DP Barcode: D167531
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To: Walter Waldrop
Product Manager PM #71
Special Review and Reregistration Division (H7508W)

From: Emil Regelman, Supervisory Chemist
Environmental Chemistry Review Section #2
Environmental Fate & Ground Water Branch/EFED (H7507C)

Thru: Henry Jacoby, Chief
Environmental Fate & Ground Water Branch/EFED (H7507C)

Attached, please find the EFGWB review of...

Reg./File # : 056502

Common Name : Pentachloronitrobenzene

Product Name : Terraclor

Company Name : Amvac Chemical Corporation

Purpose : Metabolite identification for Accumulation in Fish study (412000-01).

Type Product: fungicide Action Code: 627 EFGWB #(s): 91-0851 Review Time: 4 days

EFGWB Guideline/MRID/Status Summary Table: The review in this package contains...

161-1		162-4		164-4		166-1	
161-2		163-1		164-5		166-2	
161-3		163-2		165-1		166-3	
161-4		163-3		165-2		167-1	
162-1		164-1		165-3		167-2	
162-2		164-2		165-4	419517-01 N	201-1	
162-3		164-3		165-5		202-1	

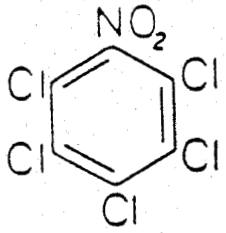
Y= **Acceptable** (Study satisfied the Guideline)/Concur
P= **Partial** (Study partially satisfied the Guideline; additional information is still needed)
S= **Supplemental** (Study provided useful information, but Guideline was not satisfied)
N= **Unacceptable** (Study was rejected)/Non-Concur

1. CHEMICAL:

Common name: PCNB

Chemical name: Pentachloronitrobenzene

Structure:



Physical/Chemical properties:

Molecular formula:	$C_6Cl_5NO_2$
Molecular weight:	295.3
Physical state:	colorless needles
Density at 25°C:	1.718
Solubility at 25°C:	0.44 ppm in water, soluble in benzene and chloroform.
Vapor pressure at 25°C:	1.13×10^{-4} mmHg
Melting point:	141-145°C

2. TEST MATERIAL:

Uniformly ring-labeled ^{14}C-pentachloronitrobenzene, radiochemical purity 98.6%, specific activity 46.4 μCi/mmole.

3. STUDY/ACTION TYPE:

Addendum to an Accumulation in Fish study (412000-01) reviewed by EFGWB on December 6, 1989. This addendum contains residue identification data and was submitted to upgrade the originally reviewed study.

4. STUDY IDENTIFICATION:

Halls, Timothy D. "Characterization of ^{14}C-PCNB Residues in Water and Bluegill (*Lepomis macrochirus*) Tissues (supplement to MRID #412000-01)." Performed by ABC Laboratories, Inc. and submitted by AMVAC Chemical Corporation. Study completed on April 8, 1991 and received by EPA on April 25, 1991. MRID #: 419517-01.

5. REVIEWED BY:

Dana Spatz
Chemist, ECRS #2
EFGWB/EFED/OPP

Date: MAY 26 1992

6. APPROVED BY:

Emil Regelman
Supervisory Chemist, ECRS #2
EFGWB/EFED/OPP

Date: MAY 26 1992

7. CONCLUSIONS:

This study presents residue identification data to support a previously reviewed Accumulation in Fish study (EFGWB review 12/6/89, MRID 412000-01) that was found to be scientifically valid, but did not satisfy the data requirement because "radioactive residues in the fish tissues and water were not characterized." Water and tissue samples from that study were stored frozen and later analyzed. The results of those analyses are presented in the current submission.

