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Date Out EFB: OCT 20 1980

To: Product Manager Jacoby (21)
TS-767

From: Dr. Willa Garner *Samuel M. Creeger (Acting Chief)*
Chief, Review Section No. 1
Environmental Fate Branch

Attached please find the environmental fate review of:

Reg./File No.: 677-313

Chemical: Chlorothalonil

(tetrachloroisophthalonitrile)

Type Product: Fungicide

Product Name: Bravo 500

Company Name: Diamond Shamrock

Submission Purpose: Add soybeans, resubmission with data

ZBB Code: ~~3c7~~ 3c7

ACTION CODE: 332

Date in: ~~03/03/80~~ 03/03/80

EFB # 393

Date Completed: ~~03/03/80~~ OCT 20 1980

Time (days)

Deferrals To:

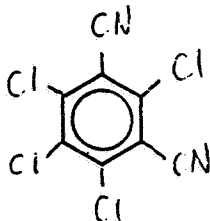
Ecological Effects Branch

Residue Chemistry Branch

Toxicology Branch

INTRODUCTION

- 1.1 The registrant requests adding soybeans to their BRAVO 500 label (EPA Reg. No. 677-313). The BRAVO 500 label contains uses on a variety of field crops and use on conifers.
- 1.2 Structure of the active ingredient (chlorothalonil)



- 1.3 See previous review of May 29, 1975.

2. DIRECTIONS FOR USE

- 2.1 SOYBEAN: Anthracnose, Diaporthe pod and stem blight, frog-eye leaf spot (*Cercospora sojina*), purple seed stain and Septoria brown spot -- Use BRAVO 500 at 2 to 3 1/2 pints per acre if two applications are scheduled or 1 1/2 to 2 3/4 pints per acre if three applications are scheduled. Apply in sufficient water to obtain complete coverage. A minimum of five gallons of water per acre should be used for aerial application. Applications should be made at 14 day intervals. Three applications should be scheduled in areas having a history of moderate to severe disease, as disease control is generally improved with the three application program. The time of first application should be determined as follows:

Determinant (Southern) Soybean Varieties -

Two application program: Make first application at early pod set (majority of pods are 1/8 to 3/8 inch in length), and the second application 14 days later. Use 2 to 3 1/2 pints per acre.

Three application program: BRAVO 500 should be applied at 14 day intervals starting at early to mid flowering. Use 1 1/2 to 2 3/4 pints per acre.

Indeterminant (Northern) Soybean Varieties -

Two application program: Make first application two to three weeks after first flowering when the largest pods are from 1 to 1 1/2 inches in length and the second application 14 days later. Use 2 to 3 1/2 pints per acre.

Three application program: BRAVO 500 should be applied at 14 day intervals starting one week after first flowering. Use 1 $\frac{1}{2}$ to 2 $\frac{3}{4}$ per acre.

2.2 DO NOT apply within 6 weeks of harvest.
DO NOT allow livestock to graze treated areas.
DO NOT feed soybean hay or threshings from treated fields to livestock.

2.3 The product contains 4.17 points ai/gallon or 500 grams per liter.

3. DISCUSSION OF DATA

- 3.1 Exposure of fish to ^{14}C -labeled chlorothalonil (DAC-2787, tech.): accumulation, distribution, and elimination of residues, prepared for Diamond Shamrock Corporation by B.H. Sleight, III, Bionomics, Inc., Accession No. 099248, p. 000050.

Procedure

Bluegill sunfish (*Lepomis macrochirus*) were acclimated for 15 days prior to testing. Accumulation and depuration were tested by a flow-through method using a continuous-flow proportional dilution apparatus and two 30-liter aquaria containing 100 fish each. Aerated well water (pH 7.1, 18 C) was used in the tests. One aquarium served as a control, whereas the other contained [^{14}C]chlorothalonil (technical grade) at a nominal concentration of 0.01 mg/liter (0.01 ppm). The duration of exposure was 28 days, followed by a 14-day depuration period in uncontaminated water. Five fish from each experimental unit and water aliquots were sampled at 0, 1, 3, 7, 10, 14, 21, and 28 days of exposure and 1, 3, 7, 10, and 14 days of depuration. Fish were eviscerated to analyze edible and nonedible parts.

