# P C B  N E W S L E T T E R

28 July 1971

MONS 206073

# ANALYTICAL METHODS

# AND

# CHEMICAL CHARACTERISTICS

MONS 206074

Judith Armour and Jerry Burke, Residue Chemistry Branch, Department of Health, Education, and Welfare, Food and Drug Administration, Washington, D. C.

Methodology for determination of PCB residues in foods is of primary concern; means of quantitation is of special interest.

I. PCB residues are currently determined as follows: PCB's are detected by electron capture GLC of 6% ethyl ether/petroleum ether Florisil eluate as described in the FDA Pesticide Analytical Manual, Vol. I. PCB's are separated from organochlorine pesticides by chromatography on silicic acid (JAOAC, 53, 761, (1970). In instances of low residue levels, instead of silicic acid chromatography, alkali dehydrochlorination of DDT and TDE may be used prior to measurement of PCB's. (GLC peaks of the olefins formed are excluded from the measured area of PCB's.) PCB residues are qualitatively verified beyond electron capture GLC, by GLC with halogen specific microcoulometric detection and stability of the residue to alkali reflux. Residues are quantitated using total area of all residue peaks versus total area of all peaks in appropriate Aroclor reference. The Aroclor with GLC pattern most similar to the residue is chosen as a reference.

II. Approximate limits of detection for PCB residues in foods using the FDA methodology are as follows:

| Food | Approx. Injected Wt. | Approx. Limit of Det.[a] |
|---|---|---|
| Fruits and Vegetables | 20 mg | 0.25 ppm |
| Fish (whole) | 25-50 mg | 0.2-0.1 ppm |
| Milk (fat) | 3 mg | 1.5 ppm |
| Milk (whole) | 75 mg | 0.06 ppm |

[a]Assumes Aroclor 1254 as residue; electron capture response of 10% full scale deflection for 5 nanograms Aroclor 1254 considered minimum detectable quantity.

III. Interlaboratory analyses were done to obtain information on the accuracy of determination of organochlorine pesticides and PCB's when present together. Rainbow trout (5.4% fat) were fortified (whole fish basis) with dieldrin (0.30 ppm), p,p'-DDT (3.0 ppm), p,p'-DDE (4.0 ppm), p,p'-TDE (1.0 ppm), and Aroclor 1254 (high group, 6.1 ppm; low group, 2.1 ppm). Residues of p,p'-DDT (0.06 ppm), p,p'-TDE (0.06 ppm), and p,p'-DDE (0.30 ppm) present in the trout were subtracted from all results for these compounds. No PCB residue was detectable in the unfortified trout. Identity and quantity of the fortification were unknown to analyzing laboratories. Methodology employed was as follows: extraction and clean-up, JAOAC, 53, 1300, (1970); separation of PCB's from DDT and analogs, JAOAC, 53, 761, (1970); determination, electron capture GLC, FDA Pesticide Analytical Manual, Vol. I; recovery of the added Aroclor 1254 was quantitated by total area versus Aroclor 1254 reference.



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Results for 12 laboratories are summarized below. Eight laboratories analyzed samples fortified with 6.1 Aroclor 1254; six laboratories analyzed samples foritified with 2.1 ppm Aroclor 1254. Pesticide levels were the same in all samples.

Results of Interlaboratory Analyses

| | | | | | Aroclor 1254 | | |
|---|---|---|---|---|---|---|---|
| | Dieldrin | p,p'-DDT | p,p'-DDE | p,p'-TDE | Total | High | Low |
| Range(%) | 70-106 | 73-105 | 64-103 | 64-107 | 63-95 | 63-95 | 66-77 |
| Average(%) | 89 | 89 | 85 | 89 | | 78 | 73 |

IV. A substance, presumed to be PCB, has been found in small quantities in some lots of Florisil. GLC retention times, response by halogen specific microcoulometric GLC detection, and stability to alkali indicated the substance to be PCB's. Quantities eluted from 25 gram columns of Florisil with 200 ml 6% ethyl ether/petroleum ether ranged up to about two micrograms for some lots of Florisil. Depending on GLC sensitivity and concentration of sample, this quantity presented a potential for false PCB findings. Elution of the Florisil column with 200 ml petroleum ether prior to use removed the contamination.

Irwin Pomerantz, Lee Miller, and Sidney Williams, Department of Health, Education, and Welfare, FDA, Washington, D. C.

The above investigators have begun a study of the photoalteration of Aroclor 1254 in solvents of differing polarity by irradiation with ultraviolet light (Hanovia 450 watt, medium pressure lamp) through various filters. Resulting solutions are being analyzed at present using GLC with electron capture and flame ionization detectors. Objective of this work is to look for presently unrecognized alteration products resulting from PCB's.

Ronald G. Webb, Research Chemist, U.S. EPA, Southeast Water Laboratory, Athens, Georgia

Chemical Identification of PCB Isomers

Our study of the chemical identities of PCB isomers is almost finished. Individual Aroclor isomers were collected by preparative GC and examined by capillary GC, IR spectroscopy, and, to a lesser extent, GC-MS. Known PCB isomers were prepared by the Gomberg or Ullman reactions and capillary GC and IR data obtained. Our best GC separations were made using 100' of SE-30 SCOT column programmed from 160 to 220° at 2°/ minute with a helium flow of about 7 ml/minute (10 psig). We used a standard 1/8" injector (PE-900) and maintained the injector and FID detector at 300°.

Aroclors 1221, 1232, 1242, and 1248 appear to have many isomers in common. Aroclor 1242 and 1248 are very similar and contain mostly dichloro through pentachloro- isomers. Aroclor 1254, in contrast, contains only tetrachloro- and larger isomers.

