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AN ABSTRACT OF THE THESIS OF John J. Salmon for the
Master of Arts in Biology presented June 9, 1972.

Title: Polychlorinated Biphenyls: Uptake by Daphnia
and Residues in Crayfish from the Willamette River.

APPROVED BY MEMBERS OF THE THESIS COMMITTEE:

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Polychlorinated Biphenyls (PCB's) are organochlorine molecules which find various industrial and product applications. PCB's are of concern to biologists because they are toxic substances which have become global contaminants. They are also of concern to biologists and analytical chemists because they interfere with the determination of some organochlorine pesticide residues. PCB's were discovered to be environmental contaminants after they showed up as unidentified peaks in pesticide analysis using gas-liquid chromatography with an electron capture detector (GLC-EC).

In the present experiment standard GLC-EC techniques

were used to assay PCB's in Daphnia experimentally contaminated in the laboratory and in crayfish from the Willamette River. Daphnia were placed in water containing 0.1, 4, 50 or 100 parts per billion (ppb) PCB for 4 to 72 hours. There was no mortality in any of the experiments. The individual PCB compounds were apparently taken up equally, since relative peak heights were similar in the standard and the residues extracted from Daphnia. Final concentrations of PCB's in Daphnia ranged from 1200 times that of the water (at water concentrations of 100 ppb) to 104,000 times that of water (at water concentrations of 0.1 ppb).

The lower concentrations used here approximate environmental PCB levels found in some areas of the U.S. and elsewhere. Assuming that chronic exposure to these lower concentrations would not strongly inhibit growth or reproduction of Daphnia, the high biological magnification found here would suggest that Daphnia and related organisms may play an important role in the accumulation of PCB's in fresh water food webs.

Digestive glands of crayfish taken from the Willamette River were analyzed for PCB's. Statistically significant regional differences were found in the concentrations of PCB's in these organisms in the lower Willamette Valley. Crayfish from the center of Portland have the highest residues (7 to 9 parts per million).

A similar distribution has been previously reported for other urban areas.

In the present investigation, however, the highest residues occurred in crayfish from the river at a point approximately in the center of the city itself, but two miles upstream from the major industrial areas. Thus, in this case, the major source of environmental contamination may have been released from manufactured goods (e.g., automobile tires, paints, etc.) rather than from industrial sewerage.

POLYCHLORINATED BIPHENYLS: UPTAKE BY DAPHNIA AND
RESIDUES IN CRAYFISH FROM THE WILLAMETTE RIVER

by
JOHN J. SALMON

A thesis submitted in partial fulfillment of the
requirements for the degree of

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1972

TO THE OFFICE OF GRADUATE STUDIES:

The members of the Committee approve the thesis of
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July 26, 1972

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INTRODUCTION

Properties and Manufacturing

Polychlorinated Biphenyls (PCB's) are organochlorine molecules which find various industrial and product applications. They consist of two phenyl groups covalently connected to each other. This leaves ten positions, any number of which can be substituted by chlorine (1). PCB's are manufactured in the United States (under the trade name Aroclor), France (trade name Phenoclor), Germany (clophen), Japan, and the Soviet Union. Aroclors are manufactured by the Monsanto Chemical Company and are designated by a four-digit number. The first two digits represent the molecular type: those designated 12 are chlorinated biphenyls; 25 and 44 are blends of chlorinated biphenyls and chlorinated terphenyls; 54 are chlorinated terphenyls. The last two digits give the weight-percentage of chlorine. Therefore, Aroclor 1254 is composed of chlorinated biphenyls containing 54% chlorine. The biphenyls commercially available from Monsanto contain from 21 to 68% chlorine (2).

In commercial PCB manufacture, biphenyl is chlorinated in towers with anhydrous chlorine, using ferric chloride as the catalyst. The degree of chlorination is determined by measuring the specific gravity of the product, which

is a mixture of several isomers (3).

The Aroclors are very stable. They are not affected by boiling with sodium hydroxide solution, and prolonged treatment (255 hours) with concentrated sulfuric acid has negligible effect. When Aroclor is subjected to a bomb test at 140 c. with 250 pounds of oxygen per square inch, there is no evidence of oxidation (4).

The Aroclors are listed by Monsanto as insoluble in water (4). Recently the solubility of Aroclor 1254 has been reported to be 2-3 mg/l in fresh and 1-1.5 mg/l in sea water. However, the dissolved fraction was richer in lower-chlorinated biphenyls than the original preparation. The sample of Aroclor 1254 used in the preceding experiment contained a relatively high amount of the lower-chlorinated biphenyls. Another sample of Aroclor 1254, with only traces of lower-chlorinated biphenyls has a solubility of 0.3-0.5 mg/l in both fresh and sea water. Solubility of Aroclor 1221 was reported as 5.0 and 3.8 mg/l in fresh and sea water respectively (5). The Aroclors are soluble in most organic solvents, solvent mixtures, and mineral and vegetable oils (1). Another important property is their low rates of vaporization, which are nevertheless measurable (4). These properties make PCB's useful in more than one hundred different industrial and product applications (4).

The companies involved in the production of PCB's

have not revealed all of their uses or annual production figures. Monsanto reported that the primary markets for Aroclors are as plasticizers in chlorinated rubber, styrene butadiene copolymers and polysulfide sealants; as insulating fluids for transformers and capacitors; and as components in heat transfer systems (6). They are also used for impregnation of cotton and asbestos for braided insulation of electrical wiring, formulation into some epoxy paints, and as protective coatings for wood, metal and concrete, as adhesives, high-pressure hydraulic fluids, specialized lubricants, gasket sealers, in carbonless reproducing paper, and ballasts for fluorescent fixtures (3).

Environmental Contamination

PCB's are of concern to biologists because they are toxic substances which, as will be shown, have become global contaminants. They are also of concern to biologists and analytical chemists because they interfere with the determination of some organochlorine pesticide residues.

Gas-liquid chromatography, using the electron capture detector (GLC-EC), has become the major analytical technique for determining organochlorine pesticides. With its development, most chemists studying pesticide residues observed unidentified peaks in their gas chromatograms (7).

Because these peaks were neither large nor sharp, investigators tended to ignore them (3). It was established that these peaks were organochlorine in nature, with a substantial chlorine content (8). In 1966 Jensen identified these peaks as PCB's (9). His work was based on analysis of 200 pike, several other species of fishes and fish spawn from numerous sites in Sweden. Human hair and feathers from an eagle were also contaminated with PCB's. He analyzed eagle feathers from museum collections and found the earliest appearance of PCB's dated to 1944. PCB's have been subsequently reported in fish, seals, birds, conifer needles, human fat, and some invertebrates in Sweden, Great Britain, Germany and The Netherlands (10-19). Residues as high as 900 parts per million (ppm) were found in herons (18).

PCB residues were determined in about 1,000 fish from lakes, rivers and seas of Sweden. Lean fish usually showed low levels. Cod liver had the highest levels, 2.2-4.9 ppm (20). In another study (21), PCB's were determined in 1,400 samples of margarine, vegetable oils, and food of animal origin. Ninety percent contained PCB's at levels less than 0.1 ppm. Twenty two samples of human milk contained from 0.01-0.03 ppm, and in Germany human milk has been reported to contain 0.103 ppm PCB, and human fat 5.7 ppm PCB (22).

A collaborative study was undertaken to determine

the residues in four species of wildlife from 11 countries of western Europe and North America (23). Four species were studied. Residues in starlings were low or undetectable, as were residues in pike. PCB's were generally present in mussels and dogfish, with levels in mussels being somewhat higher than in other samples (up to 0.47 ppm).

No PCB's were found in the soil macro-fauna at 12 agricultural sites near Huntingdon, England (24). Airborne dust collected at the Barbados Island and La Jolla, California, was also free of PCB contamination (25). However, small amounts of PCB's were present in all samples of rain water collected in the British Isles (26).