This study, taken together with study 412000-01, cannot be used to fulfill the Accumulation in Fish data requirement because the results indicate that the test organisms were not exposed to a constant concentration of the active ingredient. PCNB was found to comprise an average of only about 22% of the ^{14}C-residues in the test water sampled on days 21, 28 and 35 of the exposure portion of the experiment. The registrant proposes that the low concentration of PCNB was the result of rapid uptake and conversion by fish. EFGWB suggests that the low PCNB concentrations and high degradate levels may also have been due to diluter contamination. Or, inappropriate storage conditions could have led to the poor results. Storage stability data were not presented.

Because of the above deficiencies, a scientifically reliable bioconcentration factor for PCNB cannot be calculated.

8. RECOMMENDATIONS:

A new study is required. Valid data on PCNB's bioconcentration potential and the nature of the accumulated residues is extremely important given the information presented thus far which points towards a tendency for bluegill sunfish to bioconcentrate PCNB with relatively slow depuration. This study must be designed using a flow-through system that will accurately maintain a constant concentration of ^{14}C-PCNB in the test tank while keeping degradate levels to a minimum. Identification of the ^{14}C-residues in the test tank water must be made at each sampling interval. If tissue and water samples will be held prior to analysis, storage stability data will be required.

AMVAC CHEMICAL CORP. SATISFIED

Hydrolysis 161-1 (40865301)
Photodegradation on Soil 161-3 (41004801, 41713201)
Aerobic Soil Metabolism 162-1 (41384501, 41713202)
Unaged Leaching-Adsorption/Desorption 163-1 (41648201)
Laboratory Volatility 163-2 (41178001)

NOT SATISFIED

Photodegradation in Water 161-2
Anaerobic Soil Metabolism 162-2
Aged Leaching-Adsorption/Desorption 163-1
Field Volatility 163-3
Soil Field Dissipation 164-1
Confined Accumulation in Rotational Crops 165-1
Accumulation in Fish 165-4

RESERVED

Long-Term Soil Field Dissipation 164-5
Field Accumulation in Rotational Crops 165-2

9. BACKGROUND:

PCNB (pentachloronitrobenzene) is a fungicide registered for use on a variety of terrestrial food crop (field and vegetable) and terrestrial nonfood (ornamentals and turf) use sites. PCNB is generally used as a seed treatment, a spray using ground equipment, or a granular soil application.

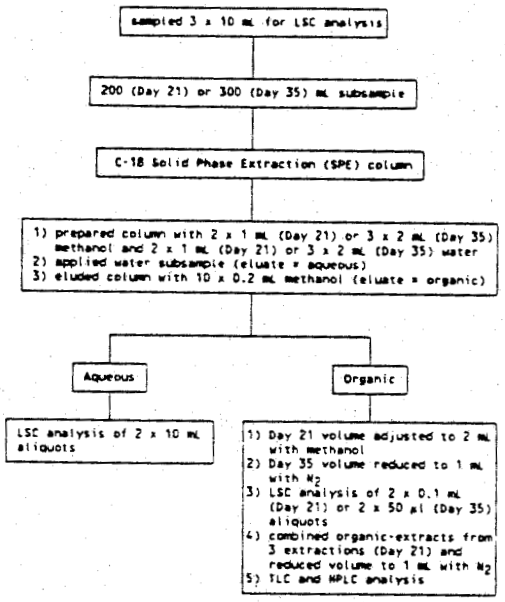
10. DISCUSSION OF INDIVIDUAL TESTS/STUDIES:

MATERIALS AND METHODS

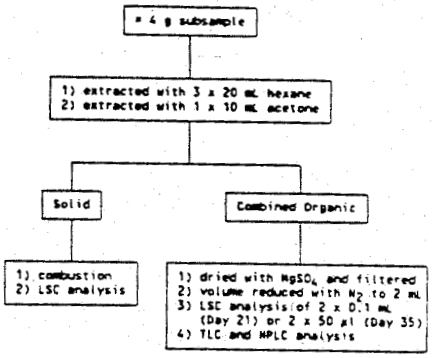
Samples of fish and water were taken at regular intervals from the time the fish were introduced to the treated system through Day 35 of the uptake phase of the test, and through Day 21 of the depuration phase. One liter water samples were taken on Days 21 and 35 (the "characterization" days). Fish samples were also taken for characterization purposes. All samples were stored at ≈ -20°C. *(see EFGWB review dated 12/6/89 of study 412000-01 for more information).*