Ann C. McCall and I will present these results in more detail at the September ACS meeting in Washington. (Ron Webb, 404/546-3185)

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Identities of PCB Isomers

| Compound | Relative Retention[a] | 1221 | Aroclor 1242 | 1254 |
|---|---|---|---|---|
| Biphenyl | | X | | |
| 2 | 18 | X | | |
| 4 | 22 | X | | |
| 2,2' | 26 | X | X | |
| 2,4' | 32 | X | X | |
| 4,4' | 42 | X | X | |
| 2,5,2' | 42 | X | X | |
| 2,4,2' | 42 | X | X | |
| 2,3,2' | 46 | | X* | |
| 2,5,4' | 55 | | X | |
| 2,4,4' | 55 | | X | |
| 2,4,3' | 55 | | X | |
| 2,3',4' | 62 | | X* | |
| 2,5,2',5' | 70 | | X | X |
| 2,5,2',4' | 72 | | X* | X |
| 2,3,2',5' | 78 | | X* | X |
| 2,3,6,2',5' | 100 | | b | X* |
| 2,4,5,2',5' | 118 | | | X |
| 2,4,5,2',4' | 120 | | | X |
| | 138[c] | | | |
| | 147[c] | | | |
| 2,4,5,3',4' | 177 | | | X |
| 2,4,5,2',3',6' | 177 | | | X |
| 2,4,5,2',4',5' | 208 | | | X* |
| | 244[c] | | | |

*IR or capillary GC shows additional isomers also present.

[a]Relative to peak 100 which at first appeared to be common to all Aroclor mixtures from 1232 through 1262. Aldrin elutes at 74; p,p'-DDE at 138.

[b]This peak in 1242 and 1254 are different materials.

[c]Major peaks of unknown identity.

Ron Webb, Continued

Quantitative Analysis of PCB's

The approximate composition of four Aroclors are:

Weight Percent

| Chlorines | Aroclor 1242 | Aroclor 1248 | Aroclor 1254 | Aroclor 1260 |
|---|---|---|---|---|
| 1 | 3 | | | |
| 2 | 13 | 2 | | |
| 3 | 28 | 18 | | |
| 4 | 30 | 40 | 11 | |
| 5 | 22 | 36 | 49 | 12 |
| 6 | 4 | 4 | 34 | 38 |
| 7 | | | 6 | 41 |
| 8 | | | | 8 |
| 9 | | | | 1 |

Each Aroclor was chromatographed on a 1/8" x 9' column of 3% SE-30 in a Tracor MT-220 GC equipped with a Coulson electroconductivity detector. This detector has a linear response to chlorine. The number of chlorines in each peak or group of overlapping peaks was known from earlier GC-MS data. A known weight of each Aroclor was chromatographed, the areas of the peaks measured with a planimeter and the composition calculated. For details please contact Al Thruston, 404/546-3185.

Several people have expressed an interest in quantitating PCB's by referencing the Aroclor area to that of a known single isomer. I can provide 10 mg samples of 2,5,2',5'-pentachlorophenyl to those who want to try this approach. (Ron Webb, 404/546-3185)

Water Solubilities of PCB Isomers

The solubilities of six of the major constituents of Aroclor 1221 in water were measured at room temperature (24±1°C). A thin film of the pure component on the inside wall of a separatory funnel was contacted with distilled water for at least 15 hours, with occasional shaking. The mixture was filtered through a sintered glass filter of fine porosity and 500 ml of the filtrate was extracted with hexane. The concentrated hexane extracts were analyzed by gas chromatography. Biphenyl, 4-chlorobiphenyl, and 4,4'-dichlorobiphenyl were also determined by uv fluorescence both of the hexane extract and of the saturated aqueous solution. Solubilities were measured in blocks of four simultaneous runs with three different solutes. One of the solutes was initially equilibrated with both hot and cold water (at least 15° above or below the final temperature). Results are summarized in the following table.

| Solute | Mean Solubility (mg/l) | Relative Standard Error of Mean | Based on Determination |
|---|---|---|---|
| Biphenyl | 4.6 | 20% | 4 |
| 2-Chlorobiphenyl | 0.9 | 20% | 4 |
| 4-Chlorobiphenyl | 0.4 | 30% | 4 |
| 2,2'-Dichloro-biphenyl | 0.9 | 19% | 5 |
| 2,4'-Dichloro-biphenyl | 0.5 | 14% | 3 |
| 4,4'-Dichloro-biphenyl | 0.06 | 22% | 5 |

Paired comparisons showed no significant differences between GC and fluorescence analyses of hexane extracts or between solubilities determined by hexane extraction and by fluorescence of the aqueous solution. Solubilities were 20% higher when hot water, rather than cold water, was used initially. Increasing the contact time from 15 to 45 hours did not alter this result. For further details, contact T. B. Hoover, (404) 546-3524.

Stephen L. Warnick, Utah Community Pesticide Study, State of Utah, Department of Social Services, Division of Health, Salt Lake City, Utah

The Utah Community Pesticide Study Laboratory reports that the fourteen Community Pesticide Projects are concerned about PCB's--mostly as they interfere with pesticide analyses. These Projects are using a new "Manual of Analytical Methods" prepared by the Environmental Protection AGency's Primate Research Laboratories at Perrine, Florida. This manual gives two methods for determining PCB's: 1) the method of Armour and Burke, JAOAC, 53, No. 4, 1970; and 2) amodification of the method developed by Mulhorn et. al. presented at the 84th meeting of the AOAC, October, 1970, in Washington, D. C.