In North and Central America, Risebrough et al. (27) reported PCB's in Peregrine falcons and their prey. One adult falcon had 1,980 ppm PCB's in its fat and 10.9 ppm in the whole carcass. They also reported PCB's in fish and birds from California, birds and a fish bat from the Gulf of California, Pacific sea birds and birds from Panama, but no PCB's were detected in eggs of Adelie penguins from Antarctica. PCB's have subsequently been reported in several places in North American aquatic organisms, birds, and human fat (7, 28-34, 35).

Ten of 36 samples of fish caught on the California coast contained PCB's (36). The bottom-dwelling coastal fish from Santa Monica Bay contained much higher residues

than did pelagic fish from the open ocean. Of 34 samples of fish from Oregon, Washington, and Alaska coastal and open ocean waters, no PCB's were found. This included a composite sample of ten Chinook salmon taken from the mouth of the Columbia River. In another study salmonids from the Big Creek Hatchery at Knappa, Oregon, had PCB levels from 0 to 0.3 ppm (average 0.13 ppm) (37). A total of 147 fish samples collected at 50 nationwide monitoring stations during the fall of 1969 all contained PCB (38). At Oregon City large-scale suckers contained 1.16 ppm, chiselmouths 0.71 ppm and white crappies 1.11 ppm (38).

PCB's have also been reported in sewage sludge dumped in British coastal waters (39) and in sewage effluents from treatment plants in California (40, 41). Sewage effluents and even tap water have been found to contain PCB's in Sweden (42), as have sewage treatment plant effluents and industrial effluents discharged into the Milwaukee River in Wisconsin (43).

Qualitative Analysis

Some doubts were expressed concerning the accuracy of PCB identification (6, 44). GLC-EC has been the most popular analytical method. Other techniques used in PCB residue analysis include the microcoulemetric detector, thin-layer chromatography, and column chromatography.

Chemical procedures have also been used, e.g., saponification, nitration, or oxidation with sulfuric or chromic acid, all of which react with some of the interfering organochlorines, but not with PCB's (10,14,20,21,23,27,28, 40,42). Mass spectroscopy has been used in a number of investigations (10,14,15,30,33,41,43,45), thus positively establishing the identity of environmental residues detected by GLC.

As previously stated, PCB's are carried through the standard organochlorine pesticide extraction and clean-up procedures. Several methods have been introduced to separate these two groups and/or eliminate the pesticides to make possible qualitative and quantitative analyses. In addition to chemical treatment as mentioned above, column chromatography has also been used (7,13,15,29,43, 44,46,47) as well as thin-layer chromatography (12,16,20, 21,31,32,48,49,50). The possibility of using carbon-skeleton chromatography has recently been suggested as a means of differentiating PCB's from DDT (51). The presence of PCB's interferes with GLC analysis of DDT and its breakdown product, DDD. However, Risebrought et al. (52) conclude that since most of the "total DDT" burden in the environment (including DDT and its breakdown products) occurs in the form of DDE (which is not obscured by PCB contamination), that earlier "DDT" determinations are not seriously in error. Results in two other papers

(7,28) were consistent with this conclusion. It was also reported (7) that analysis of heptachlor epoxide residues were unaffected by PCB residues.

Quantitative Analysis

Quantitation of PCB's from the environment is a very serious problem. Jensen et al. (14) used a combination of mass spectrometry, micro-colorimetric (sic) and electron capture detection. Even so, their estimates (based on the GLC techniques) may be correct only within a factor of two. Richardson et al. (45) used the above techniques and added GLC with flame ionization detection. They concluded that it is impossible to determine the quantity of PCB's in the environment with any degree of confidence.

Most investigators compare areas under one or more of the peaks of their environmental chromatograms with those of a commercial PCB mixture, using whichever of the commercial preparations that shows the greatest similarity. Values are then reported in terms of the commercial PCB mixture as a standard. Within any standard commercial sample of PCB's the compounds of lesser chlorination have shorter retention times in the gas chromatograph than the more heavily chlorinated PCB compounds (53). Many investigators have reported environmental residues to have a relatively higher quantity of the more highly chlorinated PCB's than is found in commercial PCB's

(7,10,12,14,15,35,43,52). Examination of the published chromatograms of environmental PCB residues reveals that, while they may have the general appearance of standard commercial PCB mixtures, the relative height of individual peaks does vary from those of the standards (7,12,15,16, 20,21,28,29,30,35,41,42,43).

Zitko et al. (53) determined that the electron-capture detector response increases strongly with increasing number of chlorine atoms. For instance, one tetrachlorobiphenyl had an electron-capture response of 0.04 (relative to DDE), while the same quantity of a pentachlorobiphenyl gave a response of 1.30, 32 times as great. Recently the structures of the compounds in Aroclor 1254 have been identified (54). Although inspection of this report reveals that none of the PCB compounds tested by Zitko et al. (53) is a major constituent of Aroclor 1254 (the preparation most commonly used as a standard), it is apparent that Aroclor 1254 includes many tetra- and pentachlorobiphenyls, hence there may be great differences in the electron-capture response of the individual chlorobiphenyls. Therefore there is a potentially large source of error in present methods of quantifying PCB's. This problem will probably only be solved when individual chlorobiphenyls are available to use as quantitative standards. A start in this direction has been made; 23 chlorobiphenyls have

now been synthesized (55).

A recently published method for quantification of PCB's by GLC-EC takes into consideration the degree of chlorination (35) but not the positions of chlorine atoms on the biphenyl ring. In regard to position effects, Zitko et al. (53) found that response to identical quantities of two different tetrachlorobiphenyls varied by a factor of 17.

Types of PCB's Reported in the Environment

In the world market there are many commercial preparations which include biphenyls or terphenyls, or sometimes both (for example, there are 12 Aroclor preparations). However, not all of these mixtures have been reported as environmental pollutants. Clophen A50 has been reported (14,23). Clophen A50 is 50% chlorine by weight and is similar to Aroclor 1254 (23). Aroclor 1254 has been reported frequently (7,18,23,28,29,31,32,34,37,39,40,43,45,52,56) as have Aroclor 1260 and Phenoclor DP6 (7,15,16,22,39,40,41). Aroclor 1260, Phenoclor DP6 and Clophen A60 appear to be identical when compared by standard gas chromatography-mass spectrometric techniques (15). Also reported in the environment are Clophen A40 (42), Aroclor 1242 (40,43), Aroclor 1248 (43), and Konechlor 400 (48% chlorine by weight) (57).

Of all the polychlorinated bi- and terphenyls manufactured, why are only biphenyls found, and why only those in a narrow range of chlorination? One reason may be simply that these are the ones manufactured and used in greatest quantity. No exact information has been made available, but of 31 specific uses recommended for the Aroclors, 13 were for 1254 (4). No information is available as to how commonly these recommendations are followed or in what quantity.

Sources of Contamination

There have been cases where PCB contamination has been traced to a single source. Two of these cases involve leaks in heat transfer systems. One leak polluted Escambia Bay in Florida with Aroclor 1254 (32). In the other, Konechlor 400 spilled into rice bran oil injured 600 people in Japan (57). In another case, death of fry at a fish hatchery was traced to a resin containing Aroclor 1254 (7). Cashew nuts were contaminated with 10 ppm PCB's (containing 40% chlorine by weight) from having been shipped in a lacquered cardboard drum. PCB's were found in pollen contaminated by storage in plastic (polyethylene) sacks (59). A herd of dairy cows was contaminated with Aroclor 1254 which came from paints used to coat the interior walls of silos (56).

Reynolds (7) suggests that the PCB's reported may simply be those most likely to be detected using standard

pesticide residue procedure. Using standard operating parameters in GLC-EC, he ran several Aroclors (1221, 1232, 1242, 1254, 1260 and 5460) and found 1254 and 1260 to be easily detected. However, the techniques are less responsive to the lower- and higher-chlorinated mixtures, the former because of low chlorine content and the latter because of long retention times.