Methodology

Trapped $^{14}\text{CO}_2$ (as carbonate) was counted by scintillation spectrometry after combustion of the fish tissues. Recovery rates for radioactivity ranged from 98 to 101%. Hexane and methanol extractions were performed by blending the edible tissues with Na_2SO_4 , mixing the solution with either hexane or methanol, filtering the extract, and assaying the filtrate radiometrically. Water samples were examined for chlorothalonil by extracting with methylene chloride and radioassaying. A radioactivity recovery level of 94% was obtained from the water samples through this method. Minimum detectable limits for these methods were 0.005 mg/kg (0.005 ppm) and 0.001 ppm for tissue and water, respectively.

Results

Only about 30% of the nominal concentration (0.01 ppm) was retained throughout the experiment, resulting in exposure of the fish to an average of 0.003 ppm. Accumulation in bluegills reached a plateau between 3 and 7 days of exposure and retained a mean residue level of 0.58 ppm from day 7 of exposure to day 28 (Table 1). The concentration in nonedible tissues was about 15 times the concentration of residues in edible tissues, with a mean residue level of 8.6 ppm. About 16% of the residues were extractable by hexane (nonpolar), and about 18% were extractable by methanol (polar). Residue levels in edible tissues had declined by about 50 and 62% after 10 and 14 days of depuration, respectively.

Table 1. Mean ^{14}C residues in the edible portion of bluegills continuously exposed to [^{14}C]chlorothalonil for 28 days.

Day	Average residue level ^a (ppm)	Standard deviation
1	0.23	0.06
3	0.48	0.10
7	0.54	0.17
10	0.72	0.34
14	0.48	0.13
21	0.56	0.20
28	0.59	0.14

^a Mean usually based on 10 radiometric analyses; the minimum number of observations was 8. Minimum detectable limit = 0.005 mg/kg. Average exposure level = 0.003 mg/liter.

Conclusions

At very low concentrations of chlorothalonil (0.003 ppm) in water, bluegill sunfish accumulate around 200 times the ambient water concentration of chlorothalonil and/or degradation products in edible tissues. Viscera tissues contain approximately 15 times more (radioactive) residues than muscle tissue. A residue plateau is reached after 3-10 days and depuration is rapid, with a half-life of about 10 days.

The exposure concentration in the water (0.003 ppm) was much lower than the intended level (0.01 ppm). This was probably due to adsorption of chlorothalonil to plastic tubing in the dilutor and/or an inaccurate dilutor, resulting in a 30% error.

It is not known whether degradation products are extracted by the methylene chloride (used to extract water samples before radioassay), and no attempt was made to characterize residues in the water or check for recovery of the degradation products.

Of the residues extracted from edible tissues, 16% reportedly was extracted by a nonpolar solvent and 18% by a polar solvent. This leaves 66% unaccounted for (probably bound residues), and certainly some of this radioactivity is due to unspecified metabolites.

3.2 Exposure of fish to ^{14}C -labeled DAC-3701: accumulation, distribution, and elimination of residues; prepared for Diamond Shamrock Corporation by B.H. Sleight, III, Bionomics, Inc., Accession No. 099248, p. 000063.

Procedure

Bluegill sunfish (Lepomis macrochirus) were acclimated for 15 days before initiation of the study. A continuous-flow proportional dilution apparatus was used to provide a flow-through accumulation test of radiolabeled 2,5,6-trichloro-4-hydroxyisophthalonitrile (DAC-3701), a major degradation product of chlorothalonil. Three 30-liter aquaria were used in the experiment, with 100 fish in each. One aquarium served as a control; the others were used for testing radiolabeled DAC-3701 at two nominal concentrations of 0.01 and 1 mg/liter (0.01 and 1 ppm). All solutions were mixed in acetone. Water and fish were sampled from all aquaria prior to exposure and at 1, 3, 7, 10, 14, 21, and 28 days of exposure. The aquarium with DAC-3701 at 1 ppm was also sampled at 35, 42, and 49 days of prolonged exposure. After 28 days of exposure, all remaining fish from the 0.01 ppm aquarium and (after 49 days) a sufficient number of fish from the aquarium containing DAC-3701 at 1 ppm were transferred to uncontaminated water for a 28-day depuration period. All fish were radiometrically analyzed at sample periods for DAC-3701 in edible tissues. The relative distributions of residues in edible and nonedible tissues were analyzed only at 28 days exposure (0.01 ppm) and 49 days exposure (1 ppm). Polar and nonpolar residues were also investigated.