For water, the samples were extracted using solid phase extraction techniques and the extracts were analyzed by LSC, TLC and HPLC. The fish tissues were extracted with a solvent scheme involving hexane, water and acetone; then, appropriate aliquots from the solvent extracts were analyzed by LSC, TLC and HPLC. The chemical nature of the radioactivity recovered from the water and tissues was qualitatively and quantitatively determined using a combination of HPLC and LSC.

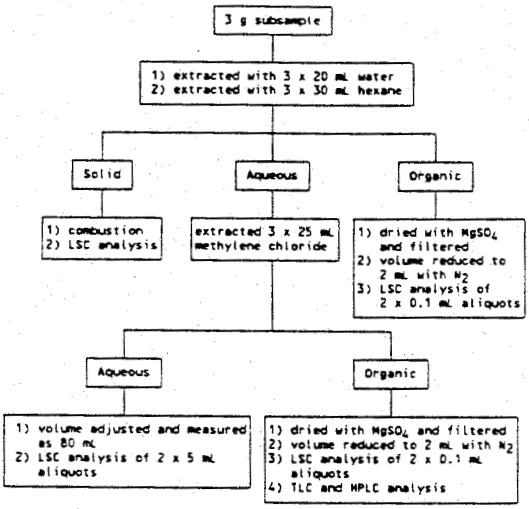
Flow Chart for the Isolation, Purification and Analysis of ^{14}C-Residues from Bioconcentration Water



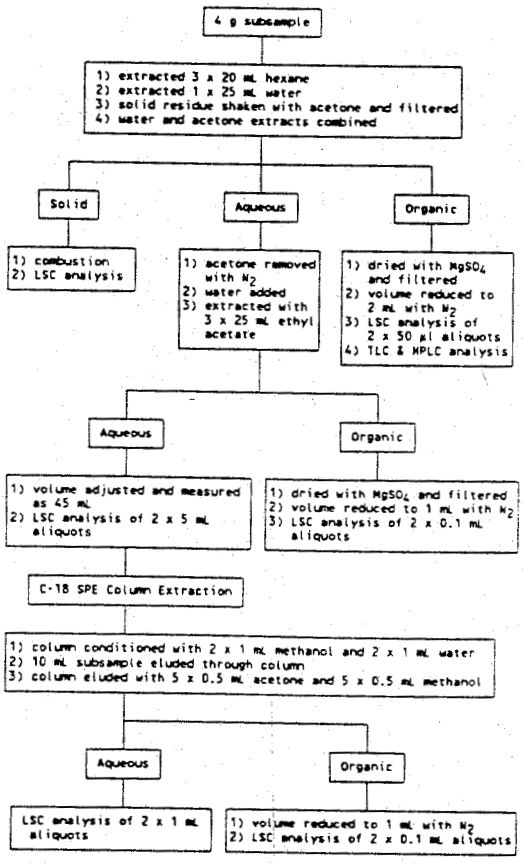
Flow Chart for the Isolation, Purification and Analysis of ^{14}C-Residues from Bioconcentration Fish Fillet



Flow Chart for the Isolation, Purification and Analysis of ^{14}C-Residues from Bioconcentration Day 21 Fish Viscera



Flow Chart for the Isolation, Purification and Analysis of ^{14}C-Residues from Bioconcentration Day 35 Fish Viscera



REPORTED RESULTS

Results from "In-Life" portion of experiment (412000-01) taken from EFGWB review dated 12/6/89.