As mentioned before, relative to commercial products, environmental samples show a higher content of the more highly chlorinated biphenyls. It has been suggested that the more sparsely chlorinated PCB's are preferentially metabolized or excreted by organisms (14). Koeman et al. (15) found the lower peaks missing in sea birds but not in roaches. They felt that the lower-chlorinated PCB's might be less persistent in birds. This was confirmed by feeding Phenoclor DP6 to Japanese quail. Chromatograms of the residues from the quail showed reduction of some of the individual peaks, particularly the lower-chlorinated ones. No disproportionate reduction of peaks was found in feeding experiments in Bengalese finches, however (18). When Aroclor 1254 was fed to rats, PCB's with lower chlorine content were found in tissues in relatively lower quantity than those of higher chlorine content (60). Chromatograms of residues from estuarine fish exposed to Aroclor 1254 duplicated those of the Aroclor except for one peak, which was reduced (61).

Risebrough et al. (52) found in organisms taken far from any known source of contamination, that one particular peak was consistently reduced. On the assumption that the PCB's were probably transported through the atmosphere, they subjected Aroclor 1254 to ultra-violet light. The same peak was selectively reduced. Another chlorinated biphenyl (hexachlorobiphenyl) has been reported to be ultra-violet labile (62).

It has been suggested that the lesser-chlorinated biphenyls are more reactive in aquatic environments than those of higher chlorine content, and are thus selectively removed from the environment. It was observed that the decrease in the concentration of Aroclor 1242 in water occurs at a rate ten times greater than that of Aroclor 1260 (43).

Among industrial products, chlorinated polyphenyls meet the general criteria of Risebrough et al. (52) as compounds potentially accumulated by organisms and dispersed around the world. These criteria are: high world production, chemical stability, insolubility in water (non-polar chemicals become concentrated in fat tissue), mobility, and aerial dispersal (PCB's have measurable, even if low, vapor pressures). That they have become pollutants is amply demonstrated, but the sources and routes of dispersal of PCB's are still unknown.

It is still unclear whether the bulk of environmental residue arises from a very few major sources (e.g., industrial effluents) or from a large number of minor sources. For instance, as discarded products are incinerated any PCB's present would not be oxidized, but vaporized (3). A general association of high PCB contamination with industrial areas has been demonstrated. Risebrough et al. (27) reported one such case, expressing PCB contamination in terms of the DDT/PCB ratio (DDT was assumed to have no regional fallout pattern). In birds from San Francisco Bay and the Gulf of Panama the rate was between one and two. In the Gulf of Panama contamination was assumed to have come from the Canal Zone and Panama City. The ratio in birds from the Farallon Islands, which are 27 miles west of the Golden Gate Bridge, was between two and five. In the Gulf of California the ratio in most cases was nine or ten. In sea birds from the Pacific the ratio was usually between five and ten. The authors feel air transport best explains the presence of PCB's in remote areas, while high levels found in industrial areas presumably result from direct discharge of industrial wastes into surrounding water, and from local fallout. Zitko (34) found PCB's in fish from Canadian lakes which received no municipal sewage or industrial waste and he assumed atmospheric contamination. Veith (43), however, checked fish in Wisconsin lakes and

found no PCB's. He concluded that agricultural runoff and air transport are not important in PCB contamination. Other populated areas in the United States (3,43) and Sweden (14) have been shown to have regionally high PCB levels.

After checking organisms from the Rhine River and the Dutch coast, Koeman et al. (15) concluded that the river was the source of contamination of the coast. A check of the waste water leaving the purification plant in Henriksdal, near Stockholm, showed it to contain 1.350 ppb PCB's (42). Aluminum sulfate precipitation of the particulates present showed that these particles contained all the PCB's, while the water itself was free from residues. Sewage sludge from Britain (39) and urban sewage outfalls in California (40) have been shown to be important sources of PCB contamination. Municipal sewage and industrial wastes were also found to be major sources of pollution in the Milwaukee River (43). Thus introduction of PCB's by both large and small municipalities throughout a river basin may reflect the widespread use of PCB's.

The Monsanto Company, in a letter to Congressman William F. Ryan, said that it would no longer sell PCB's to customers for use in general plasticizer operations where disposal of the end products cannot be controlled (3). It remains to be seen if this will substantially reduce PCB's in the environment.

Biological Magnification

No extensive environmental work has been carried out on the accumulation of PCB's through food webs. Risebrough et al. (27) found that PCB's tend to be an order of magnitude higher in marine birds than in fish. Some species of raptors did not have high levels of PCB's and this seems to be related to their diets. For example, the white-tailed kite preys primarily upon the short-lived herbivorous vole, which accumulate very little organochlorine. Jensen et al. (14) studied the simplified food chains of fish to seal, fish to guillemot, fish to heron, and fish to white-tailed eagle. For all of these, the increase from prey to predator is at least an order of magnitude. For eagles and heron it went up to two orders of magnitude (100x). In a work on Escambia Bay in Florida no PCB's were found in the water; however, shrimp averaged 2 ppm and fish 9.7 ppm (32). Sediments often had high levels and it was suggested that leaching from the sediment continued after the source of contamination was eliminated. Fiddler crabs and pink shrimp were exposed to contaminated sediment from Escambia Bay (63). They take up the PCB's and, in most cases, residue levels were directly related to the amount of PCB's in the sediment. Waters from the Firth of Clyde showed no PCB contamination; zooplankton, however, contained less than 0.03 ppm, while clupeoid fish had up to 2.0 ppm (39).

Acute Toxicity

Several experiments have been made to determine the acute toxicity of PCB's. Rats given 50 mg. of Aroclor 1265 orally every other day had a 50% mortality (64). Two oral doses of 69 mg. of 42% chlorinated biphenyl given one week apart were fatal to guinea pigs (65). Rats fed diets containing Aroclor 1254 at a concentration of 10 and 1000 ppm did not die at the 10 ppm dosage, but 4/5ths of the second group died within 53 days. Single dosages of Aroclor 1254 ranging from 100-4000 ppm (mg. per kg. body weight) produced some mortality, which, however, was not proportional to dosage (66).

Aroclors 1242, 1248, 1254, and 1260 at 1 ppm in water killed over 50% of mosquito larvae within 58 hours (67). Duda (68) reported that Aroclor 5460 was toxic to the elm leaf beetle and suggested a possible synergistic effect with lindane. Lichtenstein et al. (50) found that PCB's were toxic to fruit flies and house flies, but to a lesser extent than dieldrin or DDT. Sublethal dosages of several PCB's increased the toxicity of dieldrin and DDT. Grasshoppers (Chorthippus brunneus) when given a single topical application of 200 micrograms of Aroclor 1254, suffered more than 40% mortality. The insects were found to be most sensitive at ecdysis (69). Wildish (70), working with the crustacean Gammarus oceanicus, found that Aroclor 1254 solubilized in Corexit 7664 was lethal at

0.01 mg/l (ppm) and 1254 emulsions were lethal at 0.1 mg/l. He found that moulting, or freshly moulted, animals were particularly vulnerable.

Juvenile pink shrimp exposed for 48 hours to water containing 1.0, 10.0, or 100.0 ppb Aroclor 1254 suffered no mortality at the first two concentrations but 100% mortality at the third. A 96-hour exposure to these concentrations decreased oyster shell growth 19%, 41%, and 100% respectively. The oysters exposed at 1.0 and 10.0 ppb were transferred to PCB-free water and after four days they contained 8.1 and 33.0 ppm of Aroclor, respectively (32).

