCB kinetics. Langston[137] has speculated that such differences may be based on interspecific differences in the chemical structure of lipid pools in bivalves. If this is the case, it is possible that preferential accumulation of PCB isomers may vary not only with species, but also in any single species with season, if lipid pools vary chemically with time. In any event, it must be acknowledged that the various components of commercial PCBs exhibit very heterogeneous kinetics in bivalves; there is a case for treating each component as a separate pollutant.

Selective accumulation of PCB components has also been noted in finfish, but again no general trend is obvious from the information reported to date. Sanborn et al.[138] found that the green sunfish (*Lepomis cyanellus*) exhibited higher concentration factors for the more highly chlorinated PCB isomers when exposed to three pure PCBs in water. Concentration factors calculated were 1510 for a pentachlorobiphenyl, 460 for a tetrachlorobiphenyl, and only 54 for a trichlorobiphenyl. The fish was apparently able to metabolize the trichlorobiphenyl to a hydroxy derivative, which at least partially explains its low persistence. Several other authors[135,141] have also reported data consistent with greater uptake or longer persistence of higher chlorinated biphenyls in fish, and this pattern is considered to be due to the greater lipophilicity of these compounds compared to the lower chlorinated isomers. However, not all reports concur with such a pattern. Thus, Stalling and Mayer[142] reported preferential accumulation of lower chlorinated PCB isomers in fish, and Hansen et al.[143] found a tetrachlorobiphenyl to be more persistent in channel catfish (*Ictalurus punctatus*) than more highly chlorinated components.

In addition to such differences in kinetics of PCB components varying in chlorination, a few authors have noted, in detailed studies, that compounds of the same degree of chlorination but differing in substitution positions also vary in kinetics. Bruggeman et al.[140] investigated the uptake of PCBs containing two to four chlorine atoms by goldfish (*Carassius auratus*), using both aqueous and dietary exposure routes. They noted that the major reason for variation in biomagnification of different compounds was the variation in clearance (excretion) rates; absorption efficiencies were similar for each compound. The overall pattern (Figure 8) showed excretion rates decreased with increasing degree of chlorination, half-lives varying from 10 days for 2,5-dichlorobiphenyl to 60 days for 2,3',4',5-tetrachlorobiphenyl. Within each group of biphenyls, chlorine substitution patterns also affected retention. Chlorine substitution *para* to the phenyl-phenyl bond influenced the clearance rates much more strongly than substitution in the *ortho* position. This pattern correlated to the aqueous solubilities of the various compounds.

Similar data have been reported by Tanabe et al.[101] for PCB kinetics in carp (*Cyprinus carpio*), after dietary exposure to individual chlorobiphenyls. These authors found that absorption efficiencies of the compounds varied, from 67 to 93%, decreasing as the number of chlorine atoms increased. This pattern appeared to be based on the molecular size differences of the various PCBs; hence, when the molecular weight was taken into account, the observed differences between the compounds disappeared (Figure 9). In terms of biological half-life, Tanabe et al.[141] found that there was essentially no loss of hexa-, hepta- or octochlorobiphenyls from carp over a 90-day excretion period, but PCBs containing two to five chlorine atoms were excreted. The overall pattern showed faster excretion of biphenyls with lower chlorination, but there were large differences in half-lives of individual compounds within each chlorobiphenyl series. Table 9 shows these differences for the trichlorobiphenyls. It is clear that compounds with more *ortho* substituted chlorine atoms tend to have shorter

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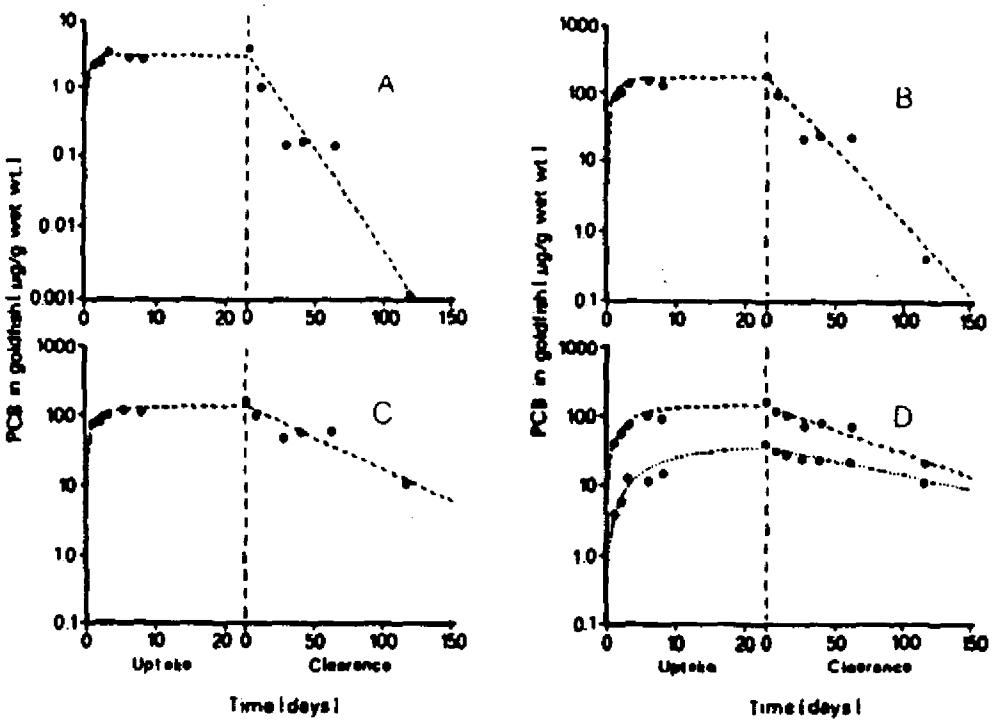

FIGURE 8. Kinetics of uptake and clearance of five individual PCBs by goldfish, *Carassius auratus*. In each case, an exposure period of 21 days was followed immediately by a depuration period of 116 days. (A) 2,5-Dichlorobiphenyl, $t_{1/2}$ of 10.5 days; (B) 2,2',5-trichlorobiphenyl, $t_{1/2}$ of 14.4 days; (C) 2,4',5-trichlorobiphenyl, $t_{1/2}$ 33 days; (D) 2,2',5,5'-tetrachlorobiphenyl with a $t_{1/2}$ of 46 days (dashed line) and 2,3',4',5-tetrachlorobiphenyl with a $t_{1/2}$ of 69 days (dotted line).[46]

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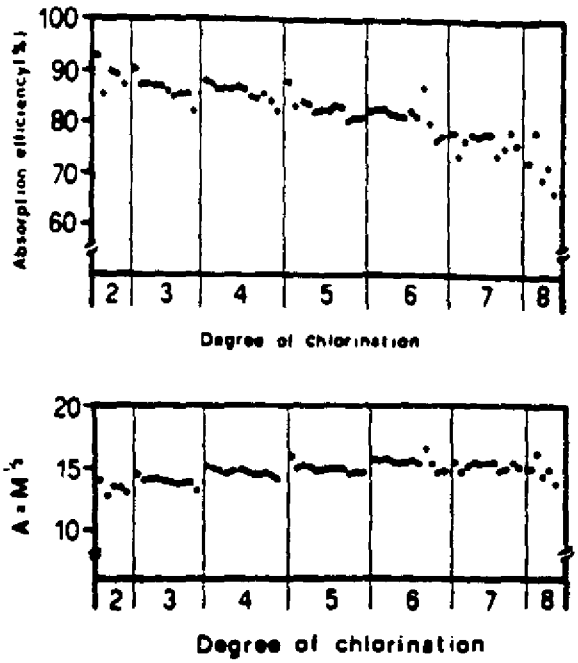

FIGURE 9 Absorption efficiencies of individual chlorobiphenyls in carp (*Cyprinus carpio*) and their variation with the degree of chlorination (top). Also shown is the absorption efficiency (A) multiplied by molecular weight $^{1/2}$ ($M^{1/2}$), showing that the variation in absorption efficiencies is a function of molecular weight differences.[141]

half-lives, i.e., are more easily metabolized. Interestingly, Sparling and Safe[144] failed to confirm this effect of *ortho* substitution on PCB kinetics in trout, although it is evident from their work that considerable differences between species exist with respect to the behavior of PCB isomers. It should be noted that the method of PCB administration (by gastric lavage) in this latter study is somewhat unusual and may influence the overall kinetics of the compounds studied.

Final mention may be made here of PCB kinetics in marine mammals, including seals and dolphins. Several authors have noted that the higher chlorinated PCB compounds predominate in seal tissues,[105,106,109] and this appears to be the case also for dolphins.[120,121] These mammals are considered by most authors to accumulate the majority of their body load of PCBs from their diet. It is likely, therefore, that the predominance of the higher chlorinated PCB isomers in seal and dolphin tissues reflects a similar profile in ingested food, coupled with a long persistence of highly chlorinated PCBs in the mammals themselves.

Several conclusions may be drawn with respect to this difficult aspect of PCB quantification in marine organisms. First, it is clear that the quantification of PCBs by methods involving a simple comparison of areas of only one or a few peaks in the sample chromatogram with a complex standard (which is essentially chosen arbitrarily) is a crude method at best. There is an urgent need for improvement in analytical techniques, and the commercial availability of pure individual PCB components would assist such improvements. Current methods commonly ignore all-important differences between organisms (or these and standards) in relative peak heights, and our understanding of PCB kinetics in organisms suffers as a result.