Methodology

Duplicate sample radioassays of fish tissues were performed after tissues were dried for 24 hours on filter disks. Combustion of fish tissues yielded $^{14}\text{CO}_2$ that was trapped as a carbonate in ethanolamine. Counting with a scintillation spectrometer of prepared sample vials resulted in a recovery level of 98-101%. Separate hexane and methanol extractions were made of fish tissues to determine polar and nonpolar residues. Dried and weighed fish were blended with Na_2SO_4 and Celite 545, filtered, and analyzed radiometrically. Levels of DAC-3701 in water were determined by extraction with methylene chloride and radioassay with a recovery level of only 34%. Minimum detectable limits were 0.75 mg/kg (0.75 ppm) for fish and 0.03 ppm for water at the high nominal concentration and 0.01 ppm for fish and 0.002 ppm for water at the lower nominal concentration.

Results

The control group suffered no mortality during the experiments, nor did the groups exposed to DAC-3701 at 0.01 ppm. The fish exposed at the high concentration became lethargic and exhibited discoloration after 16 days; about 18% mortality was observed after 28 days in the fish exposed at the high concentration. Water samples contained the compound at actual average exposure levels of around 0.005 and 0.61 ppm instead of 0.01 and 1 ppm, respectively, as shown in Table 1. Fish exposed at a nominal concentration of 0.01 ppm reached peak accumulation levels in edible tissues between the 1st and 3rd days (Table 2), with a maximum accumulation of 0.19 ppm. The maximum accumulation level of edible fish tissues exposed at the nominal concentration of 1 ppm was 48.9 ppm, reached on the 28th day of exposure (Table 1). Thereafter the accumulated concentration declined. On the average, nonedible tissues bioconcentrated five times as much as edible tissues. Depuration was rapid, with half the residues being eliminated within 1-3 days in the fish exposed at the nominal 0.01 ppm concentration (Table 1). Less than 4% of the residues in fish exposed at the nominal 1 ppm concentration for 49 days remained after 28 days of depuration (Table 3).

Of the residues remaining in the edible tissues of fish exposed to DAC-3701 at the high concentration, about 1% was extractable by hexane and about 34% was extractable with methanol. Corresponding values for fish exposed at the lower concentration were 8% (hexane) and 24% (methanol), respectively.

Table 1. ^{14}C -DAC-3701 residues measured in the water during continuous exposure of bluegill sunfish.

Day	Measured concentration (ppm)	
	(Nominal) 0.01 ppm	(Nominal) 1.0 ppm
0	0.005	0.69
1	0.004	0.77
3	0.004	0.50
7	0.005	0.52
10	0.006	0.44
14	0.005	0.49
21	0.005	0.58
28	0.004	0.45
35	--	0.50
42	--	0.42
49	--	0.44

Table 2. Mean measured ^{14}C residues (mg/kg) in the edible portion of bluegill sunfish (*Lepomis macrochirus*) during continuous exposure to [^{14}C]DAC-3701.

Day	Nominal exposure level (mg/l) ^a			
	0.01		1.0	
	Average ^b residue level	S.D. ^c	Average ^b residue level	S.D. ^c
1	0.16	0.02	2.2	0.5
3	0.19	0.02	3.1	0.7
7	0.15	0.02	6.8	1.3
10	0.15	0.02	15.6	3.6
14	0.10	0.05	18.3	6.5
21	0.09	0.02	25.2	5.0
28	0.07	0.02	48.9	11.0
35	--	--	31.9	17.0
42	--	--	20.6	9.5
49	--	--	21.2	7.6

^a Actual exposure concentration averaged 0.005 and 0.61 mg/l throughout the course of the experiment.

^b Average based on 6-10 radiometric analyses. Minimum detectable limits were 0.75 mg/kg (high concentration) and 0.01 mg/kg (low concentration).

^c S.D. = standard deviation.

Table 3. Mean measured ^{14}C residues (mg/kg) in the edible portion of bluegill sunfish during the depuration period following either 28 or 49 days of exposure to [^{14}C]DAC-3701 at 0.01 and 1.0 mg/liter, respectively.

Day	Preceding nominal exposure level (mg/l) ^a			
	0.01		1.0	
	Average ^b residue level	S.D. ^c	Average ^b residue level	S.D. ^c
1	0.04	0.01	20.0	3.4
3	0.04	0.01	24.7	7.6
7	<0.02	--	11.8	6.6
10	<0.01	--	13.5	8.8
14	<0.01	--	<3.8	3.7
21	--	--	2.0	0.6
28	--	--	<1.8	0.5

^a Actual predepuration exposure concentrations averaged 0.005 and 0.61 mg/l, respectively.