Aroclor 1242 is very toxic to fertile chicken eggs. Injection of 25 mg. per egg produced complete failure of hatching in all of 20 eggs tested (71). Fifty percent of chicks fed a diet with 400 ppm Aroclor 1242 died in three weeks. Gross pathological changes including hydropericardium, hemorrhage of internal organs, enlarged and mottled liver, and enteritis were noted (72). In another experiment a similarity was noted between chick edema disease and symptoms produced by feeding chlorinated biphenyls (73). Japanese quail given food containing 2000 ppm Phenoclor DP6 died within 6 to 55 days. The birds developed hydropericardium (15).

Vox and Koeman (74) exposed chickens to three PCB preparations: Phenoclor DP6, Clophen A60 and Aroclor

1260. These preparations are indistinguishable using standard GLC-MS techniques as mentioned above, but showed a significant difference in toxicity. Feeding 400 ppm PCB for 60 days with either Phenoclor DP6 or Clophen A60 produced 100% mortality, whereas Aroclor 1260 killed only 3 out of 20. The first two mixtures caused microscopically centrilobular liver necrosis, while chemical porphyria and atrophy of the spleen was noted with all three PCB mixtures. Residues in the liver and brain were checked. While high, they were variable and there seemed to be no correlation between residue levels and duration of survival. An explanation has been offered for the different toxicities of the seemingly identical PCB's. The two European PCB preparations were shown to contain as contaminants, polychlorinated dibenzofurans, which are highly toxic (75). Hydropericardium was ascribed to the polychlorodibenzofurans and, because there is an occasional occurrence of hydropericardium in chicks fed Aroclor (72,73,74), there may be a very small quantity of these chemicals in Aroclors. The presence of toxic contaminants makes evaluation of PCB toxicity very difficult.

In chickens fed a diet containing 250 ppm Aroclor 1254, deaths did not occur until after 13 weeks. By this time combs and testes in treated birds were significantly smaller than controls (76). Chicks fed 50 ppm Aroclor 1248 showed symptoms of depressed weight gain, edema,

gasping for breath, hyperpericardial fluid, internal hemorrhaging, depression of secondary sexual characteristics, and an increase in the weight of the liver relative to body weight (77).

Water containing Aroclor 1254 solubilized by Corexit 7664 was lethal to Atlantic salmon at concentrations between 4 and 12 mg/l (ppm) in three-day experiments (5). Aroclor 1254 had no effect on juvenile pinfish put in water containing 1, 10, or 100 ppb for 48 hours. Whole body residue analysis revealed 0.98, 3.80 and 17.00 ppm, respectively (32).

Chronic Toxicity

During 20-day exposures of juvenile pink shrimp and juvenile blue crabs to 5.0 ppb Aroclor 1254 (in water) 72% of the shrimp died. There was no mortality in the control group. The first shrimp died on the 10th day. A composite sample of the dead shrimp contained 16 ppm Aroclor 1254. The survivors contained 33 ppm. Those shrimp that died did not exhibit typical symptoms of organochlorine insecticide poisoning before death (extreme irritability, followed by loss of equilibrium). Several of the shrimp died immediately after moulting. Only 1 of 20 crabs died under the same conditions (and so did one of the controls). Concentrations of PCB in the crabs were not appreciably decreased by exposure to clean water for one week (average

22 ppm) but were reduced after 4 weeks (average 11 ppm, range 3-14 ppm) (32).

The 2 estuarine fishes, pinfish and spot, died (41% and 66%) when exposed for 45 days to 5 ppb Aroclor 1254. Fish that died did not exhibit typical organochlorine poisoning symptoms. Most fish developed fungus-like lesions on the body. Mortality was not related to tissue residue levels, which increased with time. PCB's were most concentrated in the liver, followed in decreasing order by the gills, whole fish, heart, brain and muscle. Maximum concentration was 37,000 times that in the water. After 84 days in PCB-free water, the PCB residues in the survivors declined 61% (61).

The marine diatom, Cylindrotheca closterium, was exposed to 0.01 and 0.1 ppm of Aroclor 1242 for 14 days. The 0.1 ppm dosage sharply inhibited growth (both harvest weights and cell counts), reduced RNA synthesis and the chlorophyll index. Final tissue residues were 4.7 and 109.2 ppm at the 0.01 and 0.1 ppm level, respectively. The 0.01 ppm dosage did not adversely affect growth but may have caused a slight reduction on nucleic acid levels and chlorophyll production (78).

Because PCB's are structurally similar to DDT, many investigators have checked for similar activity in organisms. One of the effects of DDT is to cause egg shell thinning in birds. Aroclor 1262 induces liver

hydroxylating enzymes which degrade oestradiol. This hormone regulates calcium metabolism in birds. Aroclor 1262 was found to have an oestradiol-degrading potential 5 times that of DDE or technical DDT (27). PCB's fed to ring doves in a diet containing 10 ppm reduces their estrogen concentration (79). The same diet caused a decrease of active transport of calcium in the oviduct in vitro (80). It was also reported that PCB's increased the period before egg-laying in the ring dove (2). The American kestrel was given 5 ppm PCB in food for 14 days. This treatment increased cytoplasmic RNA in liver cells and produced a dose dependent increase in the in vitro breakdown of oestradiol by the liver. This breakdown was independent of sex and type of PCB (Aroclor 1254 or 1262) (81).

An 18-hour glycogen response of the immature rat uterus, in vitro, was used to test a series of Aroclors for estrogenic activity. Aroclors 1221, 1232, 1242, and 1248 were estrogenic at the 8 mg level, while 1254, 1260, 4465, 5442, and 5460 were inactive. Natural and synthetic estrogens are active in the microgram and submicrogram range (82).

No effects on carbonic anhydrase levels (which help provide carbonate for the egg shell) were found in the oviducts of ring doves fed a diet with 10 ppm Aroclor 1254 for six months. Upon checking the ashed egg shell

weights of the treated birds it was concluded that PCB's do not play a significant role in the phenomenon of egg shell thinning (83). However, chickens given a diet containing 10 or 100 ppm of Aroclor 1242, or 100 ppm of 1254, showed symptoms of anorexia, loss of body weight, poor hatchability of eggs and decreased thickness of egg shells (84).

Ducklings that were fed a diet containing Aroclor 1254 at the 25, 50 and 100 ppm levels for 10 days showed no clinical effects. Five days later the birds were inoculated with duck hepatitis virus. The treated birds had significantly higher mortality than controls (85).

Ulfstrand et al. (19) tested the effect of PCB's on the nocturnal activity in caged migratory robins. The birds which ingested 55 to 60 micrograms of Clophen A50 showed higher average activity than the controls, but showed no significant differences with respect to direction or dispersion. Because of the fact that fat is rapidly mobilized during migration, and the demonstration of a correlation between restlessness and migratory distance in different warbler species, a quantitative change in restlessness is potentially of great ecological significance.

Pregnant rabbits were fed diets of 1 and 10 ppm Aroclors 1221 and 1254 during the first 28 days of gestation and enzyme induction was caused by Aroclor 1221 (86).

Rats were fed a diet contaminated with 50 and 100 ppm PCB (21 to 68% chlorine by weight). PCB's stimulated

enzyme induction. The amount of stimulation increased regularly with percentage of chlorination in the PCB's. Those PCB's containing more than 50% chlorine were as potent as inducers as DDT (87).

Rats fed Aroclors 1254 or 1260 for 18 months had increased liver weights at 100 ppm, but not at 1 or 10 ppm. Aroclor 1242 produced no effects. In a rat reproduction study there were decreased survival of pupa at 100 ppm Aroclor 1242 or 1254, and decreased mating indices with Aroclor 1242. Dogs fed 100 ppm 1254 or 1260 did not gain weight as well as controls (84).

Dermal toxicity studies of PCB preparations in rabbits established porphyria as a symptom of PCB poisoning. Various dermal symptoms were subsequently associated with the polychlorodibenzofurans which contaminated the two European PCB preparations used. A less frequent occurrence of these dermal symptoms in rabbits treated with Aroclor 1260 may indicate that this Aroclor is contaminated too, which means that all PCB toxicity data must be viewed with suspicion until more experiments are done to see what role contaminants play in toxicity (88).