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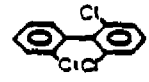
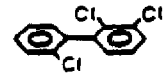
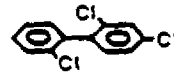
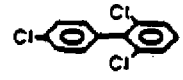
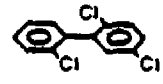
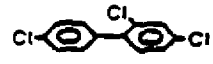
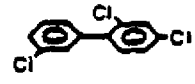
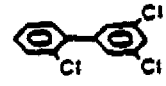
Table 9

RELATIONSHIP BETWEEN THE BIOLOGICAL HALF-LIFE AND NUMBER OF CHLORINE ATOMS SUBSTITUTED IN *ORTHO* POSITIONS OF TRICHLOROBIPHENYLS FOR CARP, *CYPRINUS CARPIO*[145]

	Peak no.	Structure	No. of ortho chlorine atoms	Biological half-life (days)
Less	310		0	94.6
	36		1	62.4
	39		1	56.8
	37		1	56.3
	38		1	46.5
Metabolism	32		2	18.2
	35		2	9.6
	33		2	8.7
	34		2	5.3
More	31		3	1.8

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Second, there can be no doubt that in some cases — perhaps the majority of cases — the uptake and excretion of PCBs by organisms (and hence the net uptake or persistence of PCBs in organisms) is defined by simple physicochemical properties of the compounds themselves. Thus, it is common observation that the more highly chlorinated PCBs are of greater persistence in biota. This correlates to their lower water solubility and higher P_{o-w} values (octanol-water partition coefficients). Within each chlorobiphenyl series, however, physicochemical differences also occur. The pattern of chlorine substitution in a chlorobiphenyl may influence the symmetry of the molecule, its molecular size, and its susceptibility to chemical (enzymic) attack. Certain authors are now beginning to probe this vital area, observing consistent differences between the kinetics of PCB isomers in biota. Shaw and Connell,[133,135] from their work on PCBs in mussels and mullet in Australian waters, have proposed the use of an empirically derived "steric effect coefficient" which permits predictions of uptake kinetics for PCBs. This is covered in greater detail in Volume I, Chapter 6.

It must also be noted that some observations of PCB kinetics in marine organisms cannot be simply explained on the basis of physicochemical properties of the compounds themselves, either in terms of lipophilicity, aqueous solubility, or steric effects. Thus, certain organisms appear to exhibit selective uptake of compounds which depend on the biological matrix rather than the physicochemical properties of the pollutant. There is a vital need for more information in this area.

Opinion thus appears to be crystallizing that the hitherto crude methods for quantifying PCBs are inadequate and must be replaced. Each PCB isomer present in an organism should essentially be considered as an individual pollutant in its own right, with its own kinetics and toxicology. While it may be some time before this is routinely possible, the current situation might be compared to the analysis of organisms for "total trace metals", and in such a situation there can be little doubt that interferences between the pollutants cloud the issues at present.

Notwithstanding the need for future improvements in analytical techniques, it is certain that the quantification of PCBs in marine organisms is required *now* as a management tool. The remainder of this chapter is therefore devoted to discussions concerning the optimal use of bioindicator organisms, as a method to quantify PCBs in marine and estuarine environments.

V. THE INFLUENCE OF LIPID

It has already been stated that both the affinity of PCBs for biota and their persistence in aquatic organisms are due to the hydrophobic, lipophilic nature of these compounds. It is therefore no surprise that the lipid content of an organism has a highly significant influence on the amounts of PCBs sequestered by that organism.

This covariance of lipid content and PCB levels within organisms has been cited by various authors as a root cause of, or partial contributor to, the following:

1. The apparent amplification of PCBs with trophic level
2. The variation between species in PCB concentrations
3. The differences in PCB concentrations present in different individuals of a given species
4. The variation in PCB levels in different tissues of a species
5. The differences found in PCB levels in various portions of a single organ or tissue in a species

The last three of the above items are highly relevant in terms of using bioindicators to monitor PCB contamination of the aquatic environment. The first two, by contrast, are of more peripheral interest and can be dealt with very briefly.

The question of whether PCBs (and other organochlorines) are amplified in concentration

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with trophic level has been discussed at length by Phillips,[35] but firm conclusions are difficult. Some authors[149,154,155] have reported that PCB concentrations in food web components show a stepwise increase at each trophic level when the contaminant concentrations are based on wet weights of the organisms, but that the differences disappear or are very much reduced if concentrations are based on lipid weights. However, other workers[156] claim more convincing profiles of trophic amplification when lipid weights are used for calculating PCB concentrations. Still other reports[27,157,158] include no evidence on either basis for an increase in PCB concentrations in ascending food chains. The argument has more recently shifted in emphasis slightly, in attempts to discover whether the food chain is in any event a significant source of PCBs to predators. The lipid-water partitioning theory of organochlorine uptake, first proposed by Hamelink et al.[159] with reference to DDT, has also received some support as a basis for PCB uptake by organisms.[160] However, this contention appears to be defensible for organisms only at lower trophic levels, as discussed by Phillips;[35] at higher trophic levels, food chain transfer predominates, at least according to an equilibrium model of trophic level transfer of PCBs constructed by Thomann.[161]

The effects of lipid on the concentrations of PCBs found in organisms are perhaps more convincingly displayed if species differences in PCB contents are considered. Many authors have noted, both for PCBs, and for other lipophilic organochlorines such as DDT and dieldrin,[162-164] that species which have high lipid contents also accumulate relatively large concentrations of organic pollutants. Thus, species differences in bioaccumulation capacities appear considerable when based on concentrations of pollutants calculated on wet weights; however, when based on lipid weights, species differences are reduced markedly. Jensen et al.[156,165] noted that data for herring (*Clupea harengus*) and cod (*Gadus morhua*) reflected this factor particularly well, PCB concentrations in the former being much higher because of the greater lipid content of herring than cod. Hattula et al.[166] confirmed this observation in extensive studies of fresh water fish from a Finnish lake. Similar conclusions have been made in other studies concerning PCBs in fish,[27,167] and other phyla also are believed to conform to this pattern.[129,168]

While the influence of lipid on trophic level amplification of PCBs or on species differences in PCB accumulation is of a certain interest with respect to bioindicator use to monitor PCBs, it is not of central importance, except perhaps as an aid to selection of species which accumulate large amounts of PCBs. Of much greater importance is the effect of lipid on PCB concentrations in different individuals of a monitoring species, or in the tissues of these individuals. Thus, bioindicator surveys involve the analysis of a number of individuals (either whole, or their tissues) from several locations to reach conclusions concerning the relative contamination of each area. The following potential effects of lipid must be recognized:

1. Individuals of the chosen species may exhibit heterogeneous levels of lipid, either within or between populations. This may give rise to a large intra-site variation in PCB concentrations, or may cause erroneous conclusions with respect to interlocation differences, or both.
2. Decisions concerning which tissue(s) of the chosen bioindicator species should be taken for analysis are influenced by the lipid levels found in the tissues (and hence the PCB distribution among the tissues), and the temporal changes in these.
3. If subsampling from a single tissue or organ is used, it is important to recognize the possibility of heterogeneity of PCB distribution within tissues, which may correlate to an uneven lipid distribution or to heterogeneous lipid chemistries in the organ.

With respect to the first of these, it is known that populations of some aquatic species may reveal highly variable lipid levels among the individuals. Thus, for example, Jensen et al.[156] reported variation of greater than an order of magnitude in lipid levels in herring

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(*Clupea harengus*) from Swedish waters, and similar variations have been recorded in other fish species.[169-174] Bivalves such as mussels are also known to exhibit considerable variability in lipid levels.[175-179] In some instances the sources of such variation are identifiable, e.g., lipid content or condition relates in most species to sexual condition and the spawning/resting/gametogenesis cycle, and may also correlate to size in some cases. However, the multiplicity of factors influencing lipid levels of a species may in some instances defy complete analysis.

This variation in lipid contents of individuals is important, as it is known that in many cases, the concentrations of PCBs (and other lipophilic pollutants) correlate to the lipid contents of the individual. Thus, where a population exhibits heterogeneity of lipid content, PCB concentrations (based on wet tissue weights) will also vary widely. This has two consequences: first, a greater number of individuals must be sampled at each location to fully characterize the PCB levels in the population, and second, interpopulation differences in PCB levels are more difficult to distinguish.[180] Both consequences are obviously undesirable in terms of bioindicator surveys, and the option of basing PCB concentrations on lipid weights of the sample must be considered.

Phillips[34,35] has argued that the use of lipid weights as a basis for organochlorine concentrations is defensible only in certain circumstances. If the kinetics of PCB accumulation by a species are such that ambient PCBs partition into body lipids until an equilibrium is attained, it is clear that basing PCB concentrations on lipid weights of an organism is perfectly valid, and will give rise to much more homogeneous intrapopulation levels and hence comparisons between PCB levels (in different populations) will be of greater validity. However, if the uptake kinetics are such that a given amount of PCB accumulated from the ambient environment is distributed about the available lipid pool in an organism, an inverse relationship between body lipid and PCB concentrations in that lipid would result. In this event, basing PCB concentrations on lipid weights would give rise to no improvements over the use of wet tissue weights as a basis, either with respect to intra- or interpopulation variability.

This argument is tantamount to that discussed above, on whether PCBs are accumulated by lipid-water partitioning or via the food chain. As previously stated, there is no consensus of opinion as yet on this subject. However, research results support both points of view in different phyla. Thus, for example, Roberts et al.[181] found that the net uptake of chlordane into the northern redhorse sucker (*Moxostoma macrolepidotum*) was directly proportional to the size of the lipid pool in the fish. Similar results were reported by Stegeman and coworkers[182,183] for petroleum hydrocarbon uptake by the oyster *Crassostrea virginica*, and also by DeFoe et al.[137] for PCB uptake by fathead minnows (*Pimephales promelas*) of different sexes and lipid pool sizes (see Section VIII). These reports support a lipid-water partitioning theory of uptake and would therefore also support the use of lipid weights as a basis for PCB concentrations. However, other data on seals reveal an inverse relationship between concentrations of DDT or PCB and blubber thickness,[184-188] and this supports the alternative concept of a defined PCB uptake being distributed about the available lipid pool. It may be that different major uptake mechanisms exist at different trophic levels, as suggested previously by Phillips;[34,35] however, no firm conclusion is possible. It is therefore advisable for researchers undertaking bioindicator work with PCBs to determine lipid levels in their samples, and to interpret analytical results using concentrations based on both wet and lipid weights. In this fashion, erroneous conclusions due to the interfering effects of sample lipids are most likely to be avoided.

