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PCB NEWSLETTER

ANALYTICAL METHODS AND CHEMICAL CHARACTERISTICS

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

A paper, by Judith Armour and Jerry Burke of this laboratory, was presented at the 1970 meeting of the AOAC and will be published in the January, 1971 issue of the JAOAC.

Behavior of Chlorinated Naphthalenes in Analytical Methods for Organochlorine Pesticides and Polychlorinated Biphenyls

Abstract

Chlorinated naphthalenes, industrial chemicals with properties similar to polychlorinated biphenyls (PCB), are recovered by the Food and Drug Administration's methods for multiple pesticide residues. These materials could interfere with the gas chromatographic determination of several organochlorine pesticides. A silicic acid column chromatographic procedure previously developed to separate PCB from pesticide residues, particularly DDT and its analogs (Armour, J. and Burke, J., JAOAC 53, #4, 761-768 (1970), has been found to also separate chlorinated naphthalenes from the common organochlorine pesticides.

Bernard M. Mulhern, Eugene Cromartie, William L. Reichel, and Andre A. Belisle, Bureau of Sport Fisheries and Wildlife, Patuxent Wildlife Research Center, Laurel, Maryland 20810

Semi-quantitative Determination of Polychlorinated Biphenyls in Tissue Samples by Thin Layer Chromatography (Accepted for publication in JAOAC, Spring, 1971)

Abstract

A method is described for the analysis of polychlorinated biphenyl (PCB) compounds in tissue samples. Cleanup by hexane-acetonitrile partitioning and Florisil column chromatography are performed on samples prior to oxidative treatment to convert DDE to DCBP. PCB's are then determined semi-quantitatively by TLC. No prior separation of PCB's from chlorinated pesticides is required. The lower limit of sensitivity is 0.2 micrograms.

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R. G. Webb, FWQA, Southeast Water Laboratory, Athens, Georgia

Solubilities of PCB's

The solubilities of the identified components of Aroclor 1221 in water at room temperature (24± 1°C) were determined. Excess of the pure component was equilibrated with one liter of 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 could also be determined by uv fluorescence. The latter technique was applicable to the saturated aqueous solution as well as to the hexane extract and provided a check on the extraction efficiency. At least four determinations were made for each compound, using a statistical design. Each block of four simultaneous runs included a pair made with the same solute but using hot and cold water for the initial equilibration. Analysis of the data is not complete but the final values are expected to be within 50% of the following values:

Biphenyl	3.0 mg/l
2-chloro-	1.6 mg/l
4-chloro-	1.0 mg/l
2,2'-Dichloro-	1.0 mg/l
2,4'-Dichloro-	0.5 mg/l
4,4'-Dichloro-	0.05 mg/l

Composition of Arochlor 1221 and 1254

The composition of Aroclor 1221 was estimated by gas chromatography on SE-30. The detector sensitivity to the individual components was checked with standard solutions of the pure compounds so far as these have been identified. The Coulson conductivity detector gave the most consistent response but did not detect biphenyl. The flame ionization detector picked up biphenyl but gave variable response to the chlorinated compounds. In the following table, the percentage of biphenyl was determined from the ratio of biphenyl to 2-chlorobiphenyl and the remaining weight percentages were derived from the Coulson results:

Biphenyl	18%
2-Chloro-	31%
4-Chloro-	19%
2,2'-Dichloro-	7%
2,4'-Dichloro-	20 %
4,4'-Dichloro-	5%

An unidentified dichlorobiphenyl elutes between 2,2'- and 2,4'- which comprises perhaps 5% but a standard is not available for calibration of the detector. The peak ascribed to 4,4'- also includes a contribution from some trichlorobiphenyl material.