A. S. Y. Chau, Department of Fisheries and Forestry, Ottawa, Canada

At present we are planning to do routine analytical analyses for PCB's, although at a later date we intend to use MS for investigation.

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# ENVIRONMENTAL

# OCCURRENCE

# AND FATE

MONS 206080

Contribution to PCB Newsletter

G. Fred Lee and Gilman D. Veith, Water Chemistry Program, University of Wisconsin, and Douglas Dube, Wisconsin Department of Natural Resources, Madison, Wisconsin.

Current studies are concerned with the sources of PCB mixtures in municipalities of Wisconsin. Field studies in Green Bay supported by the University of Wisconsin Sea Grant Program have shown that the Fox, Oconto and Menominee Rivers are major sources of PCBs to the Bay. Efforts are being made to determine the relative significance of industrial and sanitary discharge in the observed PCB levels in the rivers.

PCBs in urban drainage is being investigated in the Madison area through the monitoring of storm waters. Sampling has begun in storm sewers draining a 123 acre residential area which is gauged for other studies in the Water Chemistry Program. Because of the large quantities of tars, oil and suspended solids in the urban runoff, estimates of PCBs in the water will be less reliable than similar analyses of rivers and waste discharges.

Douglas Dube is completing his MS thesis concerning PCBs in the discharges of sewage treatment plants in southeastern Wisconsin. The effluents of eleven communities which were selected have shown that PCBs are present in small communities which have no industry. Twenty-four hour studies of the sewage treatment plant effluents were conducted to examine the daily fluctuations in transport estimates. Mass spectral analyses at the EPA Southeast Water Laboratory (Athens, Georgia) have confirmed the lesser chlorinated isomers in a limited number of samples.

## PCBs in Environmental Samples

PCBs having GC-MS patterns like Aroclor 1254 were identified in extracts of water and sediments from Escambia Bay (Florida). This analysis was conducted for the Gulfbreeze Laboratory of the Bureau of Commercial Fisheries to verify their detection of Aroclor 1254 by electron-capture GC.

Nine extracts of aqueous samples from Lake Michigan or sewage effluents emptying into the lake were analyzed by GC-MS for the Wisconsin Department of Natural Resources. Although DDT, dieldrin and other pesticides had been tentatively identified by GC, only PCBs were present in enough quantity for MS identification. (Wayne Garrison, EPA, Southeast Water Laboratory, Athens, Georgia). (404/546-3187)

Robert C. Kroner, U.S. Environmental Protection Agency, Analytical Quality Control Laboratory, Cincinnati, Ohio.

The puzzle of how polychlorinated biphenyls find their way into surface waters has been partly solved by some investigative work done by the AQCL Organics Unit. Analyses of a number of fish taken from the Great Miami River showed PCB Concentrations of more than 150 ppm. Subsequent analysis of a number of industrial effluents to the Great Miami revealed that several paper mills are currently discharging significant quantities of PCB's into the Great Miami River. The PCB materials are apparently used in paper coating processes. As an interesting sidelight, one commonly used reproduction paper, the type used in business forms, was analyzed directly for PCB and found to be "loaded". In this case the PCB's are apparently used in a microencapsulation process.

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U.S. ENVIRONMENTAL PROTECTION AGENCY
Analytical Quality Control Laboratory
Cincinnati, Ohio

# PERSISTENCE OF AROCLORS IN SURFACE WATERS

JIM EICHELBERGER

A study of the behavior of seven polychlorinated biphenyl materials (Aroclor compounds) in river water during a 16 week period has been completed. The compounds observed were Aroclor 1232, 1242, 1248, 1254, 1260, 1262 and 1268. The compounds were studied individually due to the complexity of their gas chromatographic responses.

Twenty-four one liter aliquots of water were used for each compound, eighteen dosed and six blanks. Dosing was performed using a freshly prepared 0.1% solution of the compound in acetone and injecting 10 µl into the water with a 25 µl micro-syringe giving 10 µg of Aroclor compound per liter of water.

After each liter was dosed, the Teflon-lined cap of the jar was replaced and the sample shaken vigorously to evenly distribute the pesticide in the water. Approximately one hour after dosing, three samples plus one blank were extracted with 15% ethyl ether in n-hexane. This initial extraction was designated time zero. The same procedure was carried out one, two, four, eight and sixteen weeks later. During the time of the study period the samples were stored in the sealed glass jars on a laboratory bench exposed to indirect daylight. The jars were shaken periodically to redistribute any suspended matter which might have settled out.

Quantification of the Aroclor compounds was done using a Micro-Tek DSS Model 179 gas chromatograph equipped with a tritium electron capture detector. The column was 6' long x 1/4" O.D. coiled aluminum packed with 60/80 mesh Gas Chrom Q coated with 3% DC-200 plus 5% QF-1. The instrument temperatures were: injection port 230°C, column 185°C, and detector 200°C. Carrier gas was nitrogen at a flow rate of 80 ml/min.