The second major influence of lipid on the PCB concentrations found in organisms is on the tissue distribution of PCBs. This is important in terms of bioindicator surveys, as it helps the researcher select the most appropriate tissue for analysis. Data on the tissue distributions of PCBs in aquatic organisms are available for crustaceans, molluscs, fish, and marine

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Table 10
CONCENTRATIONS OF ΣDDT BY WET AND LIPID WEIGHTS (mg/kg) IN 6 TISSUES OF A SINGLE PORPOISE (*PHOCAENA PHOCAENA*) FROM SCOTLAND[197]

Parameter	Blubber	Kidney	Liver	Muscle	Spleen	Brain
ΣDDT by wet weight	3.80	0.04	0.58	0.56	0.12	0.02
Percent lipid	67.00	1.4	13.20	6.10	5.10	8.30
ΣDDT by lipid weight	5.60	2.90	4.80	9.20	2.40	0.27

mammals. Nimmo et al.[186,187] found that the distribution of both DDT and PCB in shrimp tissues was correlated to that of lipids, the majority of the residues being accumulated by the hepatopancreas and least by abdominal muscle and the exoskeleton. Ernst et al.[188] reached similar conclusions on the effects of lipid on PCB distributions in the scallop *Chlamys opercularis* and three finfish species, and many other authors have confirmed that PCBs and lipids in finfish correlate in terms of distribution among the tissues, at least as a general rule.[79,138,171,189,191]

The tissues of marine mammals also exhibit a covariance of lipids and PCBs. Lipid-rich tissues (principally the blubber) contain the great majority of the PCB load in these animals, concentrations in the other tissues (which have much less lipid) being considerably lower on a wet weight basis.[140,150,185,192,196]

In all of the cases cited above, the very marked differences between PCB concentrations (based on wet weights) of the various tissues are substantially reduced when PCB levels are recalculated on a lipid weight basis. This indicates that the lipid distribution in an organism is a major factor in determining the distribution of PCBs.

There is therefore an argument for researchers wishing to undertake bioindicator studies for PCBs to concentrate on organs which are relatively lipid-rich. In this fashion, the concentrations of PCBs in the sample will be increased and analytical accuracy improved. In addition, it would be an advantage to select a tissue which exhibits relatively constant levels of lipids and a slow net turnover of lipids, as this would give rise to more constant PCB levels in the chosen tissue.

The final aspect requiring discussion in this section concerns the possible influence of lipid chemistry on the distribution of PCBs both between and within tissues of aquatic organisms. This subject is particularly important, as researchers commonly wish to subsample material for analysis from a single individual, and in some cases this may be extremely difficult to do in a reproducible fashion.

An early indication of the possible influence of lipid chemistry on PCB accumulation by organisms arose from work concerning the PCB distribution in tissues of marine mammals, referred to briefly above. Holden and Marsden[197] were the first authors to note that organochlorine concentrations in marine mammals revealed some interesting anomalies. They discovered that ΣDDT concentrations (i.e., levels of DDT plus its metabolites) in the porpoise *Phocaena phocaena* differed considerably among the tissues on a wet weight basis (Table 10). Conversion of the data to a lipid weight basis gave rise to much smaller differences between the ΣDDT concentrations in different tissues, except for the brain, which exhibited pollutant levels an order of magnitude lower than those of other tissues. This inability of brain lipids to sequester ΣDDT to the same extent as other tissues has since been confirmed for PCBs in other mammals, including sea lions,[150,198] various seal species,[140,185,193,199,199] and the striped dolphin *Stenella coeruleoalba*.[150] Frank et al.[198] felt that a blood brain barrier might exist, but other authors have suggested that the unusual lipid chemistry of the brain tissues is the cause. Brain tissues are exceptionally rich in phospholipids, as opposed to triglycerides which are normal constituents of lipids in other tissues and in depot fat. It is

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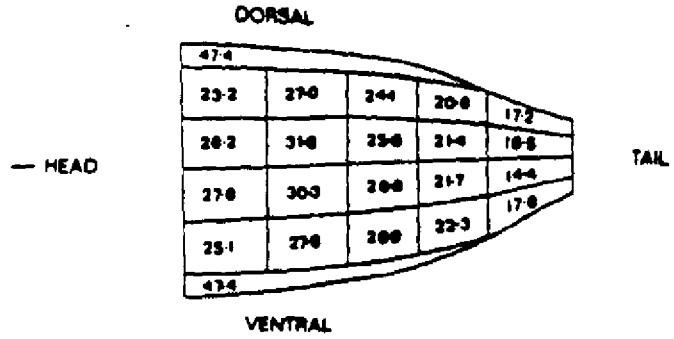

FIGURE 10. The variation in total lipids as a percentage of biochemical components in different regions of the axial muscle of herring, *Clupea harengus*. Differences between right and left muscle fillets were not observed.[88,170]

very likely that PCBs are sequestered much more efficiently by triglycerides than phospholipids, because of the lipophilic nature of PCBs (phospholipids are more polar). Tanabe et al.[150] give particular support to this hypothesis, noting that the isomers of HCH (hexachlorocyclohexane) vary in tissue distribution in the striped dolphin, the more hydrophilic α-HCH being more abundant in the brain than the β-HCH isomer. In a later paper,[151] the same authors suggested that transplacental transfer of PCBs in the dolphin to the fetus was reduced because of the existence of large amounts of phospholipids in the blood, the placenta, and the blubber of the fetus.

These reports are important, as they give substance to the supposition that the covariance of lipid and PCBs in organisms is a function not of a nonspecific affinity of PCBs for all lipids, but of specific affinities of PCBs for certain types of lipids. The paucity of PCBs in the brains of marine mammals is matched by similar profiles in finfish,[102,205,206] and this influence of lipid chemistry on PCB uptake by brain tissues may be expected to be universal. Cahn et al.[205] have noted that elasmobranchs, which exhibit unusual PCB tissue distributions compared to teleosts, also have an unusual lipid chemistry.[206] Langston[172] considered that species differences in PCB kinetics in bivalve molluscs might also be based on differences in the chemical structures and percentage compositions of their lipid pools. It is therefore important to realize that the correlations between PCB distribution and lipids in organisms, found by so many authors, are approximate only, because of the nonspecific methods employed to extract and determine lipid weights. There is considerable room for both theoretical and practical research on this aspect of PCB accumulation by biota.

Differences in lipid chemistries or lipid amounts within different portions of a tissue may be of considerable practical importance with respect to the use of bio-indicators to monitor PCBs. Figure 10 shows a profile describing the variation of total lipids in different parts of the musculature of the herring, *Clupea harengus*.[88,170] It is clear that considerable differences in lipid content occur both longitudinally and dorsoventrally in the axial muscle of this species. Other finfish species also exhibit such differences, although the sites of preferential lipid storage vary according to their ecological niche (swimming habits in particular). It is known that these variations in lipid composition of fish muscle give rise to heterogeneous distributions of ΣDDT in the tissues (see discussion of Reinert,[203] Stout et al.[206] and Reinert and Bergman,[208] in Phillips[33]), and the distribution of PCBs within the tissues will also almost certainly be affected. However, the only mention of PCB heterogeneity within finfish muscle published to date appears to be that of Shaw and Connell,[49] who mention briefly that mullet exhibit this phenomenon. More detailed information[170] reveals that the differences

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Table 11
CONCENTRATIONS (ng/g WET WEIGHTS) OF VARIOUS ORGANOCHLORINES IN BLUBBER AND MUSCLE OF A STRIPED DOLPHIN, *STENELLA COERULEOALBA*, AND THEIR VARIATION WITH AREA WITHIN EACH TISSUE[139]

Organs & tissues	PCBs	*p,p'*-DDE	*p,p'*-DDD	*p,p'*-DDT	ΣDDT*	α-HCH	β-HCH	γ-HCH	ΣHCH*	HCB
Blubber										
Front	23600	27900	1100	4800	33700	53	390	20	463	126
Center	21300	28200	1100	4600	33900	37	434	22	513	113
Rear	20800	26700	900	3500	31100	49	368	18	435	129
Mean	22600	27600	1030	4300	32900	53	397	20	470	129
Muscle										
Dorsal front	232	297	15	23	335	0.9	6.5	0.4	7.8	2.7
Ventral front	855	980	38	98	1116	2.5	18.6	1.2	22.3	9.1
Dorsal center	106	204	15	8	227	0.5	3.7	0.2	4.4	2.0
Ventral center	233	284	9	24	317	0.8	7.0	0.4	8.2	2.5
Dorsal rear	314	413	34	16	463	1.2	9.6	0.6	11.4	3.3
Ventral rear	209	287	27	12	326	0.9	7.5	0.5	8.9	2.5
Mean	335	411	23	30	466	1.1	8.8	0.6	10.5	3.1

* ΣDDT = *p,p'* DDE + *p,p'* DDD + *p,p* DDT.
* ΣHCH = α-HCH + β-HCH + γ-HCH (δ-HCH was not detected)

in PCB concentrations between different areas of the musculature of mullet are considerable, concentrations covarying with lipid over almost an order of magnitude.

Similar within-tissue heterogeneity has also been reported for seals and dolphins. Anas and Worlund[207] found that ΣDDT and PCBs in the blubber of fur seals (*Callorhinus ursinus*) were distributed heterogeneously and that different methods of subsampling from the blubber gave rise to different results. Tanabe et al.[139] also reported differences in concentrations of PCBs and other organochlorines in blubber and (particularly) muscle of the striped dolphin (Table 11).