Aroclor 1254 caused a 50% inhibition in culture growth after 48 hours in Hela cells at 63 ppm and in human skin fibroblasts at 110 ppm, which is as toxic an effect as produced by DDT. Effects on the synthesis of

DNA, RNA, and protein were not significant (89).

Various Aroclors at concentrations as low as 0.03 ppm inhibit magnesium ATPase and sodium and potassium ATPase from tissues of bluegills. There seemed to be no correlation between percent-chlorination and inhibition (90).

The present experiment deals with two aspects of PCB contamination. In the first part, uptake of Aroclor 1254 by Daphnia sp. is measured. These crustacea may be an early stage in many aquatic food chains. Similar experiments were planned for Gambusia sp. but had to be abandoned because these fish (obtained from commercial sources) were found to be previously contaminated. In the second part, PCB residues were measured in crayfish, Pacifastecus spp., from several locations in the Willamette River, to determine residue levels in this area and as a preliminary to identification of sources and routes of contamination.

MATERIALS AND METHODS

Organisms

Daphnia were purchased from Carolina Biological Supply. They were raised at room temperature in wide-mouthed gallon jars filled with stream water and fed a few drops per day of egg yolk suspension (made by thoroughly mixing one hard-boiled egg yolk with 500 ml of distilled water in a Waring blender). In experiments on the uptake of PCB's, Daphnia were placed in one liter of stream water contained in a 6-quart all-glass aquarium. Aroclor 1254, dissolved in 1 to 4 ml of acetone, was added by pipette and the water was then stirred with the empty pipette.

Gambusia were purchased from Carolina Biological Supply. They were raised in a 10-gallon aquarium filled with dechlorinated tap water and fed commercial dried fish food.

Crayfish, Pacifastecus spp., were trapped at three sites on the Willamette River. Site #1 (downstream) was adjacent to navigational light #10 on the east side of Sauvie Island. Site #2 was the Portland Police Harbor Patrol dock in the city of Portland; site #3 (upstream) included the banks by the loading docks of the Canby Ferry. Site #2 was 9 linear miles (11 river miles)

upstream from site #1, and 15 linear miles (20 river miles) downstream from site #3. Trapped crayfish were immediately taken to the laboratory and frozen (-18C). The period between removal from the trap and freezing was no more than one hour. A sample of sandy sediment was collected at site #1 with an Ekman dredge.

Clean-Up and Extraction of Polychlorinated Biphenyls

Chemicals. Sand (Mallinckrodt, washed and ignited) was used for grinding. Sodium sulfate, used for dehydration, was anhydrous granular A.R. Solvents used were acetone, hexane, and Isopropyl alcohol (all Mallinckrodt nanograde). Aroclors 1242, 1254 and 1260 were kindly provided by Monsanto Chemical Company. Standard pesticides DDD, DDE and DDT were obtained from Analabs. Florisil (Floridin 60-100 mesh) was used for chromatography. Each jar of Florisil was first checked by using PCB and pesticide standards to be sure performance was satisfactory (93).

Daphnia. At the conclusion of each experiment the Daphnia were captured in a small fine-meshed net, rinsed with glass distilled water and dried overnight at room temperature. Clean-up and extraction were carried out the next day, using the method of Reinhart (91), except that, instead of 5 mg of Daphnia being used, samples ranged from 2 to 10 mg and the amounts of solvents were varied proportionately.

Gambusia. The fish were removed from the aquarium with a small net, rinsed with glass distilled water, dried with paper tissue and weighed. Extraction and clean-up procedures followed those of Ferguson et al. (92), except that hexane was substituted for pentane, and the column chromatography method was that of Reynolds (44).

Crayfish. Animals were taken from the freezer within two weeks of collection and allowed to thaw to a state where the digestive gland could be removed and weighed without loss of body fluids. The rest of the procedure was the same as for Gambusia.

Sediment. Sediment was dried at room temperature and weighed. The rest of the procedure was the same as for Gambusia with the exception of the initial step (grinding with sodium sulfate), which was omitted.

Evaporation Procedure. The above clean-up and extraction procedures all call for evaporation of hexane containing the PCB's. In all cases evaporations were carried out by immersing the flask with the residue-containing hexane solution in a water bath (35-40 C) and blowing compressed air over the solution. The air was cleaned and dried by bubbling it through concentrated sulfuric acid and then passing it through a column of Drierite. The solution was evaporated to 5 ml and transferred to a 15 ml conical centrifuge tube and

evaporated to dryness at room temperature with compressed air. The sides of the tube were washed with 1 ml of hexane, which was evaporated. At precisely the point of dryness a known volume of hexane was added.

Checks in Extraction and Clean-Up Procedures. Daphnia and smelt, checked to be sure they were uncontaminated, were spiked with PCB's and extraction efficiencies were determined. The above clean-up and extraction procedures were first tried without a known source of PCB's. Only when this procedure showed no interfering peaks were the clean-up and extraction procedures used on experimental material.

Cleaning of Equipment. Glassware was cleaned following the procedures of Bevenue et al. (94). Teflon was cleaned in the same manner except the heat treatment was replaced with soaking overnight in acetone. Rubber stoppers were washed in detergent solution, rinsed in tap and distilled water, and wrapped in aluminum foil for use. Before the above technique for washing glassware was adopted, glassware was washed as follows: Soak in saturated dicromate in sulfuric acid solution overnight; wash thoroughly with tap water; boil in detergent solution; rinse with tap water, dilute HCl, tap water, distilled water and acetone; then dry in oven at 60 C. Because the aquariums used in the Daphnia experiments could not withstand heating, the above procedure was used

in cleaning them, except the detergent solution was not boiling, nor were they oven-dried. Both cleaning methods for glassware were checked by rinsing cleaned flasks, etc. with 10 ml hexane, evaporating the hexane to 0.1 ml and injecting a portion of the hexane into the gas chromatograph. No interfering peaks were found.

Gas Chromatography. The gas chromatograph used was a Wilkens HY-FI, Model A-600-B, the electrometer of which was modified to increase its sensitivity. It was equipped with a tritium foil concentric tube electron capture detector. The recorder was a Varian G-42A dual channel 10-inch strip chart recorder. Columns were coiled 1/8" x 6' pyrex. The conditions were as follows: The column temperature was 200 C. Injector temperature was 250 C., and the detector temperature was 200-240 C. (The higher detector temperatures were obtained by placing one or two heating pads around the detector). The carrier gas was nitrogen at 20-30 ml/min. Electrometer settings used were input impedance 10^9 , output sensitivity X1, attenuation X16 or X32. Injection volumes ranged from 2-5 ml. The recorder was set at 1 millivolt span, 9 inches full scale, and the chart speed was 2/3" per minute. Column packings were 4% SE-30/6% QF-1 on Chromosorb W (acid-washed) 60/80 mesh (theoretical plates 1540), and 5% DEGS/2% H_3PO_4 on Chromosorb W (acid-washed) 60/80 mesh (theoretical plates 960). Columns

were prepared by Varian aerograph.

In the determination of PCB's in Daphnia only the first of the above two columns were used (no confirmation was felt necessary since control organisms were not found to be contaminated with PCB's or interfering substances). Residue samples from Gambusia and crayfish were put through both columns. Residues from Gambusia were quantified on the first of the above columns and their identity confirmed on the latter. This procedure was reversed for crayfish residues. This was an arbitrary decision, either sequence would be satisfactory for both organisms.

Quantitation of PCB's was accomplished by comparing the total of the peak heights of peaks 7, 8 and 10 of the experimental chromatogram and a chromatogram produced by an injection of a known amount of PCB's (see Appendix). The response of the EC detector varies from day to day. Because of this, the residue chromatogram and the standard chromatogram were run within 30 minutes of each other and with no more than one intervening chromatogram. All quantitation was carried out within the linear range of the detector, which was determined by injecting a series of standards. Lower limit of detection was 0.01 ng Aroclor 1254 and a linear response was found to about 17 ng.