The recoveries of the compounds throughout the 16 weeks are listed in the following table:

PERCENT RECOVERY OF AROCLOR COMPOUNDS

| Aroclor Compound | Zero Time | 1 Week | 2 Week | 4 Week | 8 Week | 16 Week |
|---|---|---|---|---|---|---|
| 1232 | 100 | 75 | 72 | 60 | 37 | Can't* Quantify |

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| Aroclor Compound | Zero Time | 1 Week | 2 Week | 4 Week | 8 Week | 16 Week |
|---|---|---|---|---|---|---|
| 1242 | 87 | 76 | 73 | 60 | 53 | 43 |
| 1248 | 80 | 60 | 50 | 78 | 54 | 38 |
| 1254 | 70 | 45 | 42 | 51 | 41 | 37 |
| 1260 | Can't Quantify | 39 | 40 | 42 | 42 | 51 |
| 1262 | 78 | 31 | 33 | 32 | 57 | 33 |
| 1268 | 75 | 18 | -- | 27 | 65 | 37 |
| $\bar{X}$ | 82 | 49 | 52 | 50 | 50 | 40 |

*Interferences in the gas chromatogram made quantification impossible

All the compounds were recovered with good efficiency at zero time. However, it appears that the higher the chlorine content, which is accompanied by higher boiling range and viscosity, the faster the compound is lost. Even though the oompounds are lost at different rates, approximately the same percent of each remains after the 16 week period, e.g. 40%.

All the Aroclors except 1232 are recovered intact; that is, the proportion of each compound to the rest of the compounds in the particular Aroclor material are unchanged after 16 weeks. The loss of compound, therefore, appears to be due to irreversible adsorption onto the walls of the container or onto the silt in the sample rather than to biological degradation.

It is very unlikely that biodegradation would effect each compound in the Aroclor to exactly the same degree.

## CONCLUSIONS

The Aroclors are recovered from the raw river water with 40% efficiency after sixteen weeks. The loss does not seem to be due to biodegradation or hydrolysis because ohe Aroclors are recovered intact. Irreversible adsorption of the Aroclors onto either the walls of the sample container or the silt contained in the sample apparently is the cause of loss.

If time permits, a study will be carried out using silt-free samples, samples with medium silt content, and samples with very high silt content.

# UPTAKE, RETENTION,

# AND OTHER

# PHYSIOLOGICAL EFFECTS

MONS 206085

## Uptake and Retention

Thomas W. Duke, EPA, Gulf Breeze Laboratory, Sabine Island, Gulf Breeze, Florida.

In conjunction with our toxicity experiments, (see Toxicity section), several studies have been completed on the uptake and retention of Aroclor in various tissues of organisms.

(1) Tetrahymena pyriformis W cells exposed to 10.0 ppb in the growth medium contained 167.9 ppb (dry weight) after 7 days.

(2) Oysters reared in sea water containing 5.0 ppb Aroclor for 24 weeks accumulated 425.0 ppm of the chemical and after 16 weeks in "clean" water, the oysters still contained 37.0 ppm.

(3) Residues in the hepatopancreas of pink shrimp, the organ of greatest concentration, reached 510.0 ppm after 22 days, a $2.0 \times 10^5$ increase over the 2.5 ppb Aroclor in the test water.

(4) Spot, exposed to 1.0 ppb Aroclor reached maximum whole body levels of 37 ppb or $3.7 \times 10^4$ times after 28 days. After 84 days in PCB-free water, the relative amount (ppm) in whole fish dropped 73%; the absolute amount (µg), decreased 61%.

H. H. Yap, D. Desaiah and L. K. Cutkomp, Department of Entomology, Fisheries and Wildlife, University of Minnesota, St. Paul, Minn. 55101, R.B. Koch, Honeywell Research Center, Hopkins, Minnesota 55343.

"The Sensitivity of Fish ATPases to Polychlorinated Biphenyls" Nature, 1971, In Press.

The finding that chlorinated hydrocarbon pesticides were inhibitory to ATPases, which may be related to their mechanism of toxicity, led to the investigation of the closely related Polychlorinated Biphenyls as possible inhibitors of ATPases. Studies with Aroclors 1221, 1254, 1268, and 5460 showed that these compounds also inhibit the ATPase enzymes. There was a difference in amount of inhibition caused by the different PCBs. There was also a difference in sensitivity of the ATPases from different tissues tested (brain, kidney, liver and muscle). Enzyme responses were determined in vitro on blue gill fish, Lepomis macrochirus, tissue homogenate fractions prepared by differential centrifugation. Aroclor 1254 was the most effective inhibitor tested, followed by Aroclor 1221. Muscle tissue $Mg^{2+}$ ATPase showed the greatest sensitivity to all of the PCBs tested. The latter response was similar to that observed by the authors for DDT.

D. Desaiah, L. K. Cutkomp, and H. H. Yap, Department of Entomology, Fisheries and Wildlife, University of Minnesota, St. Paul, Minn. 55101, R. B. Koch, Honeywell Research Center, Hopkins, Minn. 55343.

"Inhibition of Oligomycin Sensitive and Insensitive $Mg^{2+}$ ATPase Activity in Fish (Blue Gill) by Polychlorinated Biphenyls." Biochemical Pharmacology - Communicated.

In vitro tests with 4 PCBs (Polychlorinated Biphenyls) on 4 different tissue homogenates of fish, Lepomis macrochirus, show prominent inhibitory effects on oligomycin - insensitive $Mg^{2+}$ ATPase, with muscle homogenate being most sensitive. Aroclors 1242 and 1254, in the intermediate range of chlorination were more effective than 1221 and 1268. $Mg^{2+}$ ATPase from mitochondria was not as sensitive to the PCBs when compared with DDT - type chlorinated hydrocarbon compounds which were more potent inhibitors of mitochondrial $Mg^{2+}$ ATPase than on oligomycin - insensitive $Mg^{2+}$ ATPase.

Some stimulation of $Mg^{2+}$ ATPase was evident from the poorest inhibitors Aroclors 1221 and 1268. $Na^{+}$ - $K^{+}$ ATPase from fish brain was inhibited by Aroclor 1242 but the dose required was several times that for $Mg^{2+}$ ATPase.