It is therefore clear that researchers wishing to subsample from a single tissue of a bioindicator species for the analysis of PCBs in a monitoring program must take particular care in terms of the reproducibility of subsampling. Thus, in all cases, tissues should be subsampled from the same site on e species, to the same depth. It is also important to note that, at least in salmonids, the PCB levels vary markedly between skinless and skin-on fillets of axial muscle;[207,209] skinless fillets are no doubt the better alternative for bioindicator work, but care must be taken upon dissection to avoid contamination from the skin.

The discussion above has been confined to the influence of lipid levels per se on PCB concentrations encountered in marine organisms. Data also exist which indicate that the amounts of lipid present in biota frequently underlie the influence of other extraneous parameters (e.g., season, size, and sex of organism) on PCB levels. Such examples will be covered in the following sections of the present review.

IV. THE EFFECTS OF SEASON/ORGANISM CONDITION

Season and organism condition are particularly important variables in bioindicator surveys. If sufficient attention is not given to correct sampling, spurious conclusions may result due to the influence of these parameters.

Several mechanisms exist by which the levels of PCBs or other pollutants in bioindicators may vary seasonally. Three major possibilities exist:

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1. Temporal fluctuations may occur in the delivery of the pollutant to coastal waters
2. Biological changes may occur within the selected bioindicator species, which may influence the accumulation of PCBs or other pollutants
3. Alterations in water quality may occur with season, and these may also influence the bioaccumulation of PCBs.

The effects of each of these three types of events will be considered here in turn.

A. Temporal Fluctuations in Pollutant Delivery

It is known that the concentrations of many organochlorines in rivers or receiving coastal waters fluctuate considerably with time. In cases where the major source of the pollutant is from its use in agriculture, this fluctuation in pollutant delivery is often regular and predictable. Hence, Butler et al.[209] showed that concentrations of ΣDDT in the oyster *Crassostrea virginica* varied seasonally in different fashions in three Texas estuaries. In two of the estuaries, a single peak in ΣDDT levels was observed in early summer; by contrast, oysters from the Laguna Madre area exhibited three peaks, in about February, July, and November. These profiles correlated precisely to DDT application in the catchments; the Laguna Madre region was multiply harvested and DDT was applied three times a year, whereas the other estuary catchments received only single annual applications of the pesticide. A similar regular variation in organochlorine abundance in aquatic ecosystems has been observed elsewhere, e.g., Yamato et al.[210,211] reported summertime peaks and winter minima in BHC and HCH levels in Japanese waters. These profiles of temporal variation are defined by the application period of the pesticide and by the timing of rainfall and subsequent run-off carrying the pollutant to rivers and then to estuaries.

The PCBs are, of course, not used as agricultural pesticides, hence their seasonal abundance is not defined by this factor. However, in certain instances, the delivery of PCBs to coastal waters may be extremely variable temporally. Studies in Sweden, notably by Olsson et al.,[173] and Södergren et al.,[180] have shown that peak abundance of PCBs occurs at the time of the spring thaw in ice cover. The data of the former authors, concerning PCBs in roach (*Leuciscus rutilus*) from Lake Roxen are shown in Figure 11. Temporal variability may also occur in instances where a continual, relatively stable PCB source is subject to dilution by run-off. In these cases, PCB levels in water vary inversely to the degree of run-off. Such an explanation was cited by Livingston et al.[213] to define seasonal variation in PCBs of biota from Apalachicola Bay in Florida.

Finally, it is evident that in many cases, the industrial sources of PCBs may fluctuate in magnitude with time. Duke et al.[203] followed the reduction in PCB concentrations in effluents receiving waters and oysters in Escambia Bay, Fla., where a single industrial source was suspected. Other authors have noted a lack of consistency in PCB levels in biota, and this is probably caused by variations in the magnitude of PCB sources, coupled with temporal changes in run-off.

B. Biological Changes Influencing PCB Accumulation

Superimposed on the temporal fluctuation of PCB concentrations in coastal waters referred to above is a range of changes which occurs in the bioindicator species themselves. These changes may be extremely important in determining the PCB concentrations present in the individuals sampled in a bioindicator survey and their effects should be taken into account during the design phase of any survey.

Biological events of importance with respect to PCB accumulation in bioindicators include the spawning cycle (and associated variation in lipid contents of bioindicators), growth, and migration. The effects of these will be discussed in turn.

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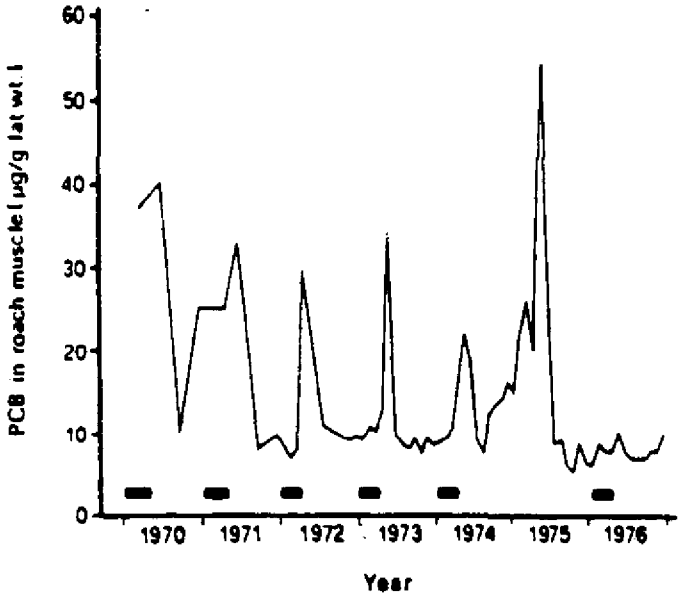

FIGURE 11. Mean concentrations of PCBs (by lipid weight) in muscle of roach, *Leuciscus rutilus*, taken from Lake Rosen in Sweden between 1970 and 1977. The bars at the bottom of the figure represent the timing of ice cover in the lake; there was no ice cover in the winter of 1974—75.[179]

1. Spawning

The gametogenesis/spawning/resting cycle is of profound importance in a bioindicator to be used to monitor organochlorines, including PCBs. This is generally considered to be caused by the variation in lipid levels of organisms with the sexual cycle. Most organisms exhibit regular and marked increases in lipid contents during gametogenesis, followed by a dramatic loss of lipid with the gametes at spawning. It has already been noted in Section V that the lipid content of organisms defines, to a large extent, their concentrations of PCBs; hence, it is evident that PCBs will vary in concentration with the sexual cycle.

Several authors have reported data showing that the mature sex organs or gametes of aquatic biota are rich in lipid and exhibit high levels of PCBs and other organochlorines. Among crustaceans, for example, it is known that DDT in particular is heavily concentrated in gametes and mature sexual organs,[214-216] and the same is almost certainly true of PCBs. Bivalves also store large amounts of organochlorines in gametes,[217-220] and whole-body levels of these compounds are reduced markedly at spawning. The loss of DDT and PCBs in finfish at spawning is also known to be considerable,[126,171,209,209,221-223] although in most cases it appears that females are much more affected than males, as the loss of organochlorines in milt is small compared to that in eggs.[130,224,225]

The importance of this dependence of PCBs in biota on their sexual condition becomes evident when one considers the simultaneous variation in condition of different populations. Figure 12 shows an example for the mussel *Mytilus edulis*, collected from different positions on the shore in two locations at close proximity to each other.[178] It is clear that even these closely sited subpopulations of mussels varied considerably in condition. If such individuals were selected for use as bioindicators, PCB concentrations would be expected to be greater

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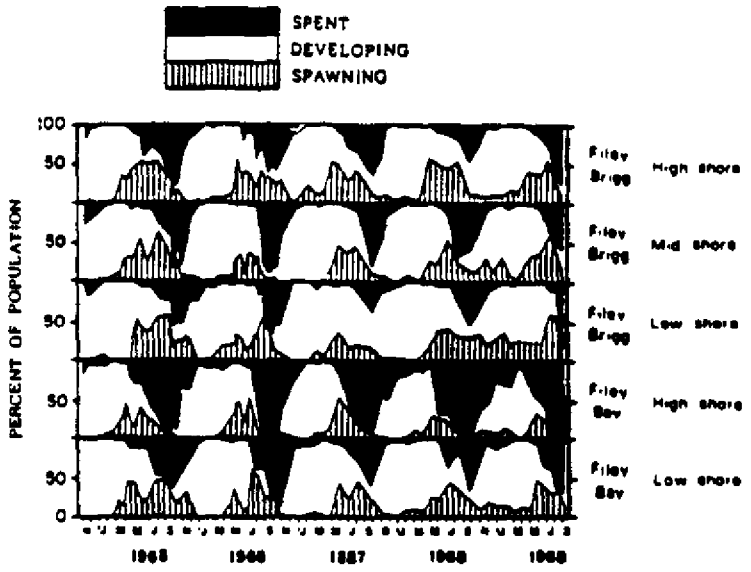

FIGURE 12. Seasonal variation in condition (expressed as percentage of population spent, developing, or spawning) for five subpopulations of the mussel *Mytilus edulis* between 1964 and 1969 in North Yorkshire waters, England.[78]

in the samples which were of greater sexual maturity, simply because of the interference of the covarying lipid parameter.

Such differences between individuals, subpopulations, or populations of organisms in sexual condition are by no means restricted to bivalve molluscs; most other species also exhibit variations in condition at any one time. The effects of this factor may be reduced by timing sampling to coincide with the period of least difference in condition between individuals, and by the use of lipid weights as a basis for PCB concentrations in the animals studied, rather than wet weights. It was mentioned in Section V that analytical results for PCBs in bioindicators should be quoted based on both lipid and wet weights of the samples to improve interpretation; this method would nicely highlight the reasons for unusual differences between samples, where these are based on concurrent variations in lipids. The best example of this concerns the seasonality of ΣDDT in coho salmon from the Great Lakes, reported by Reinert and co-workers.[163,200] These authors reported an increase in ΣDDT in salmon from Lake Michigan over the summer, but stated that the same species from Lake Erie exhibited no such seasonal variations. However, on recalculating the data based on lipid weights, Phillips[34,35] showed that the differences were a function of variation in lipid seasonality between the two locations (Table 12), and that salmon from both lakes exhibited increased ΣDDT concentrations (based on lipid weights) over the summer period.