^b Average based on 6-10 radiometric analyses. Minimum detectable limits of 0.75 mg/kg (high concentration) and 0.01 mg/kg (low concentration).

^c S.D. = standard deviation.

Conclusions

The chlorothalonil degradation product 2,5,6-trichloro-4-hydroxy-isophthalonitrile (DAC-3701) has a bioconcentration factor in bluegill sunfish of about 50 when the concentration in water is 0.005 ppm and about 110 when the concentration in water is about 0.5 ppm. At the higher concentration, the compound is toxic to fish, as a 16-18% mortality rate is observed on day 28. DAC-3701 residue levels reach a plateau in fish by 1-3 days at concentrations in water of ~0.005 ppm and only after 28 days in fish exposed at ~0.5 ppm. Depuration is rapid; 50% of the residues are lost within 3 days at both treatment levels.

Actual concentrations of DAC-3701 in the water in this study are questionable because a 34% recovery rate from the water was reported. The report used the nominal water concentrations for calculations of bioaccumulation factors, thus yielding false data.

No hydrolysis or metabolism studies are included in this package. Thus, it is not clear what metabolites are expected and no characterizations of the residues were attempted.

3.3 Adsorption of chlorothalonil (2,4,5,6-tetrachloroisophthalonitrile, DS-2787), M.B. Szalkowski, Diamond Shamrock Corporation, Accession No. 099248, p. 000078.

Procedure/Methodology

Samples of six soils (Table 1), muck, sea sand, and montmorillonite clay in flasks were treated with a mixture of ^{14}C ring-labeled chlorothalonil and unlabeled chlorothalonil (Diamond Shamrock Corp., purity 99.5-99.7%) to yield concentrations of 0.1, 0.2, 0.3, 0.4, and 0.5 ppm. The soils were equilibrated for 20 hours and then were shaken and centrifuged. The supernatant was analyzed by liquid scintillation counting.

Results

The clay and organic matter contents of the soils were the major factors affecting chlorothalonil adsorption. The adsorption constant (K) was correlated to the soil organic matter content. The "K" values for the six soils tested ranged from 11.8 in the Michigan loma sand to 471.8 in the Iowa peat loam (Table 2). The adsorption of chlorothalonil residues to soil reduces its potential for mobility in most soils.

Table 1. Characteristics of soils used in a study of chlorothalonil adsorption.

Location	Soil type	pH	Organic matter (%)	Sand (%)	Silt (%)	Clay (%)
Texas	Clay loam	7.5	0.8	44.6	27.8	27.6
Ohio	Sandy loam	5.6	1.6	61.3	30.4	8.3
Nebraska	Silt loam	5.6	1.9	1.4	71.5	27.1
Iowa	Peat loam	7.0	7.2	29.7	50.0	20.3
Minnesota	Silt loam	5.6	3.4	13.7	63.7	22.6
Michigan	Loamy sand	6.6	1.8	79.0	14.0	7.0

Table 2. Freundlich isotherm constants and adsorption coefficients for chlorothalonil adsorbed onto various soils.

Media	K ($\mu\text{g/g}$)	1/n	Q ($\mu\text{g/g}$)
Sand	0.33	0.69	-- ^a
Michigan loamy sand	11.83	0.75	629
Texas clay loam	15.96	0.72	1,995
Ohio sandy loam	22.88	1.01	1,404
Nebraska silt loam	43.38	0.61	2,271
Minnesota silt loam	111.35	1.05	3,275
Muck	201.09	1.26	-- ^a
Iowa peat loam	471.85	1.15	6,590
Clay	1,342.76	0.69	-- ^a

^aQ = K x 100/% organic matter; Q was not calculated for adsorbing media containing no organic matter or for which the organic matter content was unknown.

Conclusions

The adsorption of chlorothalonil to soil cannot be determined from the data because the soil was sieved to 60 mesh, thus removing the medium and coarse sand and altering the soil characteristics. Soils whose characteristics have been altered in this manner will have higher "K" values than unaltered soil. Therefore, the results of this study are not representative of actual environmental conditions.

The peat loam soil was not properly classified. A peat soil has an organic matter content of greater than 50% (Glossary of Soil Science Terms, Soil Science Society of America, October 1979). Since the peat loam soil had an organic matter content of only 7.2%, it should be classified as a loam soil.