Since the electron detector dose-response plot does not go precisely through the origin, and since its slope

may vary slightly from day to day, the quantity of standard PCB solution injected was adjusted to give a peak height within $\pm 20\%$ of the unknown sample.

RESULTS AND DISCUSSION

Extraction Efficiencies

Two extraction efficiency tests with Daphnia gave recoveries above 90%; three tests with smelt gave recoveries above 85%. Unspiked samples were run at the same time and were free of significant interfering peaks. As is usual in organochlorine-residue analysis, no correction for extraction efficiency was made.

Checks for Interfering Peaks

At first these tests showed that there was a source of serious contamination. This contamination was traced to Kontes type "M" o-ring used in the chromatographic column. On advice from Kontes their "Viton" o-ring was substituted and it worked satisfactorily. The company also suggested using a "Buna-N" o-ring, but this was not tested. With the "Viton" o-rings the clean-up and extraction procedures were repeated with no interfering peaks being produced.

Daphnia

Uptake of Aroclor 1254 by Daphnia is presented in Table I. There was no mortality in any of the experiments. The individual peaks were apparently taken up equally, since relative peak heights were similar in the standard

and the Daphnia tests. Those Daphnia grown at 0.1 ppb PCB took up 10% of the PCB's present in the aquarium. Since this was the largest percentage uptake, the present experiments are comparable to experiments using continuous-flow systems in which the PCB levels of the medium were constant.

The uptake of PCB's by invertebrates and diatoms has been reported previously (32,63,67,68). However, comparing PCB-uptake of Daphnia with uptake by other organisms must be done with caution. Rate of uptake and final residue levels may be influenced by such factors as surface to volume ratio, percentage of fat, permeability of exterior tissues and metabolic rate. In an earlier investigation (32), shrimp exposed for 48 hours to 100 ppb Aroclor 1254 contained 3.9 ppm (presumably on a wet weight basis). This is a much lower biological magnification than occurred in Daphnia in the present investigation (Table I) even assuming that the Daphnia lost 90% of their weight in drying out overnight. Shrimp mortality at the 100 ppb level after 24 hours was 80% and after 48 hours was 100%. Exposure at the 1 and 10 ppb level resulted in PCB residue levels of 0.14 and 1.30 ppm, respectively, with no mortality at the end of the 48-hour experiment (32). Diatoms raised for two weeks in 0.1 and 0.01 ppm Aroclor 1242 concentrated the Aroclor 1,093- and 470-fold, respectively (78). In this case the

biological concentration factor went up as the PCB concentration went up.

Uptake of some organochlorine pesticides by Daphnia has also been reported. Reinhert (91), using a continuous-flow system, exposed Daphnia to 2.1, 4.5, and 12.8 ppb dieldrin for six days. At the first two concentrations residues rose to 35 and 62 ppm (dry weight) during the first three days, then showed little change. At 12.8 ppb residues increased until the fourth day and leveled off at 180 ppm. These results indicate that at 4 ppb dieldrin is accumulated by Daphnia about three times as much as are PCB's. For the three concentrations he used, the concentration in the organism was proportional to that in the water. The accumulation factor for PCB's, however, was smaller at higher concentrations (Table I) so that, at higher concentrations, biological magnification could be much greater for dieldrin than for PCB's. Johnson et al. (95), using a continuous-flow system, found a biological concentration of 114,000 in Daphnia (based on dry weight) raised three days in 80.3 ± 13.7 ppt (ng/liter) DDT and 141,000 for those raised three days in 16.7 ± 0.37 ppt aldrin. No leveling-off of residue build-up was observed during the three-day experiments. These results indicate that DDT concentration is magnified about as much as PCB's were in the present experiment.

There are significant differences between the

TABLE I
UPTAKE OF AROCLOR 1254
BY DAPHNIA

Concentration of PCB's in test water	PCB residues (ppm) in <u>Daphnia</u> at various exposure times				Biological magnification times 1,000
	<u>4 hrs</u>	<u>24 hrs</u>	<u>48 hrs</u>	<u>72 hrs</u>	
0.1 ppb	10.4	-	-	10.3	104.
4 ppb	-	22	21	-	5.
50 ppb	-	68	-	-	1.4
100 ppb	-	-	118	-	1.2

results of the present experiment and those of Johnson et al. (95) and Reinhert (92) in the time taken to reach the concentrations reported. From Table I it appears that in Daphnia maximum concentrations of PCB's may be reached in as little as four hours. In the other two studies Daphnia pesticide residue levels continued to rise for at least three days.

Aroclor 1254 at 100 ppb is not acutely toxic to Daphnia, nor would levels this high generally be reached in the environment. Maximum residue levels in the Escambia River, which was badly contaminated, were 0.1 ppb (32). No studies have been done on possible chronic effects, however. If we assume chronic exposure of Daphnia to environmental levels of PCB's will not inhibit growth and reproduction, the high biological magnification figures

in Table I would suggest that Daphnia and related organisms may play an important part in the biological magnification of PCB's in fresh water food webs.

A study similar to the Daphnia experiments was planned on the uptake of PCB's by Gambusia. The Gambusia were to be divided into two groups, one to be raised in water containing PCB, the other to be raised in PCB-free water. Both of these groups were to be divided into two subgroups, one subgroup to be fed PCB-free Daphnia, the other to be fed Daphnia contaminated with PCB's. The purpose was to determine which would be more important in contaminating the Gambusia, the water or the Daphnia. However, this was found to be impractical because the fish were already contaminated with PCB's. The average concentration in the fish was 1 ppm, making it impossible to perform experiments at environmental residue levels. This background level of PCB's completely obscured any uptake of PCB's by fish exposed for 24 hours to 0.1 ppb Aroclor 1254 in water.

Crayfish

PCB residue levels found in digestive glands of crayfish taken from the Willamette River are shown in Table II. Digestive glands were chosen for analysis because of the probability that they would contain the highest residue levels. Localization of DDT in the body

TABLE II
PCB RESIDUES IN THE DIGESTIVE GLANDS
OF CRAYFISH FROM THE WILLAMETTE
RIVER

Site	Wet weight of the crayfish in grams	PCB's ppm, wet weight
1	23.7	1.0
1	18.0	1.0
1	40.3	1.5
1	49.3	1.9
2	45.1	7.4
2	21.1	8.6
2	26.1	7.2
2	33.6	7.1
2	37.8	9.2
2	18.8	8.0
3	87.6	0.5
3	46.6	0.5
3	36.1	0.5
3	31.6	0.2
3	20.9	0.1
3	28.2	0.1

organs of pink and white shrimp showed that the highest levels were in the hepatopancreas, which is homologous to the crayfish digestive gland (96). Separate analyses of crayfish tails and viscera show a selective accumulation of both aldrin and dieldrin in the viscera (97).

Chromatograms of crayfish residue were quantified by reference to Aroclor 1254 as a standard. However, in all cases the peaks of the lower-chlorinated biphenyls were missing or greatly reduced in the tissue. While the method used is a standard one considerable error may be

introduced because these lower-chlorinated peaks may make up a significant portion of Aroclor 1254. Even though the results are approximate they do permit evaluations of relative concentrations.

Sandy sediment at collection site #1 contained 0.15 ppm PCB. Unlike the PCB residues in the crayfish, the sandy sediment contained those PCB's less rich in chlorine. Indeed, chromatograms of the latter resemble the Aroclor 1254 standards.

Aroclor 1254 can be absorbed from sediments by fiddler crabs and pink shrimp. The individual peaks maintained their relative height in both the sediment and tissues of the test animals (63). As shown above, Daphnia have also been shown to take all peaks up equally well. If crayfish take up PCB's from sediments in the same manner as the other crustaceans, then the fact that their PCB residues contain proportionately less of the lower-chlorinated biphenyls could mean that crayfish:

1. have a mechanism for selectively metabolizing and/or excreting the lower-chlorinated biphenyls,
- or 2. sediment is not a major source of PCB's for these crayfish.