2. *Growth*

The influence of the growth of an organism on its content of PCBs merits brief mention here. It is well known that in temperate waters, organism growth commonly occurs mainly during the spring and summer periods of warm water temperatures. This could produce seasonal fluctuations in contaminant levels, if the uptake of pollutants were constant throughout the year and the body load were distributed throughout the body tissues or body lipid

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Table 12
SEASONALITY OF ΣDDT RESIDUES IN WHOLE COHO SALMON (*ONCORHYNCHUS KISUTCH*) FROM LAKES MICHIGAN AND ERIE, AND THE INFLUENCE OF LIPIDS ON THE TEMPORAL CHANGES[34,35,200]

Lake	Year-class and date of collection	No. of fish	Weight (g)	ΣDDT (wet wt)	Oil (%)	ΣDDT (lipid wt)
Michigan	1965 year-class					
	21—22 April 1968	5	1,054	3.3	7.6	44.6
	4—7 May 1968	3	1,180	3.5	5.3	66.0
	21 August 1968*	10	3,514	12.3	13.2	93.2
	18 October 1968*	10	3,486	12.6	7.1	177.5
Michigan	1966 year-class					
	1 May 1969	4	1,328	3.5	9.0	38.9
	3—11 September 1969	10	3,903	11.8	11.6	101.7
Michigan	1967 year-class					
	4 May 1970	5	967	2.9	6.9	42.0
	21—28 August 1970	13	3,663	14.0	13.8	101.4
Michigan	1968 year-class					
	17 April 1971	12	1,029	1.8	7.6	23.7
	28 August 1971	15	4,254	9.9	13.0	76.2
Erie	1966 year-class					
	6 April 1969	6	811	1.3	10.8	12.0
	5 May 1969	5	1,050	2.1	10.0	21.0
	7—8 October 1969	10	2,027	2.2	5.2	42.3

* Two 25-mm thick steaks and the brain had been removed from these fish.

pool. Niimi and Cho[226] have discussed the influence of growth on contaminant kinetics in finfish, and have in particular emphasized that biological half-life data for contaminants are often calculated without recognizing the influence of growth dilution of pollutants on the data. While organism growth is not considered a major variable in defining PCB seasonality in aquatic biota, it is nevertheless of potential significance. It is also of interest that various authors have found PCBs to influence growth rates of biota in laboratory studies, at least in the case of certain crustaceans,[227] bivalve molluscs,[218,228] and some finfish.[130,143,229-232] It is more than possible that this effect is linked to an influence of PCBs on steroid hormones,[224,225] but whether it exists at PCB concentrations likely to be encountered in natural waters is uncertain.

3. Migration

Migration is the final biological factor which may lead to seasonality of contaminants, including PCBs, in aquatic biota. Unfortunately, most authors ignore this factor almost completely in designing bioindicator surveys, despite its enormous importance, particularly in estuaries and in studies of finfish. However, several authors have recognized the possible influence of migration of finfish on organochlorine surveys.[101,168,198,226,227] Many coastal species of finfish display annual migration subsequent to sexual maturity, spawning in estuaries, and moving away from shore at other times. As noted by Phillips,[35] the concentrations of a pollutant in a migratory animal are a complex function of its uptake through both space and time. It is presently impossible to interpret data from surveys employing migratory bioindicators, at least where the species is not sampled more than once. The basic prerequisite that a bioindicator should be sedentary in order to represent its area of sampling (see Section III above) has been much abused by authors, to the detriment of their own research.

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C. Seasonal Variations in Water Quality

The final major factor which may give rise to seasonality of PCBs in aquatic biota concerns various aspects of water quality, the most important of which are salinity and temperature. Research on trace metal uptake by bioindicators has frequently shown an influence of these parameters on metal uptake by aquatic biota, although data for organochlorines are much harder to find.

However, it is known that salinity is an important factor in determining the uptake of DDT by finfish, at least in some cases.[139] While no data showing a direct influence of salinity on PCB uptake by organisms are known, several authors have suggested that the partitioning of PCBs between water and sediments (or suspended inorganic particulates) may be influenced by salinity.[66,67] Further research on this aspect, and its importance in terms of defining uptake of PCBs by biota, is required as an adjunct to aiding the interpretation of bioindicator surveys.

Water temperature is also known to affect the uptake of organochlorines by finfish. Murphy and Murphy[139] reported faster uptake of DDT by mosquitofish (*Gambusia affinis*) at 20°C than at 5°C, and a similar increase in DDT uptake at higher temperatures was noted by Reinert et al.[140] for rainbow trout (*Salmo gairdneri*). More recently, Powell and Fielder[141] have shown that the sea mullet *Mugil cephalus* also takes up DDT residues to a greater extent at higher temperatures. Edgren et al.[142] reported data on the influence of temperature on the uptake of DDT and two individual PCBs (2,3',4',5-tetrachlorobiphenyl and 2,2',4,4',5,5'-hexachlorobiphenyl) by perch, *Perca fluviatilis*. Once again, increased temperatures were accompanied by greater accumulation of each organochlorine. However, no consistent effects of temperature on the elimination of these pollutants could be confirmed. By contrast, Langston[123] noted faster rates of both accumulation and elimination of 4,4'-dichlorobiphenyl in the cockle *Cerastoderma edule* at higher temperatures (Figure 13). Half-lives for excretion of this isomer were 7 days at 8°C and 5 days at 15°C.

The influence of temperature on PCB and DDT uptake may also be based, at least partially, on an effect on the partitioning of these chemicals between solution and suspension;[66,67] it is known that organochlorines exhibit greater solubilities in water at higher temperatures.[143] Certain authors have also theorized that the increase in respiration rate of finfish at higher temperatures may also be significant.[139,142]

D. Conclusions on the Effects of Season/Organism Condition

Seasonal fluctuations in the concentrations of PCBs in aquatic biota can be severe in some instances. The causes of such fluctuations are multiple and may include temporal changes in PCB abundance (correlated to source emissions or run-off), biological effects from the bioindicator itself (particularly lipid changes correlating to the sexual cycle), and variations in water quality (particularly salinity and temperature).

A bioindicator survey for PCBs seeks, ideally, to take a "snap shot" of relative pollution conditions at various sites at any one time, in order to reach conclusions on the areas and relative magnitudes of pollutant sources or abundance. This is most efficiently and accurately achieved where:

1. Samples are taken over as short a period as possible
2. The period of sampling is selected such that no major changes in pollutant availability, water quality, or organism biology (particularly lipids), are occurring
3. The organism selected for study is not migratory

The period corresponding to that of least change, noted in item (2) above, will depend on the nature of both the area and the species studied. In temperate waters, it has been suggested that the best period for sampling bivalves for organochlorine analysis would be about 2

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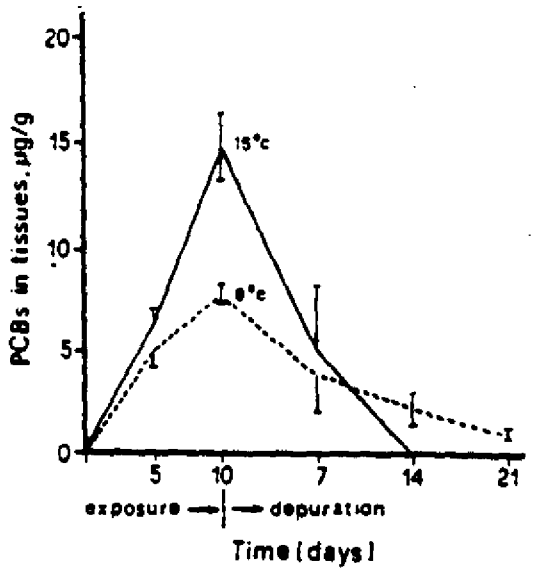

FIGURE 13. The uptake and clearance of 4,4'-dichlorobiphenyl by the cockle *Cerastoderma edule* at different temperatures. Bars represent standard errors, and tissue concentrations are in µg/g wet weight. Half-life calculations suggested a $t_{1/2}$ of 5 days at 15°C and 7 days at 8°C.

months prior to spawning.[88] However, while this has advantages in that the levels of lipid should be high and relatively similar between individuals and populations, other factors (e.g., gross variability in source emissions or water quality at this period) may outweigh these advantages. It is clear from the literature that some species exhibit only very minor seasonality of PCBs. Thus, for example, studies in the U.S. Musselwatch Program have consistently revealed only small changes in the concentrations of PCBs or other organochlorines with season.[190,344,345] However, other authors have found considerable seasonal fluctuations, sometimes over short periods, using similar or identical species.[175,313,346] While this is likely to depend on the stability or otherwise of inputs of PCBs, and the extent and rapidity of spawning of the species studied, no general rule may be drawn in advance of any detailed knowledge. It is therefore recommended that researchers take account of the potential influence of seasonal variation on the results of bioindicator surveys, by sampling as proposed above.

VII. SIZE (WEIGHT, AGE) OF INDIVIDUALS SAMPLED

The most important variable influencing PCB levels in aquatic biota, after lipid levels and season of collection or animal condition, is animal size. Bioindicator surveys must take account of the potential influence of organism size on the PCB contents of the samples studied; again, much can be done at sampling to reduce the influence of this variable on survey results.

There are various basic mechanisms whereby PCB levels might alter with organism size within a species:

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- Uptake of PCBs may be a size-related phenomenon, i.e., small individuals may attain higher PCB concentrations during episodic exposure to PCBs because of their higher surface area/volume ratio. (This assumes PCB uptake is dominated by uptake from solution across the body surface of an organism.)
- Individuals of different size of a species may exhibit differences in lipid contents, leading to variation in PCB levels with size.
- If PCB excretion is very slow in a species, these pollutants may be accumulated throughout the lifetime of a species, leading to size-dependent (or, more strictly, age-dependent) variations.[247]
- Various changes which occur with an increase in individual size or age of a species might incidentally influence PCB accumulation. Two major changes of potential importance in this respect concern diet and migration, but in some instances other factors may also be significant, particularly the attainment of sexual maturity.