Gas chromatography of Aroclor 1254 on 6' x 1/4" of 6% SE-30 using a Coulson detector gives nine measurable peaks. From the area of each

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peak and the number of chlorines in each peak (determined independently by mass spectrometry), the weight percentage of each material can be calculated:

Peak	#Cl	Wt.%
1	4	9
2	5	12
3	5	18
4	5	23
5	6	14
6	6	12
7	6	9
8	6	2
9	6	1

Detector Response for Components of Aroclor 1221

The Coulson conductivity detector appears to respond linearly with the amount of chlorine in each PCB. Fluorescence results are highly variable with some components giving no response at all. Our data also indicates that the relative response of the flame ionization detector (based on biphenyl = 100) and electron capture detectors (2,4'- response = 100) varies considerably from compound to compound:

Compound	FID	ECD
Biphenyl	100	0
2-ClBP	70	20
4-ClBP	60	7
2,2'-DCB	36	19
2,4'-DCB	24	100
4,4'-DCB	13	17

For additional information on solubilities, percentage composition, or detector response, please contact Dr. Tom Hoover, 404-546-3185.

Chemical Identification of Individual PCB Isomers

In the last newsletter we reported the identity of several components of Aroclor 1221. We also assigned these materials a relative retention index based on their retention times on 6' x 1/4" of 8% SE-30 at 200°. For example, the index for 4,4'-dichlorobiphenyl is 0.41 under these conditions. We postulated that the peak at 0.32 was 2,4'-. This has been confirmed. We have also shown by comparison of the IR spectra of knowns and the Aroclor fraction that in 1242 the peak at 0.41 contains at least three components: 4,4'-; 2,5,2'-; and 2,4,2'-. The peak at 0.46 contains 2,3,2'- and probably another component as well. There is reasonably good GC and IR data to suggest that 0.54 contains 2,5,4'- and 2,4,4'-. Capillary GC-MS shows that the peak at 0.58 contains at least three separate materials, two tetra-chloro isomers and a trichloro isomer. This latter material has been positively identified as 3,4,2'-. The GC retention times also indicate that 2,3'-; 3,4'-; 2,4,2',4'-; and 2,4,5,2',5'- may be present in peaks 0.32, 0.41, 0.69, and 1.19 but no IR data is available to confirm this yet.

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R. G. Webb (Continued)

Capillary GC of the Aroclors mixed with synthetic PCB's shows that 2,3,4'-; 3,4,4'-; and 2,4,6,2',4',6'- are not in Aroclor 1248.

Work is in progress to identify some of the tetra and pentachloro-isomers.

A few of these materials have been purified and are available in one milligram or larger quantities. If lack of pure PCB isomers is the major obstacle in anyone's program, please contact Dr. Ron Webb at 404-546-3185.

ENVIRONMENTAL OCCURRENCE AND FATE

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

Gilman D. Veith completed his Ph.D. thesis on "The Environmental Chemistry of the Chlorobiphenyls in the Milwaukee River" in August, 1970. Copies of Dr. Veith's thesis may be obtained for short term loan by writing to G. Fred Lee. Microfilm copies may be obtained from the Ann Arbor Microfilming Service. This thesis provides data on the amounts of PCB compounds found in the Milwaukee River water, plankton, and fish. Also, limited data is provided on concentration of PCB in municipal and industrial waste water effluents.

Current studies on PCB include work of Dr. Veith on the major sources of PCB's in the Green Bay region of Lake Michigan and compositional changes of the commercially prepared mixtures of the PCB's in the environment. In particular, the chemical and biological degradation of the PCB's will be evaluated in an effort to predict the fate of the compounds in the environment. Additional studies have been initiated in the development of chemical confirmation methods for the PCB's.

INTERACTIONS AND UPTAKE STUDIES

Dr. Daniel W. Anderson, Bureau of Sport Fisheries and Wildlife, Davis, California

I am planning some PCB research with Bob Risebrough this spring and will be involved in brown pelican research. We are interested in PCB-DDE interactions, re: eggshell thinning, etc.

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

We have been successful in preparing radioactive ^{36}Cl-Aroclor 1243 and 1254 by neutron irradiation. Using these materials to evaluate the cleanup procedure reported by Armour and Burke (JAOAC, 53, No. 4, pp. 751-768, 1970) less than 2 percent of the PCB's were present in the pesticide fraction. Fish PCB uptake studies are currently underway using the radioactive materials. Whole body residues of 2-4 µg/g resulted from 3-day exposures of channel catfish to 4-10 µg/l of each PCB. These data are from a six week PCB exposure of bluegill and channel catfish to determine residue changes upon uptake and to examine the possibility of metabolism by fish. Data from PCB feeding studies is not yet available.