It could be that these crayfish obtained most of their PCB's by ingesting vertebrates. Many studies of PCB residues in vertebrates have mentioned that the lower-chlorinated peaks were reduced or missing (7,10,12,14,15, 35,42,52). In two of these studies an invertebrate was

included; one had data on mussels (14), and the other on roaches (15). In neither case were the lower-chlorinated biphenyls reduced or missing as they were in vertebrates analyzed. Being on different trophic levels then might explain the difference in the nature of the residues of the crayfish and those of the mussels and roaches.

The results of Table II indicate that there are regional differences in distribution of PCB's in the lower Willamette Valley. Crayfish from the center of Portland have the highest residues. Results were significant at the 0.05 level. There is no significant relationship (at the 0.05 level) between body weight and residue level. Sewage effluents have previously been shown to be a source of PCB contamination (39,40,41,42,43). Between Portland and Canby there are seven municipal sewage outfalls and two industrial outfalls. In Portland, north of the city of Milwaukie, most sewage is taken by interceptor sewer to the Columbia Boulevard treatment plant, whose outfall is located on the Columbia River, and thus would not normally contribute to Willamette contamination. However, individual sewers carry sewage and storm waters to interceptor sewers which run parallel to both sides of the Willamette River, and this system is designed to carry a maximum of three times the dry weather volume. When rain water swells the volume to over three times this volume, the excess is discharged directly into the Willamette River.

This discharge occurs at over fifty outfalls on the Willamette River. The results in Table II suggest that these regular and/or intermittent outfalls may be a source of pollution.

However, aerial fallout could also be a possible source of contamination. Rain water from seven widely distributed sites in the British Isles contained small amounts of PCB's (26). Air transport was suggested as the best explanation for the presence of PCB in remote areas (27). Zitko (34), finding PCB in eels taken from lakes receiving no industrial effluents or domestic sewage, suggests the possibility of pollution by aerial fallout. On the other hand, Veith (43), who found no PCB's in Wisconsin lakes, suggests aerial transport may not play an important role in PCB distribution.

Illegal discharges could account for a significant but unknown amount of contamination. Research at Escambia Bay has shown that a leak in a heat transfer system of a single factory can cause large-scale contamination in the downstream-eco system (32).

With the results presented in Table II it is impossible to delineate the sources of the PCB contamination found in the Willamette River. Residues are higher in the Portland area. This sort of distribution, relative to urban areas, has been shown previously (3,14,27,43). The fact that the industrial section of Portland starts about two miles down-

stream of collection site #2 is very interesting. This could mean that the high contamination at site #2 is produced by release of PCB's that had been put into products such as paints, auto tires, etc., rather than industrial sewerage.

Samples from more sites will be needed to substantiate these speculations. Residue levels should also be determined on a body of water close to the city which has no sewerage input. This would help to assess the contribution aerial fallout plays in contamination. Also, some outfalls should be tested directly.

It is difficult to estimate what PCB levels to expect in other organisms on the basis of the crayfish levels found in this experiment. In another study crayfish DDT residue levels were about one-half of those found in fish (97). However, these crayfish were much smaller (four to eight grams) than crayfish studied in the present experiment (Table II) and may represent contamination at a different trophic level. It has not been possible to determine whether the crayfish in this experiment were more often predators or scavengers. In regard to organisms preying upon the crayfish, several large fish have been shown to ingest crayfish (98). PCB residues are known to increase one or two orders of magnitude between prey and predator (14). However, it is not known whether crayfish of the size studied in the present

investigation form a substantial part of any predator's diet.

LITERATURE CITED

1. Penning, C. H., Ind. Eng. Chem., 22, 1180 (1930).
2. Peakall, D. B. and J. L. Lincer, BioScience, 20, 958 (1970).
3. Gustafson, C. G., Env. Sci. Tech., 4, 814 (1970).
4. Monsanto Technical Bulletin O-FF/1.
5. Zitko, V., Bull. Environ. Contam. Toxicol., 2, 279 (1970).
6. Risebrough, R. and V. Brodine, Environment, 12, 16 (1970).
7. Reynolds, L. M., Residue Rev., 34, 27 (1971).
8. Roburn, J., Analyst, 90, 467 (1965).
9. Anonymous, New Sci., 32, 612 (1966).
10. Widmark, G., J. Assoc. Offic. Anal. Chem., 50, 1069 (1967).
11. Holden, A. V. and K. Marsden, Nature, 216, 1274 (1967).
12. Holmes, D. C., J. H. Simmons and J. O'G. Tatton, Nature, 216, 227 (1967).
13. Fluczynski, V. D. and V. Werdland, J. Ornithol., 109, 462 (1968).
14. Jensen, S., A. G. Johnels, S. Olsson, and G. Otterlund, Nature, 224, 247 (1969).
15. Koeman, J. H., M. C. Ten Noever de Brauw and R. H. deVos, Nature, 221, 1126 (1969).
16. deVos, R. H. and E. W. Peet, Bull. Environ. Contam. Toxicol., 6, 164 (1971).
17. Presst, I. and D. J. Jefferies, Bird Study, 16, 168 (1969).
18. Presst, I., D. J. Jefferies and N. W. Moore, Environ. Pollut., 1, 3 (1970).

19. Ulfstrand, A., A. Sodergren and J. Rabol, *Nature*, 231, 467 (1971).
20. Westoo, G. and K. Noren, *Var Foda*, 9-10, 93 (1970).
21. Westoo, G. and K. Noren, *Var Foda*, 2-3, 9 (1970).
22. Acker, L. and E. Schulte, *Naturwissenschaften*, 10 (1970).
23. Holden, A. V., *Pesticides Monit. J.*, 4, 117 (1970).
24. Davies, B. N. K., *Ann. Appl. Biol.*, 61, 29 (1968).
25. Risebrough, R. W., R. J. Huggett, J. J. Griffin and E. D. Goldberg, *Science*, 159, 1233 (1968).
26. Tarrant, K. R. and J. O'G. Tatton, *Nature*, 219, 725 (1968).
27. Risebrough, R. W., P. Rieche, D. B. Peakall, S. G. Herman and M. N. Kirven, *Nature*, 220, 1098 (1968).
28. Anderson, D. W., J. J. Hickey, R. W. Risebrough, D. F. Hughes and R. E. Christensen, *Can. Field Natur.*, 83, 91 (1969).
29. Armour, J. A., and J. A. Burke, *Journal of the AOAC*, 53, 761 (1970).
30. Bagley, G. E., W. L. Reichel and E. Cromarite, *Journal of the AOAC*, 53, 251 (1970).
31. Mulhern, B. M., W. L. Reichel, L. N. Locke, T. G. Lamont, A. Belisle, E. Cromarite, G. E. Bagley and R. M. Prouty, *Pesticides Monit. J.*, 4, 141 (1970).
32. Duke, T. W., J. I. Lowe and A. J. Wilson, Jr., *Bull. Environ. Contam. Toxicol.*, 2, 317 (1970).
33. Biros, F. J., A. C. Walker and A. Manbery, *Bull. Environ. Contam. Toxicol.*, 2, 317 (1970).
34. Zitko, V., *Bull. Environ. Contam. Toxicol.*, 6, 464 (1971).
35. Rote, J. W., and P. G. Murphy, *Bull. Environ. Contam. Toxicol.*, 6, 377 (1971).
36. Duke, T. W. and A. J. Wilson, *Pesticides Monit. J.*, 5, (1971).