With the possible exception of the third of these, evidence exists to suggest that each single mechanism, or a combination of more than one, may be effective in certain circumstances in defining size-related variation in PCB concentrations in a species.

The available data relating PCBs to size, weight, or age of individuals in any given species mostly concern finfish. However, a limited amount of information is available with respect to other phyla, and this will be considered first.

Several authors have reported that the concentrations of either DDT or PCBs accumulated by zooplankton (mainly planktonic crustaceans) are size related.[30,60,246-250] Smaller organisms accumulate greater concentrations of the pollutants, and the authors have theorized that this is due to their higher surface area/volume ratio compared to larger animals. Neff and Giam[247] cited a similar hypothesis to explain differential uptake of Aroclor® 1016 by two groups of horseshoe crabs (*Limulus polyphemus*) differing in size. The explanation assumes that these populations do not attain equilibrium conditions on exposure to PCBs, either because the exposure period is episodic in nature or for other reasons. McLeese et al.[70] have reported that accumulation of PCBs by the polychaete *Nereis virens* and the shrimp *Crangon septemspinosa* varied with size of individual; in both cases, smaller individuals took up greater concentrations of PCBs.

Some information is also available for molluscs. Ernst et al.[188] found that PCB concentrations in mantle musculature of the squid, *Loligo forbesi*, from the English Channel, tended to decrease with increased animal weight, using either a wet or lipid weight basis for concentrations. However, these trends were not statistically significant and the sample size was small. The same authors found no influence of size on PCB concentrations in digestive gland or adductor muscle of the scallop *Chlamys opercularis*. Other negative reports also exist with respect to size effects on PCBs in bivalves; no difference in PCB concentrations with size could be found for *Acanthocardia tuberculata* (foot or digestive gland),[251] or *Mercenaria mercenaria* (whole soft parts).[130] In addition, Vreeland[168] noted that PCB concentrations in oysters (*Crassostrea virginica*) used in long-term uptake experiments in the laboratory stayed constant after equilibration, even though the PCB body burdens increased to keep pace with oyster growth. This consistency of PCB levels in bivalves with variations in individual size is matched by other organochlorines. Cowan[177] for example, could find no influence of size on dieldrin concentrations in mussels, *Mytilus edulis*, from Scottish coastal waters, and no reports of variations in DDT levels of bivalves with size of individual are known. Phillips[33] has theorized that the major reason for this difference between the bivalve molluscs and finfish (see below) is that bivalve molluscs do not exhibit consistent increases in lipid levels with age or size.

By contrast to the above, there are numerous reports indicating that organochlorine concentrations vary with size of individuals for many finfish species. The majority of these

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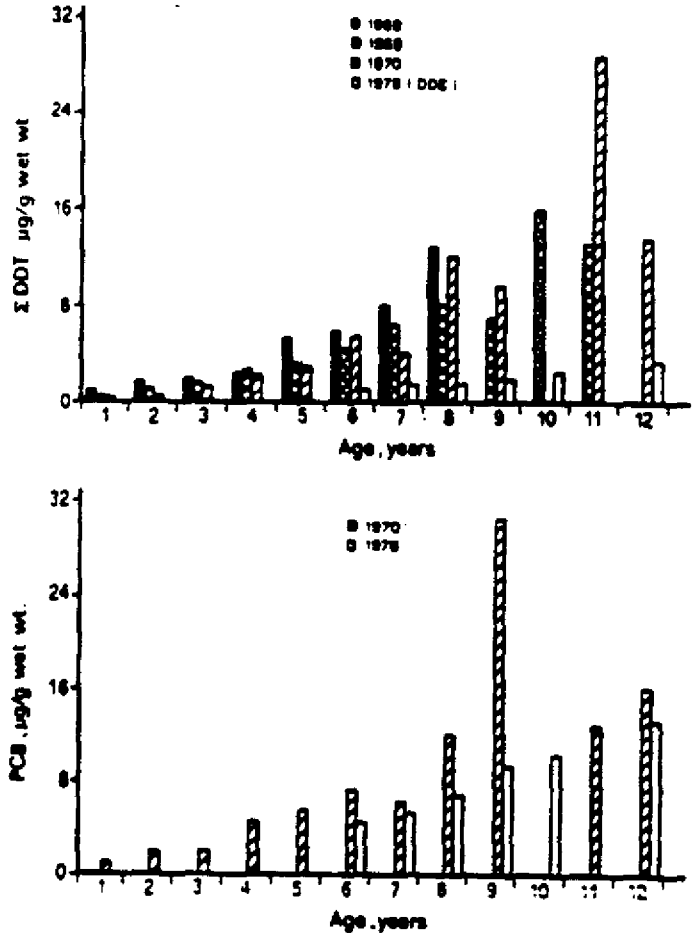

FIGURE 14. Concentrations of ΣDDT (1968, 1969, 1970) or DDE (1978) and PCBs in lake trout *Salvelinus namaycush* from Cayuga Lake, New York State, and their variation with age. Data from 1968, 1969, and 1970 are for whole eviscerated trout and are redrawn from Youngs et al.[225] for ΣDDT and Bache et al.[225] for PCBs. Data from 1978 are for the edible flesh only and are from Wszolek et al.[226]

reports concern DDT and its metabolites rather than PCBs, and these will not be discussed here, except in isolated instances where comparative profiles for DDT and PCBs are instructive.

Perhaps the most comprehensive information on the relationship of PCB concentrations in finfish with size or age, at least in terms of length of study, is that concerning lake trout (*Salvelinus namaycush*) from Cayuga Lake in New York State. Bache et al.[225] first reported that PCB concentrations (based on wet weights) in these trout varied with age. A different but concurrent report from the same group[227] showed that DDT residues followed a similar pattern. More recently, Wszolek et al.[226] produced data for DDE and PCBs in trout taken from the same lake 8 years later. These data are shown in Figure 14. It is notable that the PCB levels reported changed little over the study period; by contrast, the concentrations of

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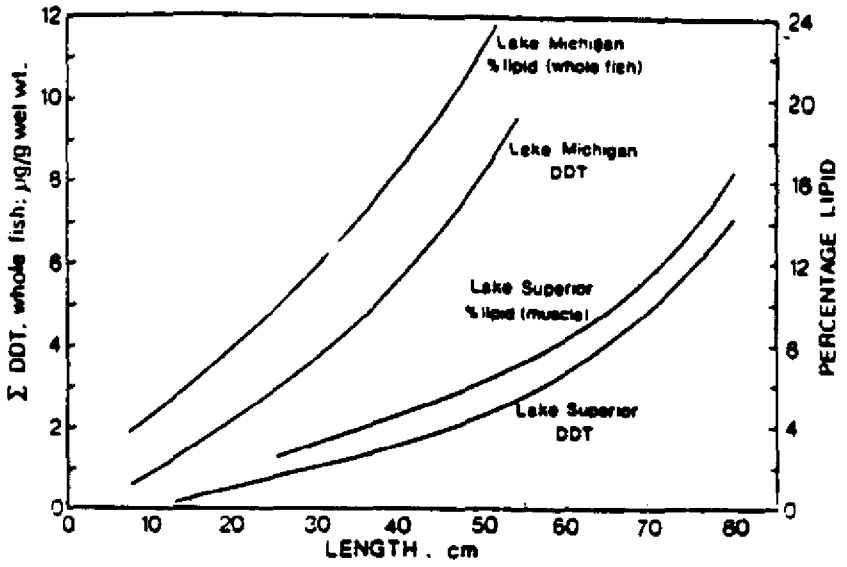

FIGURE 15. Concentrations of ΣDDT and percentage lipid in lake trout *Salvelinus namaycush* from Lakes Michigan and Superior, and their variation with fish length. Data for lipids in Lake Superior fish are from Eschmeyer and Phillips;[226] all other data are from Reinert.[163]

DDE in the later study (while still exhibiting significant increases with fish size), were considerably lower than ΣDDT levels in the previous investigations. Unfortunately, none of these reports included data on lipid levels of the fish analyzed; hence, it is impossible to know whether this effect of size on organochlorine levels is due to increases in lipid contents of the fish with age. A similar problem exists in other studies which have reported data for PCBs or hexachlorobenzene in lake trout.[226,227] However, Reinert and co-workers,[163,228] who worked on DDT accumulation in the same species from the Great Lakes, reported most convincing evidence showing that the increased DDT concentrations with length in lake trout are caused by a similar variation in lipid levels with fish length (Figure 15). It may therefore be quite safely assumed that this mechanism underlies the change in PCB levels in lake trout from Lake Cayuga.

Other fresh water finfish species are also known to exhibit size- or age-dependent variations in PCB concentrations in the field, including coho salmon (*Oncorhynchus kisutch*) from Lake Ontario,[223] carp (*Cyprinus carpio*) from Lake Huron,[227] yellow perch (*Perca flavescens*), white bass (*Morone chrysops*) and smallmouth bass (*Micropterus dolomieui*) from Lake Erie,[170] and yellow perch from the Ottawa River.[230] The data of Hattula et al.[160] are of particular note, as they involve more than 1700 individuals of 12 species from Lake Päijänne in Finland. Both pike (*Esox lucius*) and bream (*Abramis brama*) exhibited a direct dependence of lipid content on fish lengths; PCB concentrations were also length dependent as a result. In addition, studies of PCB uptake by sheepshead minnows, *Cyprinodon variegatus*, have revealed size-dependent uptake.[229] In many of these instances, the underlying basis for the increase in PCB levels with age is known to be a concomitant increase in lipid levels.