PCNB residues (uncharacterized) accumulated in bluegill sunfish exposed to ^{14}C-PCNB at a nominal concentration of 1.0 ppb for 35 days under flow-through conditions. The maximum mean bioconcentration factors were 400x in the edible tissues, 1800x in the nonedible tissues, and 1100x in the whole fish. Maximum mean concentrations of total ^{14}C-residues occurred at 35 days and were 350 ppb in edible tissues, 1600 ppb in nonedible tissues, and 930 ppb in the whole fish. The mean concentration of ^{14}C-residues in the water during the exposure period was 0.88 ppb. After 21 days of depuration, 78% of the accumulated ^{14}C-residues were eliminated from the edible and nonedible tissues. Throughout the study, the water temperature ranged from 20 to 22°C, the pH ranged from 7.7 to 8.2, and the dissolved oxygen content ranged from 6.6 to 8.3 mg/L. Total ^{14}C-residues in the treated water ranged from 0.47 to 1.2 ppb during the exposure period.

WATER

Recovery of radioactivity for the water extractions averaged 83.5%, with an average of 99% of this recovered radioactivity in the organic-extractable fraction of the water samples from Days 21, 28, and 35. The remaining radioactivity was not accounted for after SPE. This loss may be due to the volatility of PCNB. Normal and reverse phase TLC of these extracts showed that the majority of the extracted radioactivity was polar, and that PCNB was generally found at a low level. Approximately 22.5% of the water residue was PCNB, while 11.0% was found as an unknown metabolite with a 4 minute retention time and 56.3% was found as an unknown metabolite at a retention time of about 13 minutes.

Since PCNB represented <25% of the water residue, the degradation of PCNB under test conditions was further investigated. Day 0 and 1 water samples were extracted and qualitatively analyzed. Day 1 water showed only polar materials while Day 0 was primarily PCNB (approximately 72% of the Day 0 water residue). Attempts to characterize the polar material, including GC/MS, were unsuccessful.

FILLET

Extraction of the fillet tissue allowed isolation of 98.3 and 98.7% (representing 0.295 and 0.346 ppm relative to the original residue levels) of the Day 21 and 35 fillet residues, respectively, as organic-extractable material.

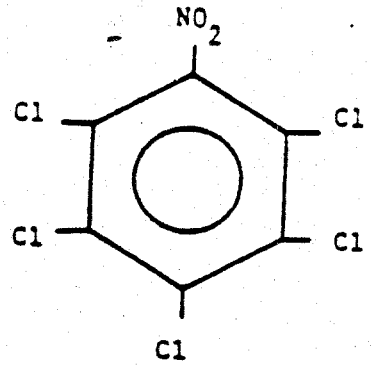
TLC analysis of the organic-extractable fractions indicated that a material which coincided with PCTA (based on cochromatography) was the predominant component of the residue. HPLC analysis also indicated the presence of PCTA as the major fillet residue.

VISCERA

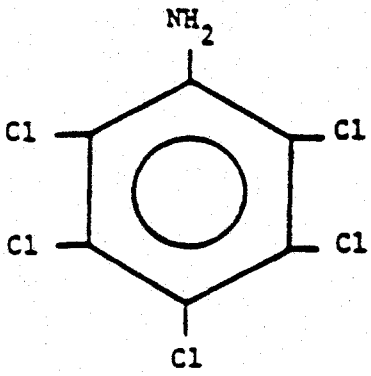
The total organic-extractable fractions contained 87.1 and 75.5% of the viscera residues (representing 1.09 and 1.21 ppm ^{14}C-PCNB equivalents relative to the original viscera residue), respectively. Aqueous-soluble residues represented 11.3% and 23.1% (0.142 and 0.370 ppm) of the total Day 21 and 35 viscera residues, respectively.

The extract contained predominantly PCTA. Co-chromatographic corroboration of PCTA as the major metabolite was done using the Day 28 viscera organic extract, unlabeled PCTA standard, and two dimensional TLC.