TOXICITY

R. G. Heath, J. W. Spann, J. F. Kreitzer, and C. Vance, Bureau of Sport Fisheries and Wildlife, Patuxent Wildlife Research Center, Laurel, Maryland

"Effects of Polychlorinated Biphenyls on Birds" Presented at the 15th International Ornithological Congress, The Hague, Netherlands, Sept. 1970.

Abstract

Toxicities of six technical polychlorinated biphenyl (PCB) compounds (Monsanto Company's Aroclors 1232, 1242, 1248, 1254, 1260, and 1262) were studied on penned mallards (*Anas platyrhynchos*), pheasants (*Phasianus colchicus*), bobwhite (*Colinus virginianus*), and Japanese quail (*Coturnix coturnix*) at the Patuxent Wildlife Research Center, Laurel, Maryland. LC_{50}'s expressed as ppm of PCB in dry feed, were determined for 2-week-old birds fed treated diets for 5 days. Aroclor toxicity, generally less than that of DDT, was found to be positively correlated with chlorine percentage (last two digits of Aroclor number). The joint toxicity of Aroclor 1254 and DDE on Japanese quail was additive, not synergistic. Low dietary levels (25 and 50 ppm) of Aroclor 1254 produced no measurable reproductive effects on mallards and bobwhite.

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

We have made progress in the PCB toxicity testing and are including three tables which summarize our findings for both acute and chronic bioassays with fish and invertebrates. (See following 4 pages.)

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Bureau of Sport Fisheries and Wildlife
Fish-Pesticide Research Laboratory
Route 1, Columbia, Missouri 65201

November 12, 1970

Aroclor® and DDT toxicity to invertebrates

Compound	Organism[a]	Bioassay type[b]	Exposure (days)	TL_{50} (µg/l)
Aroclor® 1242	Crayfish	static	7	30
Aroclor® 1254	Crayfish	static	7	100
Aroclor® 1254	Crayfish	continuous-flow	7	80
DDT	Crayfish	static	4	100
Aroclor® 1242	Scud	continuous-flow	4	10
Aroclor® 1242	Scud	continuous-flow	10	5.0
Aroclor® 1248	Scud	static	4	52
Aroclor® 1254	Scud	static	4	2,400
DDT	Scud	static	4	3.2
DDT	Scud	continuous-flow	5	0.6
Aroclor® 1254	Glass-shrimp	continuous-flow	7	3.0
DDT	Glass-shrimp	static	5	1.0
DDT	Glass-shrimp	continuous-flow	5	1.3
Aroclor® 1242	Dragonfly	static	7	800
Aroclor® 1254	Dragonfly	static	7	1,000
Aroclor® 1242	Damselfly	continuous-flow	4	400
Aroclor® 1254	Damselfly	continuous-flow	4	200
DDT	Damselfly	static	4	56

[a] Crayfish (Orconectes nais), Scud (Gammarus fasciatus), Glass-shrimp (Palaemonetes kadiakensis), Dragonfly (Macromia sp.), Damselfly (Ischnura verticalis).

[b] Temperature, 15.6°C; alkalinity, 35 ppm; pH, 7.1

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Acute toxicity[a] of several Aroclors® to selected fishes.

Aroclor®	Species	Temperature (C)	96hr TL_{50} (µg/l)
1221	Cutthroat	8.9[a,b]	1,170
1232	Trout	8.9	2,500
1242		8.9	5,400
1248		8.9	5,750
1254		8.9	42,500
1260		8.9	60,900
1262		8.9	50,000
1268		8.9	50,000
4465		8.9	50,000
5442		8.9	50,000
5460		8.9	50,000
1248	Channel catfish	18.3[a,c]	6,000
1254		18.3	12,000
1248	Bluegills	18.3[b,c]	278
1254		18.3	2,740

a. Static Bioassay.
b. Alkalinity, 159 ppm; pH, 7.6
c. Alkalinity, 35 ppm; pH, 7.1

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Chronic toxicity of Aroclors® to three fishes[a].