H. H. Yap, D. Desaiah and L. K. Cutkomp, Department of Entomology, Fisheries and Wildlife, University of Minnesota, St. Paul, Minn. 55101. R. B. Koch, Honeywell Research Center, Hopkins, Minn. 55343.

"In vivo effects of Polychlorinated Biphenyls on ATPases of fathead minnow, Pimephales promelas." Unpublished data.

Fathead minnows were chronically exposed at the National Water Quality Laboratory, EPA, Duluth, Minn., to several PCBs. Enzyme tests were with minnows treated with Aroclor 1242 and Aroclor 1254 and exposed for about 4 months at concentrations of 0.31, 0.93, 2.8 and 8.3 ppb using continuous flow serial diluters. Four tissues, brain, kidney, liver and muscle were analysed for ATPase activities. Preliminary results indicate that Aroclor 1242 was a better inhibitor than Aroclor 1254 on fish brain and kidney ATPases. Aroclor 1242 selectively inhibited mitochondrial $Mg^{2+}$ ATPase from brain and kidney, the range of inhibition being from 28 - 54% and from 45-75% respectively. Oligomycin - insensitive $Mg^{2+}$ ATPase and $Na^{+}$ -$K^{+}$ ATPase activities were inhibited to a lesser extent. Aroclor 1254 inhibited only mitochondrial $Mg^{2+}$ ATPase of brain tissue at all concentrations tested. On kidney tissue, Aroclor 1254 inhibited only $Na^{+}$ - $K^{+}$ ATPase at all concentrations except the lowest, 0.31 ppb.

G. Fred Lee and Gilman D. Veith, Water Chemistry Program, University of Wisconsin, Madison, Wisconsin.

A review of PCB levels in fish from the Milwaukee River and Lake Michigan near Milwaukee and pertinent physiological data was presented by G. Fred Lee and G. D. Veith at the 14th Great Lakes Research Conference in April 1971. Analyses of a limited number of eggs from coho salmon indicated that the PCB concentration (13 µg/gm net weight as Aroclor 1254) are much higher than the concentrations which increased egg mortality in other studies.

David L. Stalling, Bureau of Sport Fisheries and Wildlife, Fish-Pesticide Research Laboratory, Columbia, Missouri.

PCB residues and composition were determined in bluegills and channel catfish exposed for 11 weeks to Aroclor® 1248 and 1254 at concentrations of 6-14 µg/l.

| Species | Aroclor | Exposure level (µg/l) | Residue (µg/g) | Concentration Factor[1/] |
|---|---|---|---|---|
| Bluegills | 1248 | 14.1[2/] | 703[3/] | 52,000 |
| Channel catfish | 1248 | 6.2 | 2.91 | 47,000 |
| Bluegills | 1254 | 7.3 | 192 | 26,300 |
| Channel catfish | 1254 | 6.7 | 376 | 49,500 |

[1/] Concentration factor = PCB residue (ng/g)/PCB water concentration (ng/ml).

[2/] Mean of four water analyses over 11-wk exposure period.

[3/] Mean of a sample of four fish after 11-wk exposure.

The composition of the PCB residues from these exposures was examined by gas chromatography. The composition of each of the PCB residues were similar in each species. The relative composition of the early eluting GLC peak of Aroclor® 1248 was lower by approximately 60 percent when compared to the composition of the standard material. However, the later eluting PCB peaks of the residues of Aroclor® 1248 were present at higher relative concentrations. Conversely, the relative composition of the early eluting peaks of the Aroclor® 1254 residues in both fish species was approximately 20 percent greater.

No new components of either Aroclor 1248 or 1254 were detected. After terminating PCB exposure, the elimination rate and composition changes of PCB residues in fish are being determined. Channel catfish began to die after 8-weeks exposure to 6.2 µg/l Aroclor® 1248.

Blake Grant, Bureau of Sport Fisheries and Wildlife, Fish-Pesticide Research Laboratory, Columbia, Missouri.

The following data are summaries of experiments to determine the physiological effects of Aroclors on fish.

I. Aroclor® 1232, 1248, 1254, and 1260: channel catfish, immature, loading $\lesssim$ 3 gm/l, turnover $\sim$ 7 hr.

Dose: 0, 1.2, and 12 ppm in food, each Aroclor dosed separately (0, 60, and 600 µg/kg body weight per day).

Parameters studied: mortality, growth, and 72-hr radioiodine fixation after 187 days of exposure and 80 days after stopping exposure.

Summary: None of the Aroclors caused mortality, nor was growth affected. Only 1254 and 1248 caused a detectable stimulation of thyroid activity. The percent of iodine uptake for the controls was 5.33 $\pm$ 0.82 ($S_{\bar{x}}$), low dose of 1254 = 8.9 $\pm$ 1.7, high dose of 1254 = 12.2 $\pm$ 1.9; low dose of 1248 = 5.9 $\pm$ 0.9, high dose of 1248 = 7.3 $\pm$ 1.2. Thyroid activity was stimulated 63% (low 1248), 150% (high 1248), 71% (low 1254), and 181% (high 1254) over the controls after 80 days of Aroclor-free diet.

II. Aroclor® 1248: Lake trout, immature, Mar-Aug 9° C, loading $\sim$ 1 g/l, turnover $\sim$ 1 hr.

Dose: 0, 1.2, 3.8, and 12 ppm in food (0, 3.6, 11.4, and 36 µg/kg body weight per day).

Parameters studied: 72-hr radioiodine uptake, growth, mortality, and serum cortisol levels after 170 days.