Soil characteristics for the muck soil were not presented.

3.4 Quantitation and characterization of the biotransformation products of 2,4,5,6-tetrachloroisophthalonitrile (chlorothalonil, DS-2787) in soil,
M.B. Szalkowski and J.J. Mannion, Diamond Shamrock Corporation,
Accession No. 099248, p. 000114.

Procedure

Soil metabolism. A sandy loam soil (61.3% sand, 30.4% silt, 8.3% clay, 1.6% organic matter, pH 5.6) was air dried and sieved to 0.59 mm. The soil was treated with [^{14}C]chlorothalonil (Diamond Shamrock Corp., purity 99.5%) to yield 10 ppm, mixed, and moistened with water. The soil was then stored at 24 C in a sealed container. The soil was re-treated with 10 ppm [^{14}C]chlorothalonil each week for 13 weeks and maintained at a moisture content of 15%.

Leaching. A sample of aged treated soil was air dried and added to a chromatographic column (inside diameter 2.5 cm) and eluted with 800 ml of distilled water.

Methodology

Soil metabolism. Samples of the treated soil were air dried and combusted. and the released $^{14}\text{CO}_2$ was analyzed by liquid scintillation counting (LSC).

Leaching. An aliquot of each leachate fraction was analyzed by LSC. The remainder of the leachate was evaporated to dryness. The residue was dissolved in methanol and analyzed by LSC. The leachate was also characterized by thin-layer chromatography (TLC) and autoradiography. The residues were scraped from the TLC plate and assayed individually by combustion and LSC as described above. In addition, the TLC spots were rechromatographed to obtain pure compounds for analysis by mass spectrometry.

The soil was extracted by stirring with acidified acetone. The mixture was filtered and the filter cake was reextracted twice. The combined extracts were analyzed by LSC. The extracted soil was air dried and analyzed by combustion and LSC as described above.

Another sample of treated soil was column chromatographed with distilled water, and the eluate was analyzed by TLC.

Results

After aging, the chlorothalonil-treated soil contained 86.3% of the applied residues, which were present as chlorothalonil, five degradation products, and soilbound residues (Table 1).

After elution with water, 40% of the residues leached through a soil column, 27% were soilbound, and 28% were extractable. The extractable residues consisted primarily of chlorothalonil, whereas the leachate contained primarily metabolite I (Table 2).

Table 1. Characterization and quantitation of [^{14}C]chlorothalonil residues in aged soil.

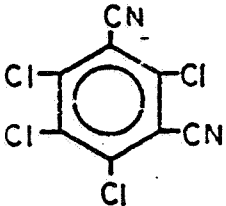
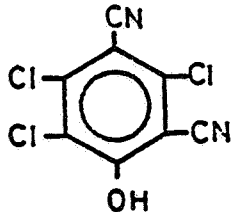
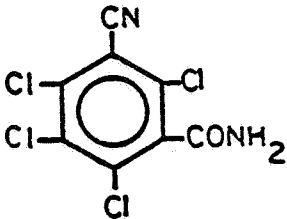
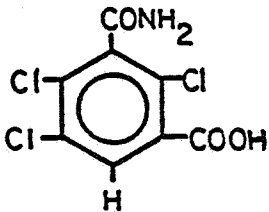
Chemical structure	Chemical name	Percent of applied chlorothalonil
	Unextractable residues	26.8
	2,4,5,6-Tetrachloroisophthalonitrile (chlorothalonil)	15.5
	4-Hydroxy-2,5,6-trichloroisophthalonitrile (Metabolite I)	22.3
	3-Cyano-2,4,5,6-tetrabenzamide (Metabolite II)	10.4
	Trichloro-3-carboxybenzamide (or other isomeric form) (Metabolite III)	4.3

Table 1. Characterization and quantitation of [^{14}C]chlorothalonil residues in aged soil. (continued)

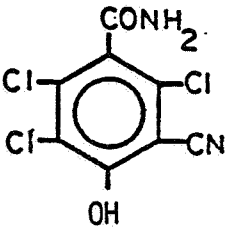
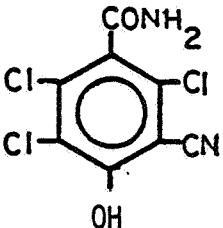
Chemical structure	Chemical name	Percent of applied chlorothalonil
	3-Cyanotrichlorohydroxybenzamide (or other isomeric form) (Metabolite IV)	3.8
	3-Cyanotrichlorobenzamide (or other isomeric form) (Metabolite V)	3.2

Table 2. Chromatographic distribution of radioactivity in aged, leached soil and leachate.