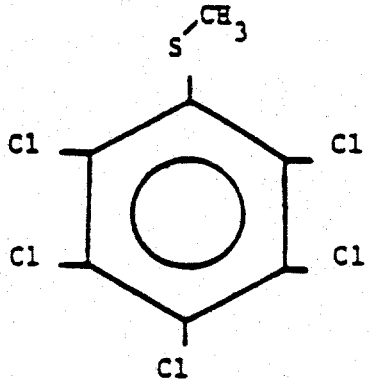
Chemical Structures of PCNB, PCA, PCTA, PCB and HCB



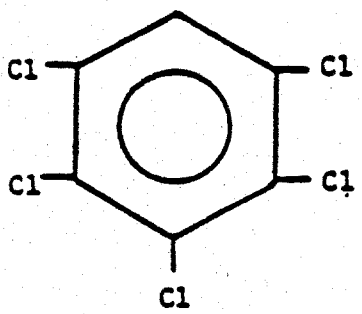
PCNB (Pentachloronitrobenzene)



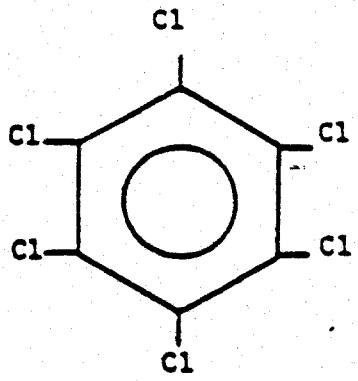
PCA (Pentachloroaniline)



PCTA (Pentachlorothioanisole)



PCB (Pentachlorobenzene)



HCB (Hexachlorobenzene)

Table I Total ^{14}C-Radioactivity Calculated as ^{14}C-PCNB in Test Water and Fish Tissue During 35 Days Exposure and 21 Days Depuration with Bluegill[a]

	Total ^{14}C Concentration as ^{14}C-PCNB[b]							
	Water		Fillet		Whole Fish		Viscera	
Day	Actual µg/l	Running Mean	µg/kg	BCF[c]	µg/kg	BCF[c]	µg/kg	BCF[c]
Uptake								
0	0.94[d]	---	---	---	---	---	---	---
0.17	0.47	0.71	12	17X	73	100X	110	150X
1	0.68	0.70	29	41X	230	330X	320	460X
3	1.1	0.80	72	90X	270	340X	450	560X
7	1.0	0.84	150	180X	570	680X	950	1100X
14	1.2	0.90	210	230X	670	740X	1000	1100X
21	0.71	0.87	300	340X	580	670X	1200	1400X
28	0.86	0.87	330	380X	930	1100X	1400	1600X
35	1.0	0.88	350	400X	930	1100X	1600	1800X
Depuration								
1	0.20	---	270	---	840	---	1200	---
3	---[e]	---	300	---	750	---	1100	---
7	---[e]	---	220	---	560	---	790	---
10	---[e]	---	190	---	510	---	650	---
14	---[e]	---	95	---	380	---	430	---
21	---[e]	---	78	---	170	---	360	---

[a] Table taken from in-life study, ABC Report 36872, MRID #41200001.

[b] All values have been rounded to represent two significant figures following ABC SOP #8.7.

[c] Daily bioconcentration factor (BCF) obtained by dividing the tissue concentration by the mean measured water concentration up to and including the respective sampling day (running mean).

[d] Samples taken immediately prior to addition of fish.

[e] Below minimum quantifiable limits of 0.0655, 3.13, 3.19 and 3.26 ppb for water, fillet, whole fish and viscera, respectively.