Summary: No mortality observed; negative correlation between growth and Aroclor dose during first three months; cumulative growth was 100% in controls, 93% in low dose, 90% in intermediate dose, 72% in high dose. Growth during second three months was 100% in controls, 94% in low, 98% in intermediate, and 63% in high. Thyroid uptake was 3.59 $\pm$ 0.44 ($S_{\bar{x}}$) and 3.57 $\pm$ 0.37% in controls, and 4.02 $\pm$ 0.69, 4.9 $\pm$ 1.1, 6.7 $\pm$ 1.1% in low, intermediate, and high doses, respectively. Serum cortisol was inhibited 33-46% below controls in the dosed groups at autopsy.

III. Aroclor® 1248 and 1254: Channel catfish, immature, Dec-Jan, 18.5° C, loading $\sim$ 0.04 gm/l, turnover $\sim$ 9 hr.

Dose (diluter): 1248 at 0, 6.7, 11.8 µg/l
1254 at 0, 3.5, 5.3 µg/l
} 77 days

Parameter measured: $^{125}$I-uptake (% of injected dose) at 72 hr.

Summary: Percent uptakes for control, and for the low and high exposures to 1248 were 3.8 ± 0.6 ($S_{\bar{x}}$), 5.7 ± 1.1, and 12.0 ± 2.5, respectively. Uptakes for control, low, and high exposures to 1254 were 4.4 ± 0.6 ($S_{\bar{x}}$), 6.3 ± 1.6, and 9.0 ± 2.2, respectively. Both Aroclors stimulated thyroid activity; 1248 was about half as effective as 1254.

IV. Aroclor® 1254: Coho salmon, immature, Sept-May, 16-17° C.

Dose: Diet, 0, 1.45, 14.5, 145, 1450, and 14500 µg/kg body weight per day (0.048 - 480 ppm in food).

Parameter studied: $^{125}$I-uptake (% of injected dose) at 72 hr.

Summary: Percent uptakes for control, 1.45, 14.5, 1450, and 14500 µg/kg were 3.1 ± 0.2 ($S_{\bar{x}}$), 3.2 ± 0.4, 4.7 ± 0.5, 4.2 ± 0.3, 5.8 ± 0.6, and 6.8 ± 1.3, respectively. Mortality occurred only the last few days of exposure, and then, only in the highest-dosed group. Growth was similar in all groups.

Louise Merkens and William Kinter, Departments of Pharmacology and Physiology. Upstate Medical Center, SUNY, Syracuse, New York 13210.

Work will begin this summer on the effect of some of the Aroclor mixtures on the intestinal water absorption and $Na^+ - K^+$ ATPase activity in marine fish. Aroclors 1221, 1248, 1254, and 1268 will be used. If results of the Aroclor 1221 appear interesting some of the isolated compounds will also be tested. (The studies will be done at Mount Desert Island Marine Biological Laboratory, Salibury Cove Maine 04672).

TOXICITY

Jack T. Garrett, Ind. Hygiene, Monsanto Company, St. Louis, Missouri. Toxicologic Studies with Polychlorinated Biphenyls. M. L. Keplinger, Otis E. Fancher, and J. C. Calandra, Industrial Bio-Test Laboratories, Inc., Northbrook, Illinois.

Abstract

Polychlorinated biphenyl compounds (PCB) have been reported to be in the environment, and the chronic toxicologic properties have not been reported. PCB compounds with 42, 54, or 60% chlorine (1242, 1254, and 1260) are being fed to rats and dogs, and rat and chicken reproduction studies are being conducted at 1, 10 and 100 ppm. After 18 months in rats (interim sacrifice and histology at 3, 6, and 12 mo), compound 1242 produced no effects. Rats fed compounds 1254 or 1260 had increased liver weights at 100 ppm but not at 1 or 10 ppm. No other effects have been observed. Dogs fed 1242 have shown no adverse effects, while those fed 100 ppm 1254 or 1260 have not gained weight as well as controls. In the rat reproduction study there was decreased survival of pups at 100 ppm of 1242 or 1254, and decreased mating indices with 1242. No adverse effects at any of the 3 levels of 1260 or at 1 or 10 ppm of the other two compounds have been observed. There was anorexia, loss of body weight, decreased thickness of egg shells, and poor hatchability of eggs from chickens fed 10 or 100 ppm of 1242, or 100 ppm of 1254. At 1, 10 and 100 ppm of 1260 and at other levels of 1242 and 1254 there were no effects.

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Feeding experiments were conducted by the Bureau of Sport Fisheries and Wildlife, and University of California at Davis, California to determine if an interaction occurs between p,p' -DDE and a PCB (Aroclor 1254) regarding eggshell thinning in mallards. Using these compounds, no additive effect could be observed. Diets of 40 ppm (dry weight) DDE and PCB and combinations of them disclosed no additive effect from Aroclor 1254. Shell thinning in the DDE group averaged around -15% for both DDE and DDE and PCB groups. Additional data are being obtained regarding egg production, laying dates, residue buildup, and resultant chromatographic pattern. Egg-eating was observed in the mallards which laid thin-shelled eggs and broke them. Normal eggs were not broken.

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Aroclor$^{R}$ 1254 has been found in the Pensacola estuary. Consequently, we are investigating in the laboratory the acute and chronic effects of this chemical on estuarine and marine biota as well as monitoring residues in the local estuary. Continuing studies by most of the laboratory staff include toxicity tests, absorption and loss rates, residue magnification, and pathological examination of exposed organisms.