Aroclor®	Species	TL_{50} (ug/l) 5 day	10 day	15 day	20 day	25 day	30 day
1242[1]	Rainbow trout	109	39	-	-	-	-
1248[1]		51	-	-	-	-	-
1254		156	8	-	-	-	-
1260		-	240	94	21	-	-
1242	Bluegills	154	72	54	-	-	-
1248		307	160	76	10	-	-
1254		-	443	204	135	54	-
1260		-	-	-	245	212	151
1242	Channel catfish	-	174	107	-	-	-
1248		-	255	127	-	-	-
1254		-	-	741	300	113	-
1260		-	-	-	296	166	137
1248[b]	Bluegills	137	76	-	-	-	-
1248[b]	Channel catfish	-	94	57	-	-	-
DDT	Rainbow trout	2.26	0.87	0.26	-	-	-

[a] Temperature, 20°C; alkalinity, 260; pH, 7.4 — Constant-flow bioassay

[b] Temperature, 27°C

[1] Preliminary bioassays

Estimated time for DDT and Aroclor® 1242, 1248, 1254, and 1260 to kill 50 percent of selected fishes.

Chemical	Concentration (µg/l)	Estimated time for 50% mortality (days) Rainbow trout 20°C	Bluegills 20°C	Bluegills 27°C	Channel catfish 20°C	Channel catfish 27°C
Aroclor® 1242	50	7.2[1]	11.7	-	15.8	-
Aroclor® 1248	50	5.2[1]	18.0	12.9	22.7	16.8
Aroclor® 1254	50	8.3	27.5	-	27.6	-
Aroclor® 1260	50	16.8	36.4	-	59.3	-
DDT	2	5.0	-	-	-	-

[1] Preliminary bioassays (Constant Flow)

Chronic effects of Aroclor® 1248 on Lake Trout (work unit 3)

The exposure of immature lake trout to Aroclor® 1248 will continue for three more months. Growth continues to be better in the control lots than in the Aroclor®-treated lots. During the first 7 months, lake trout receiving control food grew 1.6 times more than those receiving relatively high amounts of PCB in their diets. Mortality was also higher in the PCB-treated lots. The iodine (^{125}I) uptake by lake trout, after 6 months of exposure, suggests that Aroclor® stimulated their metabolic rates. Serum cortisol concentrations in all treatment groups were depressed 33 to 46%. These results imply that dietary doses of 1.2 to 12 mg/kg of Aroclor® 1248 in natural or synthetic diets may well influence osmoregulation in fish, and their ability to tolerate stress.

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Alan V. Nebeker, FWQA, National Water Quality Laboratory, Duluth, Minnesota

Testing the effects of PCB's on survival and reproduction of Daphnia magna is essentially completed. Eight Aroclors®, 1221-1268, were used in static testing. The Daphnia were tested from newly-born young (± 12 hours old) through to reproduction and growth of their young (3 weeks) with new solutions changed once a week. Safe levels for survival and reproduction were determined: 1254 was the most toxic with a safe level near 20 ppb. The toxicity decreased for 1260 with a safe level near 35 ppb. Aroclor 1262 had a safe level near 40 ppb, and 1268 was safe at 250 ppb. Toxicity also decreased below 1254. Aroclor 1248 was safe near 30 ppb with 1242 being safe at 50 ppb. Aroclor 1232 was safe at approximately 75 ppb. Aroclor 1221 was the least toxic with a safe level near 300 ppb.

Continuous-flow testing with Aroclors 1242 and 1254 with Daphnia magna indicate that 30 ppb 1242 and 15 ppb 1254 are safe levels. Gammarus pseudolimnaeus have survived and reproduced at 1242 levels of up to ca. 15 ppb. The fathead minnow survived 100 ppb for one month but was obviously stressed. (The concentrations given above are approximations since we have not yet developed analytical methods to measure these levels.)

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