Table II. Distribution of ^{14}-PCNB Equivalent Residues in the Organic-Extractable and Water-Soluble Fractions of Bioconcentration Test Water

	Day-21[a]		Day-28[b]	
	Percent	ppm	Percent	ppm
Total in Water	100.0	0.0007	100.0	0.0009
Organic-Extractable[d]	81.9	0.0006	84.7	0.0008
Water-Soluble	2.6	0.0001	0.0	0.0
Average Recovery[e]	84.5		84.7	

	Day-35[c]		Average of Days 21, 28 and 35	
	Percent	ppm	Percent	ppm
Total in Water	100.0	0.0010	100.0	0.0009
Organic-Extractable[d]	81.2	0.0008	82.6	0.0007
Water-Soluble	0.0	0.0	0.9	0.0
Average Recovery[e]	81.2		83.5	

a Average of three extractions on a total of 400 ml of Day-21 test water.

b Average of five extractions on a total of 490 ml of Day-28 test water.

c Results from a single extraction of 200 ml of Day-35 test water.

d Isolated by C-18 solid phase extraction.

e It is very likely that the recovery loss was due to volatility of PCNB.

Table III. Distribution of ^{14}C-PCNB Equivalent Residues in the Combined Organic-Extractable Fractions of Bioconcentration Test Water as Determined by HPLC Analysis

	Combined Organic Extracts[a]		
	%	ppm	dpm/g
Total in Water	100.0	0.0010[b]	51.6
Organic-Extractable	100.0	0.0010	51.6
Unknown 1 (4 minutes)	11.0	0.0001	5.2
Unknown 2 (13 minutes)	56.3	0.0006	31.0
PCNB	22.5	0.0002	10.3
Unaccounted for	10.3	0.0001	5.2

a Water samples from Days 21, 28 and 35 were extracted and the organic-extractable fractions were combined.

b Day 35 contained the highest residue level (0.0010 ppm) of the three water samples extracted. Thus, the Day 35 residue level is taken to represent the mixture of three study days.

Table IV. Distribution of ^{14}C-PCNB Equivalent Residues in the Organic-Extractable and Water-Soluble Fractions of Fish Fillet Tissue as Determined by HPLC Analysis

	Day 21			Day-35		
	%	ppm	dpm/g	%	ppm	dpm/g
Total in Fillet	100.0	0.300	15480	100.0	0.350	18060
Organic-Extractable[a]	98.3	0.295	15222	98.7	0.346	17854
PCNB	ND	ND	ND	2.9	0.010	516
PCTA	98.3	0.295	15222	95.9	0.336	17338
Tissue-Unextractable	1.7	0.005	258	1.3	0.004	206

[a] See Figure 2 for extraction method.

Table V. Distribution of ^{14}C-PCNB Equivalent Residues in the Organic-Extractable and Water-Soluble Fractions of Day 21 Viscera Tissue as Determined by HPLC Analysis

	%	ppm	dpm/g
Total in Viscera	100.0	1.250	64500
Organic-Extractable[a]	87.1	1.089	56192
Methylene Chloride	84.6	1.057	54541
PCNB	16.3	0.204	10526
PCTA	68.3	0.853	44015
Hexane	2.6	0.032	1651
Water-Soluble	11.3	0.142	7327
Tissue-Unextractable	1.5	0.019	980

[a] See Figure 3 for extraction method.

Table VI. Distribution of ^{14}C-PCNB Equivalent Residues in the Organic-Extractable and Water-Soluble Fractions of Day 35 Viscera Tissue as Determined by HPLC Analysis

	%	ppm	dpm/g
Total in Viscera	100.0	1.600	82560
Organic-Extractable[a]	75.5	1.209	62384
Hexane	62.9	1.007	51961
PCNB	1.4	0.022	1135
PCTA	61.6	0.985	50826
Ethyl Acetate	12.6	0.201	10372
Water-Soluble	23.1	0.370	19092
SPE - organic fraction	3.9	0.062	3199
SPE - aqueous fraction	19.2	0.308	15893
Tissue-Unextractable	1.3	0.022	1135

[a] See Figure 4 for extraction method.