We examined the acute toxicity (48-96 hr) of Aroclor 1254 to five species. Growth rates of Tetrahymena pyriformis W in axenic cultures were reduced 2.9% by 0.1 ppb (µg/l) to 11.4% by 100.0 ppb within 96 hours. Population density, another measure of effect, was reduced 13.0% by 0.1 ppb; 18.8% by 100.0 ppb. All juvenile pink shrimp, Penaeus duorarum, died after 48 hours exposure to 100 ppb of Aroclor 1254 in the water and oysters, Crassostrea virginica, failed to grow when exposed to this amount of PCB for 96 hours. Recent experiments showed that Aroclor 1254 is not acutely toxic to juvenile pinfish, Lagodon rhomboides, and adult mosquitofish, Gambusia affinis. In 48 hours, pinfish survived concentrations to 100.0 ppb; mosquitofish, to 10,000 ppb.

Most of our effort has been expended on the chronic toxicity of this PCB. For example, the ciliate, T. pyriformis W, continuously exposed to 1.0 ppb and 10.0 ppb Aroclor 1254 for 8 weeks showed evidence of developing resistance to the chemical because growth rates and population sizes of experimental cultures, depressed in the first week of exposure, were greater in the eighth week, exceeding those of control cultures. Growth of young oysters, Crassostrea virginica, reared in flowing sea water with 5.0 ppb of the chemical was retarded. After 24 weeks, control oysters outweighed the PCB-exposed group by 8.0 grams and shell growth of controls was greater by 12.0 mm. In contrast, oysters exposed to 1.0 ppb Aroclor 1254 did not appear to be affected after 24 weeks. In flowing-water chronic bioassays, 0.94 ppb Aroclor 1254 killed 51% of juvenile pink shrimp (2.5 to 3.8 cm long) within 15 days. Adults were less sensitive than juveniles: exposure to 3.5 ppb for 35 days killed 50% of a group of adult shrimp (9.5 to 12.5 cm long). Pinfish, and spot, Leiostomous xanthurus, died when exposed for 14-45 days to 5.0 ppb, but spot appeared unaffected by exposure to 1 ppb for periods up to 56 days. Onset of death in both species exposed to 5.0 ppb was delayed; significant mortality typically did not occur until the second week of exposure. Some fish that survived exposure to 5.0 ppb of Aroclor 1254 died later when placed in flowing water free of this PCB.

Symptoms exhibited by fish and shrimp prior to death are not those typical of poisoning by the chemically-related organochlorine pesticides. Pinfish exposed to the PCB usually develop fungus-like lesions on the body, more pronounced around the mouth. Hemorrhaging also occurs around the mouth. Spot, usually cease feeding and become emaciated, with lesions on the body and ragged fins. Pink shrimp, that have been exposed to acute levels of DDT, within a few hours show nervous impairments such as tremors, loss of equilibrium, and finally paralysis. Regardless of the Aroclor concentration in the water, shrimp show delayed mortality and die at a rate of one to two per day with no apparent symptoms of poisoning.

Tissues from oysters, shrimp and fish chronically exposed to Aroclor 1254 are being examined by consulting pathologists. Results are incomplete at this time.

Plans for further studies include more pathological examinations of oysters, shrimp and fish, and experiments to determine effects of low concentrations of Aroclor 1254 on oxygen consumption of certain estuarine fishes. Studies of the metabolism of the chemical by organisms and the effects of the breakdown products in various tissues in addition to toxicity tests with other Aroclors are being planned.

## Publications

A polychlorinated biphenyl (Aroclor 1254 R) in the water, sediment, and biota of Escambia Bay, Florida. T. W. Duke, J. I. Lowe, and A. J. Wilson, Jr. Bull. Environ. Contam. Toxicol. 5: 171-180. (1970)

Chronic toxicity, uptake, and retention of Aroclor R 1254 in two estuarine fishes. D. J. Hansen, P. R. Parrish, J. I. Lowe, A. J. Wilson, Jr., and P. D. Wilson, Bull. Environ. Contam. Toxicol. 6: 113-119. (1971)

Polychlorinated biphenyl absorbed from sediments by fiddler crabs and pink shrimp. D. R. Nimmo, P. D. Wilson, R. R. Blackman and A. J. Wilson, Jr. Nature (London) 231: 50-52. (1971)

Alan V. Nebeker and Frank A. Puglisi, EPA, National Water Quality Laboratory, Duluth, Minnesota.

Continuous-flow testing to determine the effects of Aroclor 1248 on the survival and reproduction of Daphnia magna indicate that levels above 5 µg/liter are not safe for the species (19°C). Aroclor 1248 is the most toxic of the Aroclor series. Further testing with D. magna is under way to determine which fractions (compounds or isomer) of 1248 are most toxic.

Continuous flow testing of 1248 with the scud Gammarus pseudolimnaeus is completed: the safe level for survival and reproduction is near 5 µg/liter also. Good survival and reproduction occurred at 3.3 µg/liter but distinct reduction in numbers and biomass of young occurred at 10 µg/liter (19°C).

Testing of the fathead minnow with 1242 and 1254 are continuing. Continuous-flow tests with 1242 and 1254 to determine the effects of PCB's on survival, reproduction, egg hatchability, and survival and growth of young have been underway since Jan., 1971. Fish less than 24 hours old were used for testing; they have matured and are now spawning. All have died above 8.3 µg/liter in both tests (25°C.).

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Reproduction and egg hatching has occurred at and below 8.3 µg/liter 1242; and at and below 2.8 µg/liter 1254. These tests will be completed by Sept. 1, 1971.

Good survival and adult emergence of the midge Tanytarsus dissimilis occurred at 10 µg/liter 1248, with total inhibition of larval growth and survival at 30 µg/liter (19°C). Good survival and emergence also was evident at 10 µg/liter 1254 with complete inhibition of growth and survival at 30 µg/liter.