Some data are also available for marine and estuarine finfish species. Zitko[169] noted a possible influence of size on PCB concentrations in herring *Clupea harengus* from Canadian

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waters. Jensen et al.[163] confirmed this effect in extensive studies of herring and cod in Sweden. Herring revealed moderate increases of PCB levels in axial muscle with age even when data were based on lipid weights; by contrast, cod (*Gadus morhua*) exhibited no such age-dependence of pollutant concentrations, and a later report from the same group[261] showed no age dependence of PCBs (based on lipid weights) in pike, *Esox lucius*. Ernst et al.[166] reported data for PCBs in various tissues of the yellow gurnard, *Trigla lucerna*, from the English Channel. Significant correlations between PCB concentration and animal weight were found only for visceral adipose tissue when the concentration data were based on wet tissue weights; when concentrations were based on lipid weights, no significant size effect could be found. Stout[167] noted significant correlations between lipid and size, and between lipid and PCB concentrations for red snapper and king mackerel from the Atlantic Ocean and Gulf of Mexico, but size/PCB concentration relationships were apparently not significant. Young and Mearns[156] found a direct relationship between fish length and PCB concentrations for mullet (*Mugil cephalus*) from Californian waters; lipid levels were also greater in the larger individuals. Similar data correlating size, lipid levels, and PCBs have been reported for striped mullet, *Mullus barbatus*, from five areas in the Saronikis Gulf, off Greece.[241]

By contrast to the above reports which show that PCB concentrations increase with size in several finfish species, some authors have reported either no correlation of size to PCB concentration, or an inverse relationship. Thus, Van den Broek[199] found that PCBs did not vary significantly with size in muscle of whiting *Merlangus merlangus*, but tended to decrease with length in plaice, *Pleuronectes platessa*. Fowler and Elder[29] reported that size did not influence PCB levels of the vertically migrating fish *Myctophum glaciale* from the Mediterranean Sea, although ratios of PCBs to either ΣDDT or DDE altered with fish size. Hattula et al.[168] found that the lipid levels of perch, *Perca fluviatilis*, from Lake Päijänne in Finland decreased with increased fish length in some samples; this gave rise to an inverse relationship between PCB concentrations and length for this species. Similarly, Klauda et al.[263] noted a trend toward an inverse relationship between PCB levels and size in Atlantic tomcod (*Microgadus tomcod*) from the Hudson River Estuary, although the data were significant statistically only for residues in the liver.

We may conclude from the above review that there is no general rule governing the relationship between PCB concentrations and animal size in aquatic biota. While some species (particularly of finfish) exhibit direct relationships between size and PCB levels, others (notably most bivalve molluscs, but also some finfish) exhibit no such correlation. In a few cases, inverse relationships have been noted. It is clear that size-dependent variations in lipid levels are commonly a major factor in determining the size/PCB relationships found, but other variables such as dietary changes and migration may also play a part in certain instances. Researchers undertaking bioindicator surveys would do well to attempt in all cases to reduce the potential influence of this variable; this is most simply achieved by sampling individuals of a restricted size or age range and by determining the lipid contents of the individuals analyzed. A case can again be made for basing the PCB concentrations quoted on both wet and lipid weights of tissues, as this aids interpretation of data markedly.

It should be mentioned finally in this section that age-dependent variation in PCB levels has also been noted for marine mammals. However, this variability is intimately connected with the sex of the individuals studied; hence these data are discussed in the following section.

VIII. SEX OF INDIVIDUALS SAMPLED

In certain instances, it is known that the accumulation of PCBs by potential aquatic bioindicator species depends on the sex of the individual concerned. It is clear that such an effect may be based upon the differential ability of male and female gonads to accumulate

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Table 13
CONCENTRATIONS OF VARIOUS ORGANOCHLORINES (MEANS ± SE, µg/g DRY WEIGHTS) IN 6 MALE AND 6 FEMALE COMPOSITES OF MUSSELS (*MYTILUS CALIFORNIANUS*) FROM 2 SITES IN CALIFORNIAN WATERS[364]

Compound	Royal Palm (outfall region)		Point Sal (control region)	
	Male	Female	Male	Female
Total DDT	4.100 ± 0.700	6.40 ± 0.320	0.110 ± 0.0040	0.110 ± 0.0070
p,p'-DDT	0.040 ± 0.005	0.06 ± 0.005	0.008 ± 0.0005	0.007 ± 0.0006
o,p'-DDT	0.030 ± 0.003	0.04 ± 0.004	0.004 ± 0.0001	0.004 ± 0.0005
p,p'-DDE	3.500 ± 0.640	5.40 ± 0.280	0.077 ± 0.0030	0.067 ± 0.0030
o,p'-DDE	0.410 ± 0.060	0.73 ± 0.050	0.015 ± 0.0010	0.026 ± 0.0070
p,p'-DDD	0.110 ± 0.010	0.18 ± 0.007	0.009 ± 0.0006	0.008 ± 0.0004
Total PCBs	0.640 ± 0.060	0.94 ± 0.040	0.044 ± 0.0060	0.054 ± 0.0020
1242 PCB	0.120 ± 0.010	0.19 ± 0.010	0.016 ± 0.0030	0.019 ± 0.0010
1254 PCB	0.530 ± 0.060	0.76 ± 0.040	0.028 ± 0.0040	0.035 ± 0.0030

Note: Each composite was 5 individuals of shell length 40—50 mm; both samples were collected in late June 1974.

PCBs, or on differences between the amounts of body weight or lipids lost by the sexes at spawning, or on other such mechanisms. It is also evident that differences between the sexes may vary with season: the most marked divergence might be expected in ripe, prespawning individuals.

The majority of the data relating sex to organochlorine content of biota involve finfish and marine mammals, particularly seals. Very little information is available for molluscs or crustaceans. In the case of molluscs this may be because of their unusual sex lives in many cases; a large number of species are either functional or successive hermaphrodites. The reason for such a dearth of data on crustaceans is unclear, but is probably a function of the overall paucity of studies on organochlorines in this phylum.

The only report known concerning possible sex effects on PCBs in molluscs is that of Young et al.,[364] involving the mussel *Mytilus californianus*. These authors reported data for DDT and its metabolites, and PCBs, in male and female mussels from two locations on the coast of California. The results for mussels from the relatively uncontaminated area showed very little influence of sex on the organochlorine concentrations encountered, although both *o,p'* DDE and PCBs tended to be greater in females (Table 13). These trends were even more evident in the results for mussels from the polluted Palos Verdes area. However, the authors stated that additional (unpublished) data showed no statistically significant influence of sex on organochlorine uptake. It is possible that changes in sexual maturity of the samples occurred during the course of the uptake studies, thus masking a sex effect; further studies would be needed to clarify this.

With respect to crustaceans, no data could be found directly showing a difference between the sexes in PCB accumulation. Vernberg et al.[365] noted that there was no sex-based difference in the toxicity of Aroclors® 1016 and 1254 to fiddler crabs (*Uca pugilator*). By contrast, the accumulation and tissue distribution of kepone in blue crabs (*Callinectes sapidus*) is known to be heavily sex-dependent.[366,367]

Some finfish species are known to exhibit sex-dependence of PCB concentrations, although this is by no means the rule. Authors who have failed to find significant differences between the sexes for PCBs include Hattula et al.,[168] studying various species, Olsson and Jensen,[241] who noted significant differences in levels of DDT and its metabolites with sex of the pike

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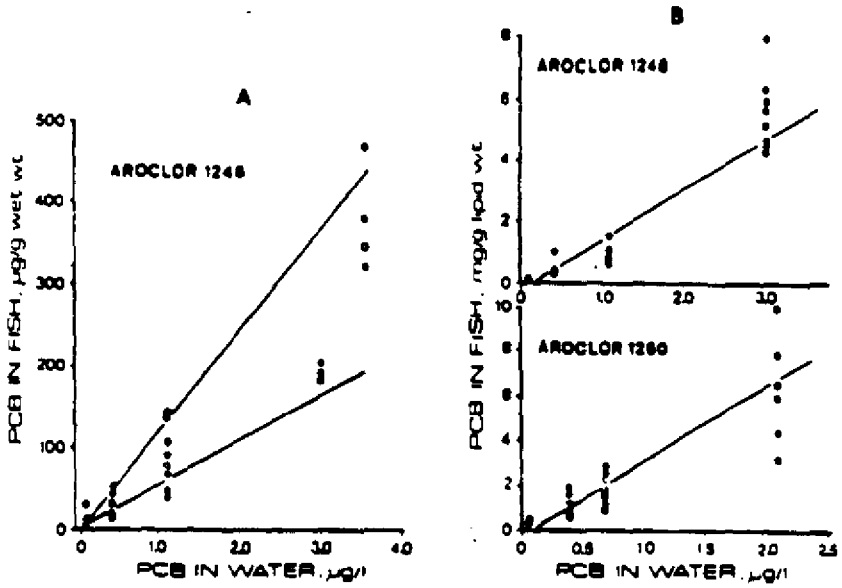

FIGURE 16. Uptake profiles for Aroclors® 1248 or 1260 in male (closed circles) and female (open circles) fathead minnows, *Pimephales promelas*, exposed to various PCB levels in water for 250 days. The profile in (A) is based on wet weights of the fish, whereas those in (B) are based on lipid weights.[127]

Esox lucius but no such variation for PCBs, and Kaiser,[188] for PCBs in the lake whitefish *Coregonus clupeaformis*. However, the levels of PCBs have sometimes been found to vary between testes and ovaries of fish in detailed tissue distribution studies.[171,203] It has been pointed out that differences generated thus are exacerbated because female finfish lose a greater percentage of total body weight at spawning than do males.[130] Such effects no doubt underlie the sex-based variation observed in certain species, e.g., by Zabik et al. for carp from Lake Huron.[237]

The most impressive example of sex-based variation in PCB accumulation by finfish, however, is based on an influence of total-body lipid differences. Thus, DeFoe et al.,[127] working with the fathead minnow *Pimephales promelas*, showed that females took up about twice as much PCBs as males because of the greater lipid contents of the former sex. Concentration factors (based on wet tissue weights) for the uptake of Aroclor® 1248 were 60,000 and 120,000 for males and females, respectively; for Aroclor® 1260 the respective concentration factors were 160,000 and 270,000. These differences between the PCB kinetics in the two sexes were almost completely eliminated by basing the tissue levels of PCBs on lipid weights (Figure 16), indicating that the effect is due almost totally to sex-based differences in lipid content. The residual differences may be caused by divergent lipid chemistries in the sexual organs of this species.