Residue	Percent of total radioactivity on chromatogram	
	Soil	Leachate
Chlorothalonil	49.3	4.3
Metabolite I	16.0	43.9
Metabolite II	11.6	17.8
Metabolite III	2.9	8.6
Metabolite IV	4.5	6.3
Metabolite V	3.6	5.3
Other	12.1	13.8

Conclusions

Chlorothalonil is degraded in soil to 4-hydroxy-2,5,6-trichloro-isophthalonitrile, 3-cyano-2,4,5,6-tetrabenzamide, trichloro-3-carboxybenzamide, 3-cyanotrichlorohydroxybenzamide, and 3-cyanotrichlorobenzamide. However, the rate of degradation is not known.

No conclusions can be made regarding the susceptibility of chlorothalonil to leaching because the soil was sieved to 0.59 mm, thus removing the coarse sand and altering the soil characteristics. Also, the length of the soil column is unknown and the soil was eluted with an excessive amount of water (the equivalent of 163 acre-inches).

65 ? "

3.5 The effect of chlorothalonil on populations of soil microorganisms,
Lankow, R.K., et al., Diamond Shamrock Corporation. Accession No. 099248,
p. 000165.

Procedure/Methodology

Effect of chlorothalonil on microbial populations. Sandy loam soil (pH 7.1, organic matter 1.9%) was maintained in pots under moist conditions in a greenhouse for 30 days as a pretreatment. Treatments were then applied as follows: Bravo 6F (flowable formulation of chlorothalonil) at 10 µg ai/g soil; sucrose at 1% (w/w) in soil; or a combination of the above.

At sampling dates of 0, 1, 2, and 3 weeks, soil was removed from individual pots and extracted with water, and serial dilutions were made in sterile, deionized water. From the two highest dilutions, 0.1-ml aliquots were spread on plates of rose bengal agar (for fungal population estimates) and soil extract agar (for bacterial and actinomycete population estimates). The culture plates were incubated at 24 C for 6 days. Colonies were counted and the number of propagules per gram dry weight of soil was calculated.

Effect of chlorothalonil on soilborne populations of *Trichoderma harzianum*. Isolates of *Trichoderma harzianum* (T-1191) were routinely cultured on V-8 juice agar. Spores were harvested by using 0.01% Tween 20. The spore density was determined with a hemacytometer and the spores were added to sterile (autoclaved) sandy loam soil samples in flasks to yield 10^5 conidia/gram soil. The treatments applied to the soils in the flasks were identical to those used in the previous experiment. Water was added to produce a 20% moisture content in the samples, which were incubated at 27 C and 95% relative humidity.

At weekly intervals, soil samples were removed, suspended in water, and blended for two 30-second intervals. Serial dilutions were made of the extract and 0.1-ml aliquots were plated on rose bengal agar. Plates were incubated at 24 C and colonies were counted after 4 days.

In vitro sensitivity of *T. harzianum* to chlorothalonil was assessed by measuring radial growth on potato dextrose agar (PDA) amended with chlorothalonil at various levels. Inhibition of spore germination was determined by seeding chlorothalonil-amended PDA with spores of *T. harzianum* and observing the amount of spore germination after 24 hours of incubation at 25 C.

Effect of chlorothalonil on soil acid phosphatase activity. Soil treated with chlorothalonil, sucrose, or both (as described before) was suspended in 50 mM sodium maleate buffer. After equilibration, p-nitrophenyl phosphate was added and the suspension was incubated for 2 hours at 37 C. The suspension was then centrifuged. An aliquot of the supernatant was analyzed for p-nitrophenyl by measuring its absorbance at 405 nm.

Results

As shown in Table 1, chlorothalonil-treated soil produced no effect on the soil fungal population as compared with the water control soil. With the addition of sucrose, a 20-fold increase in soil fungi was observed the 1st week after treatment; this increase was limited to 5-fold in samples treated with chlorothalonil. However, 3 weeks after treatment, there was no significant difference between the fungal populations of the control and chlorothalonil-treated sucrose-amended soils.