S. Bailey, Ministry of Agriculture, Fisheries and Food, Pest Infestation Control Laboratory, Hook Rise South, Tolworth Surbiton, Surrey ENGLAND.

The third meeting of the UK. PCB Discussion Group was held on April 7, 1971. The hosts for this meeting were the Unilever Research Laboratories at their Laboratory at Welwyn, Hertfordshire.

The meeting opened with the paper "Structural Identification of Polychlorinated Biphenyls in Commercial Mixtures by GLC, NMR and Mass Spectrometry" jointly presented by Mr. G. M. Telling, Mr. D. Sissons and Mr. D. Welti of Unilever Research Laboratories, Colworth House, Sharnbrook, Bedfordshire.

SUMMARY

The major constituent polychlorinated biphenyls in Aroclor 1254 have been characterised by high resolution Nuclear Magnetic Resonance and Mass Spectrometry following separation by liquid-solid and gas-liquid chromatography. The retention indices of these compounds together with those of forty synthesised PCBs have been used to predict the structures of the remaining Aroclor 1254 constituents, together with all those of Aroclor 1242 and 1260.

The paper has now been accepted for publication in the Journal of Chromatography.

Mr. J. H. Koeman, Instituut voor Veterinaire Farmacologie en Toxicologie Der Rijksuniversiteit Te Utrecht, then read a short paper on the "Environmental Levels of PCBs in the Netherlands." He spoke of the difficulties of quantifying PCB residues but by using one peak Rf 1.45 relative to Dieldrin PCB levels had been determined. Levels in Plankton were around 0.25 ppm and in Muscles up to 1.7 ppm. Determination of residues along the coast shows a gradient reaching a maximum at the Rhine. The position has not varied during the period 1966-1970. Sandwich terns which have been shot showed a residue of up to 11 ppm total body weight while Eider ducks found dying contained levels up to 100 ppm. Of freshwater samples monitored, using the roach, the Rhine and Waal show the highest level (4ppm).

Mr. Koeman then spoke of the increased toxicity of continental mixtures of PCBs. Separation on alumina revealed a tetra - and a pentachlorodibenzofuran and chloronaphthalenes as minor constituents of the mixtures. Cormorants found dead contained 100-500 ppm of PCB but a careful investigation failed to reveal furans although naphthalenes were present.

Dr. J. E. Portman, Ministry of Agriculture, Fisheries and Food, Fisheries Laboratory Burnham-on-Crouch, Essex then described the results of a three year surveillance for organochlorine pesticide and PCB residues in fish around the British coast.

The fish analyzed were basically inshore fish and samples consisted of bulked liver and muscle tissues from 10 specimens of plaice, cod, whiting, mackerel and herring.

Results show increasing levels as one moves from north to south along the east coast of Britain decreases along the south coast and increases to a peak in the Irish sea - Clyde area, decreasing again around the north of Scotland. Highest levels of PCBs in cod liver (43 ppm) and plaice liver (48 ppm) in Clyde estuary. Samples also contained DDT (4.8 and 0.6 ppm respectively). Muscle levels were 10-100 times lower. Along the east coast levels in cod liver were of the order of 5 ppm and plaice 0.5 ppm. The high levels were probably attributable to the dumping of sewage sludge in the area concerned. The PCB patterns obtained did not normally correspond to any commercial formulation, although the 54% chlorine mixtures were most closely copied. The quantification of residues was by summation of 3 major peak heights. The work was carried out in conjunction with the Department of Agriculture and Fisheries for Scotland.

The meeting continued with a talk by Mr. J. G. Vos of Veterinaire Pathologisch Instituut der Rijksuniversiteit Utrecht outlining the work which had resulted in four papers being published, or about to be published. [Toxicol. Appl. Pharmacol 17, 656-668, 1970, Fd. Cosmet. Toxicol. 8, 625-633, 1970., and two papers in paess in Toxicol. Appl. Pharmacol. 1971.]

This work is concerned with the difference in toxicity between Phenoclor DP6, Clophen A60 and Aroclor 1260 and resulted in the identification and toxicological studies of chlorinated dibenzofurans.

The meeting concluded with two papers from the Ministry of Agriculture, Fisheries and Food's Pest Infestation Control Laboratory, Hook Rise South, Tolworth Surbiton, Surrey on PCBs in Birds. The first paper on "Some Studies on Residues" was read in the absence of Mr. S. Bailey by Dr. P. J. Bunyan and was followed by Dr. Bunyan's own paper "Some Biochemical Aspects."

The work referred to, consisted of feeding pigeons with wheat dressed with Aroclor 1242 at 500 ppm for 28 days and then sacrificing at 0, 1, 2 and 6 months after return to normal diet. The residue work consisted of measuring the levels of PCB in liver, muscle, brain and fat and also of a demonstration of the change in pattern of residue with time. The composition of the residues bore no resemblance to the starting material.

Measurement of the hepatic cytochrome $P_{450}$ in these birds was also reported and related to total residue levels. $P_{450}$ was still more than twice its normal level at the end of the experiment when residues were of the order of 1 ppm. A more thorough study of drug-metabolising enzymes was reported in quail fed Aroclor 1242 and Aroclor 1254 at 250 ppm for 20 days and sacrificed immediately. Residue levels were also measured in these birds and it was noted that whilst there were extensive changes in the composition of the residue with 1242, changes were not so marked in 1254. Induction of hepatic microsomal enzymes was noted in both cases but found to be considerably higher in those fed 1254.

The work reported is now being submitted for publication.

It is proposed to hold the next meeting of the group in late September at Chelsea College, University of London. Particulars may be obtained from Mr. S. Bailey at the address above.