Very little information is available on PCB differences between the sexes in elasmobranchs, which is unfortunate, as the sex-based differences for mercury in these species are particularly interesting. Forrester et al.[206] and Walker[209] have shown that mercury accumulates slower with length in females than males of elasmobranchs, and this is thought to be due to the faster growth rate of females. Similar studies of PCBs in elasmobranchs would be of particular interest, as the results would indicate whether these compounds are taken up from food or from water at this trophic level. It is in any event likely that sex-based differences exist

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with respect to PCBs in viviparous species of elasmobranchs, as PCBs are known to be transferred from the mother to the ovarian egg and the mature fetus.[270]

The transfer of PCBs to the offspring, noted above for viviparous elasmobranchs, is matched by marine mammals, although transplacental transfer of PCBs in mammals is not particularly high due to the predominance of polar lipids (phospholipids) in the blood, placenta, and fetus.[131] After birth, marine mammals suckle their young and organochlorines are again lost from the mother to the young by this route,[271] although once again a partial barrier appears to exist, possibly for the same reason.

The loss of PCBs (and other organochlorines) by female marine mammals to the fetus during gestation and to the young by suckling gives rise not only to sex-based differences in these species after sexual maturity, but also to divergences in the age/concentration relationships for the sexes. Thus, data from various authors[146,149,184,193,271,272] show that young seals or other marine mammal species are born with relatively low levels of PCBs, derived transplacentally from the mother during gestation. After birth, PCB concentrations rise, initially because of their transfer in milk, and later because of high PCB levels in the solid diet (fish, etc.). Prior to the attainment of sexual maturity there is no evidence of any difference in PCB concentrations of the two sexes. However, once the breeding age is attained, females are able to lose more PCBs than males because of pollutant transfer to the fetus, and later suckling. Thus, the levels of PCBs in breeding females may level off at the attainment of sexual maturity, or may go on increasing, but at a slower rate than PCBs in the males. An exception to this pattern occurs if the females are not breeding successfully. Helle et al.[273-275] reported studies of ringed seals (*Pusa hispida*) from the Baltic Sea: PCBs are believed to have caused pathological changes in the uterus of ringed seal females, eliminating any chance of breeding in a large percentage of the population. Female seals exhibiting these changes had higher PCB levels than those without such pathology or those which were pregnant, showing clearly that parturition and lactation are major routes of PCB loss for female marine mammals. A similar problem may exist with California sea lions.[184]

In a sense, the dramatic sex differences in PCB accumulation seen in marine mammals are merely an extension of such effects in finfish, as both are a function of the greater involvement of the female in species propagation. The influence is, of course, greater for the mammals because of the entire mammalian habit of bearing live young and suckling the young. Viviparous elasmobranchs might be considered to be in an intermediate position in this respect.

Whatever the mechanisms behind sex-based divergence in PCB levels, it is clear that in some cases this factor should be taken into account when sampling animals for bioindicator surveys. In these instances, researchers should strive to stratify samples for sex, either by collecting animals of only one sex from all study locations, or by maintaining a given ratio of male to female animals in all samples. As noted above, such a requirement is likely to be particularly important if sampling takes place at a time when the individuals are ripe, i.e., just prior to spawning.

IX. VERTICAL POSITION OF SAMPLING

The final variable which merits attention here because of its potential influence on the results of bioindicator surveys is the depth of sampling of biota, or more strictly, the vertical position of sampling in relation to both the surface and the sea bed.

Three potential effects exist with respect to this variable:

> The water column itself may be vertically stratified. In such instances, either the absolute amounts of PCBs present may vary vertically, or the factor causing the stratification may influence the bioaccumulation of the PCBs present.

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- Organisms living intertidally may be exposed to unusual concentrations of PCBs compared to those living subtidally.
- In some instances, bottom sediments may be a major source of PCBs and the levels of PCBs available to biota may depend on their vertical distance from the bottom sediments.

With respect to the first of these, it is well known that certain types of waters exhibit vertical stratification. Thus, estuaries draining large catchments will commonly exhibit salinity-based stratification, with a more-or-less well-developed halocline between an underlying salt wedge and an overlying brackish or freshwater layer. In enclosed embayments with little freshwater inflow, thermal stratifications may occur. Either of these may vary seasonally if large differences exist throughout the year in run-off or in ambient temperature and insolation.

This vertical stratification of the water column may give rise to vertical heterogeneity of PCBs. Thus, in the case of estuarine areas, the brackish water may contain greater amounts of PCBs (from terrestrial sources, delivered to the estuary in run-off) than the underlying salt wedge. Such a profile may also be produced in areas where the major source of PCBs is via wash-out of airborne contaminants, as these too will be introduced at the water surface.[274] In these and similar instances,[275] biota which are sessile may be expected to reflect the vertical variation in abundance of PCBs in the water column, i.e., concentrations of PCBs in a bioindicator will vary with depth.

A second possibility also exists in areas of vertical stratification based on water salinity or temperature. As noted in Section VI.C, both salinity and water temperature may influence the bioavailability of PCBs. Thus, even in areas where the absolute ambient levels of PCBs in the water column do not vary vertically, differences with depth may occur with respect to PCBs in a sessile bioindicator because of direct effects of salinity or temperature on its accumulation of PCBs.

The second mechanism whereby depth of biota may influence their PCB concentrations is in a sense a special case of vertical stratification. It has already been mentioned in Section III.A that surface films may be exceedingly rich in PCBs compared to subsurface waters.[12,51,65-67] A sessile bioindicator living in the intertidal zone may be exposed to PCBs in surface films, either if it irrigates the gills close to the water surface (e.g., as the tide recedes), or if the film is broken up and dispersed vertically by wave action on the shore. This mechanism could give rise to enhanced accumulation of PCBs by bioindicators in the intertidal zone compared to the same species living subtidally.

Finally, the influence of sediments must be considered. PCBs exhibit a high affinity for particulates, and sediments are considered to be a major environmental sink for these contaminants. In the absence of biodegradation, the burial of PCBs in marine sediments is essentially the sole mechanism of their loss from the ecosystem. However, evidence has been accumulating for some years that sediments might also be viewed as a source of PCBs for biota. Laboratory experiments by various authors have shown that particulate-associated or sediment-associated PCBs may be available for uptake by phytoplankton,[89] crustaceans such as fiddler crabs and shrimp,[70,81,276] polychaetes,[74-76,78] and fish.[277] Similar conclusions have been reached in field studies of a wide variety of organisms, notably in Californian waters.[69-84,153,268] Perhaps the most convincing data are those reported by Young et al.[268] concerning uptake of DDT and PCBs in mussels (*Mytilus californianus*) transplanted from a relatively noncontaminated area to various depths at Palos Verdes, Calif. These authors found that both pollutants were taken up to a far greater extent by mussels closer to the bottom sediments (Figure 17). It is known that the sediments at Palos Verdes are particularly contaminated by DDT and PCBs, and are probably now the major source of these contaminants in the region; hence, this is possibly an extreme example. However, it is clear that

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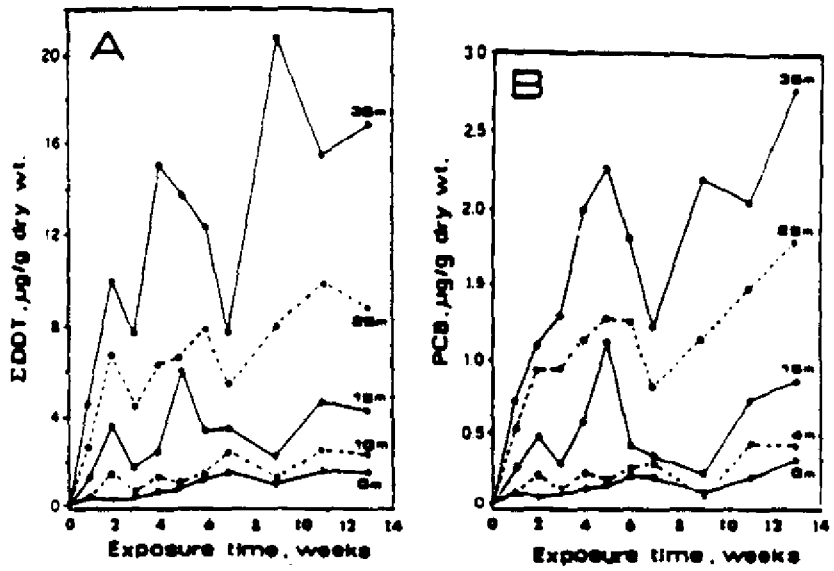

FIGURE 17. The net uptake of (A) ΣDDT and (B) PCBs by whole soft parts of mussels (*Mytilus californianus*) transplanted from Point Sal to various depths in the water column at Palos Verdes, Calif.[84]

bottom sediments may act as a particularly important source of PCBs, and this may also give rise to vertical variation of PCBs in bioindicators.

It may be concluded from the above that several mechanisms exist whereby bioindicators (particularly sessile or sedentary species) may exhibit depth-dependent variations in PCB accumulation. Researchers wishing to employ such species in monitoring surveys should take this into account and should sample at similar regions in all study locations with respect to tide marks and/or distance above the sediments. In instances where it is suspected that vertical heterogeneity may exist, it may be worthwhile to sample individuals at various depths in the water column.

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