37. Cornwall, A. C., Private Communication (1971).
38. Henderson, C., A. Ingilis and W. L. Johnson, Pesticides Monit. J., 5, 1 (1971).
39. Holden, A. V., Nature, 228, 1220 (1970).
40. Schmidt, T. T., R. W. Risebrough and Franklin Gress, Bull. Environ. Contam. Toxicol., 6, 235 (1971).
41. Bonelli, E. J., Amer. Lab., 27 (Feb. 1971).
42. Ahling, B. and S. Jensen, Anal. Chem., 42, 1483 (1970).
43. Veith, G. D., Ph.D. Dissertation, Univ. Wisconsin, 193 pp. (1970).
44. Reynolds, L. M., Bull. Environ. Contam. Toxicol., 4, 128 (1969).
45. Richardson, A., J. Robinson, A. N. Crabtree and M. K. Baldwin, Pesticides Monit. J., 4, 169 (1971).
46. Holden, A. V. and K. Marsden, J. Chromatogr., 44, 481 (1969).
47. Snyder, D. and R. Reinert, Bull. Environ. Contam. Toxicol., 6, 385 (1971).
48. Fehringer, N. V. and J. E. Westfall, J. Chromatogr., 57, 397 (1971).
49. Mulhern, B. M., E. Cromarite, W. L. Reichel and A. A. Belisle, Journal of the AOAC, 54, 548 (1971).
50. Lichtenstein, E. P., K. R. Schultz, T. W. Fuhremann and T. T. Liang, J. Econ. Entomol., 62, 761 (1969).
51. Asai, R. I., F. A. Gunther, W. E. Westlake and Y. Iwota, J. Agr. Food Chem., 19, 396 (1971).
52. Risebrough, R. W., P. Reiche, and H. S. Olcott, Bull. Environ. Contam. Toxicol., 4, 192 (1969).
53. Zitko, V., O. Hutzinger and S. Safe, Bull. Environ. Contam. Toxicol., 6, 160 (1971).
54. Sissons, D. and D. Welte, J. Chromatogr., 60, 15 (1971).

55. Hutzinger, O., S. Safe and V. Zitko, Bull. Environ. Contam. Toxicol., 6, 209 (1971).
56. Fries, G. F., G. S. Marrow, Jr., and C. H. Gordon, Paper 130 presented at the Annual Meeting of the American Dairy Science Assoc. (1971).
57. Nishizumi, M., Arch. Environ. Health, 21, 620 (1970).
58. Bailey, S., P. J. Bunyan and F. B. Fishwick, Chem. Ind., 20, 705 (1970).
59. Mestres, R., S. Illes and C. Chevallier, Trav. Soc. Pharm. Montpellier, 30, 227 (1970).
60. Grant, D. L., W. E. J. Phillips and D. C. Villeneuve, Bull. Environ. Contam. Toxicol., 6, 102 (1971).
61. Hansen, D. J., P. R. Parrish, J. I. Lowe, A. J. Wilson, Jr. and P. D. Wilson, Bull. Environ. Contam. Toxicol., 6, 113 (1971).
62. Safe, S. and O. Hutzinger, Nature, 232, 641 (1971).
63. Nimmo, D. R., P. D. Wilson, R. R. Blackman and A. J. Wilson, Jr., Nature, 231, 51 (1971).
64. Bennett, G. A., C. K. Drinker and M. F. Warren, J. Ind. Hyg. Toxicol., 20, 97 (1938).
65. Miller, J. W., U. S. Pub. Health Rec., 59, 1085 (1944).
66. Tucker, R. K. and D. G. Crabtree, Handbook of Toxicity of Pesticides to Wildlife, U. S. Dept. of Interior, Bureau of Sport Fisheries and Wildlife Resources, Publication No. 84 (1970).
67. Doener, C. C., H. A. Jones and H. H. Incha, J. Econ. Entomol., 39, 459 (1946).
68. Duda, E. J., J. Econ. Entomol., 50, 218 (1957).
69. Moriarty, F., Ent. Exp. and Appl., 12, 206 (1969).
70. Wildish, D. J., Bull. Environ. Contam. Toxicol., 5, 202 (1970).
71. McLaughlin, J., Jr., G. P. Marliac, M. J. Verrett, M. K. Mutchler and O. G. Fritzlaugh, Toxicol. Appl. Pharmacol., 5, 760 (1963).

72. McCune, E. L., J. E. Savage and B. L. O'Dell, Poultry Sci., 41, 295 (1962).
73. Flick, D. F., R. G. O'Dell and V. A. Childs, Poultry Sci., 44, 1460 (1965).
74. Vos, J. G., and J. H. Koeman, Toxicol. Appl. Pharmacol., 17, 656 (1970).
75. Vos, J. G., J. H. Koeman, H. L. van der Maas, M. C. ten Noever de Braux and R. H. deVos, Food Cosmet. Toxicol., 8, 625 (1970).
76. Platonow, N. S. and H.S. Funnell, Vet. Rec., 109, (Jan. 23, 1971).
77. Rehfeld, B. M., R. L. Bradley, Jr. and M. L. Sunde, Poultry Sci., 50, 1090 (1971).
78. Keil, J. E., L. E. Priester and S. H. Sandifer, Bull. Environ. Contam. Toxicol., 6, 156 (1971).
79. Anon. C and EN, 31 (March 16, 1970).
80. Peakall, D. B. and J. L. Lincer, Amer. Zool., 10, 515 (1970).
81. Lincer, J. L. and D. B. Peakall, Nature, 228, 783 (1970).
82. Bitman, J. and H. C. Cecil, J. Agr. Food Chem., 18, 1108 (1970).
83. Peakall, D. B., Bull. Environ. Contam. Toxicol., 6, 100 (1971).
84. Keplinger, M. L., O. E. Fancher and J. C. Calandra, Toxicol. Appl. Pharmacol., 19, 402 (1971).
85. Friend, M. and D. O. Trainer, Science, 170, 1314 (1970).
86. Villeneuve, D. C., D. L. Grant, W. E. J. Phillips, M. L. Clark and D. J. Clegg, Bull. Environ. Contam. Toxicol., 6, 120 (1971).
87. Street, J. C., F. M. Urry, D. J. Wagstaff and A. D. Blau, Abstracts of Papers, 158th National Meeting, American Chemical Society, 1969.
88. Vos, J. G. and R. B. Beems, Toxicol. Appl. Pharmacol., 17, 617 (1971).

89. Herest, L., C. L. and E. P. Lichtenstein, Arch. Environ. Health, 22, 454 (1971).
90. Yap, H. H., D. Desai, L. K. Cutkomp and R. B. Koch, Nature, 233, 61 (1971).
91. Reinhert, R. E., Ph.D. Dissertation, Univ. of Michigan, 66 pp (1967).
92. Ferguson, D. E., J. L. Ludke and G. C. Murphy, Trans. Amer. Fish Soc., 95, 335 (1966).
93. Bevenue, A., and J. N. Ogata, J. Chromatogr., 50, 142 (1970).
94. Bevenue, A., T. W. Kelley and J. W. Hylin, J. Chromatogr., 54, 71 (1971).
95. Johnson, B. T., C. R. Saunders, H. O. Sanders and R. S. Campbell, J. Fish Res. Bd., Canada, 28, 705 (1971).
96. Nimmo, D. R., A. J. Wilson, Jr. and R. R. Blackman, Bull. Environ. Contam. Toxicol., 2, 333 (1970).
97. Bridges, W. R., B. J. Kallman and A. K. Andrews, Trans. Amer. Fish Soc., 92, 421 (1963).
98. Carlander, K.

APPENDIX

5 ng Aroclor 1254
gods with fish c
QF-1, SE-30

2.1 μ l -.5 ng/ μ l 1254
goes with crayfish a
5% DEGS, 2% H_3PO_4
60/80 w a.w. 6 x 1/8 pyrex

TE 240

TO about 215

col 4% SE-30 6% QF-1

60/40 chrom w AW

pyrex 6' x 1/8"

ATT B

R 1

2.9 μ l Daphnia
QF-1, SE-30

Shows bases from which
Peak h's measured