The bacterial population was slightly affected by the chlorothalonil treatment for the first 2 weeks after treatment, though no effect was observed after 3 weeks. Sucrose amendment resulted in rapid bacterial growth that was actually greater in the chlorothalonil-treated soil (Table 2).

Chlorothalonil inhibited the growth of *T. harzianum* in soil by as much as 60% during the 1st week of treatment (Table 3). After 1 month, however, growth in treated soil was equal to that in untreated soil. The inhibitory effect did not occur in soil amended with sucrose.

In vitro and spore germination studies with *T. harzianum* demonstrated that the organism was moderately sensitive to chlorothalonil. ED₅₀ values of 11.6 and 15.5 ppm were reported (no data given) for growth and spore germination, respectively.

It was reported that the acid phosphatase activity in soil directly paralleled the microbial population (figure referenced not included). Chlorothalonil did not cause inhibition of soil acid phosphatase activity in the absence of sucrose, but there was some suppression in activity after 1 week with the sucrose addition. By the 2nd week, no differences were noted (Table 4).

Table 1. Effect of chlorothalonil treatments on soil fungal populations.

Soil treatment	Colony forming units $\times 10^4$ /gram soil at days after treatment			
	0	7	14	21
H ₂ O control	2.0	2.2	2.2	1.2
Chlorothalonil	2.2	1.6	1.6	0.9
Sucrose	2.1	40.8	48.5	33.5
Chlorothalonil + sucrose	2.1	11.1	21.4	30.2

Table 2. Effect of chlorothalonil treatments on soil bacterial populations.

Soil treatment	Colony forming units $\times 10^6$ /gram soil at days after treatment			
	0	7	14	21
H ₂ O control	4.1	4.7	21.1	8.2
Chlorothalonil	4.2	3.1	15.1	9.4
Sucrose	3.6	68.8	24.9	119
Chlorothalonil + sucrose	4.2	105	832	172

Table 3. Effect of chlorothalonil treatments of soil on the population of Trichoderma harzianum.

Soil treatment	Colony forming units x 10 ⁴ /gram soil at days after treatment ^a				
	0	7	14	21	28
H ₂ O control	0.25a	41.5a	32.3a	46.4a	30.4ab
Chlorothalonil	0.92a	15.5c	28.1a	30.5b	29.9b
Sucrose	0.75a	24.5b	36.4a	36.8b	31.8a
Chlorothalonil + sucrose	0.50a	25.6b	31.0a	37.0b	30.2b

^aWeekly values followed by the same letter not significantly different at the 95% level.

Table 4. Effect of chlorothalonil on soil acid phosphatase activity.

Soil treatment	Phosphatase activity at days after treatment ^a			
	0	7	14	21
H ₂ O control	0.134	0.122	0.111	0.098
Chlorothalonil	0.125	0.123	0.118	0.102
Sucrose	0.137	0.322	0.224	0.139
Chlorothalonil + sucrose	0.134	0.277	0.236	0.139

^aActivity expressed as milligrams phosphate/hour/gram dry soil.

Conclusions

The results show that chlorothalonil has a measurable effect on soil microbial populations but that the populations recover in 2-3 weeks. In the presence of a ready nutrient source (such as sucrose), soil fungal populations show a greater sensitivity to chlorothalonil. The compound at an estimated 12-15 ppm will reduce the in vitro mycelial growth and spore germination of T. harzianum by 50%.

3.6 Absorption and translocation of 2,4,5,6-tetrachloroisophthalonitrile (chlorothalonil) metabolites in soil by leafy, root and fruiting crops - a laboratory rotational crop study, Szalkowski, M.B., and D.E. Stallard, Diamond Shamrock Corporation, Accession No. 099248, p. 000182.

Procedure

A sandy loam soil (62.2% sand, 6.8% clay, 31.0% silt, 3.2% organic matter, pH 6.0) was sieved to 590 μ m, amended with [14 C]chlorothalonil (99.7% purity) to yield a concentration of 10.4 ppm, and aged for 14 weeks, after which < 3% of the test material remained as intact [14 C]chlorothalonil. Transfer of soil (1.3 kg) was made to six plastic planting pots, two each for Grand Rapids lettuce, Nantes carrots, and Tendergreen beans. Petri dishes were placed under the pots to catch any leachates resulting from daily watering. Plants were maintained at 27-29 C during daylight and 16-24 C during a 10-hour dark cycle. Plant tissue samples were taken at 15, 30, 45, and 63 and/or 90 days after planting. Both soil and plant tissues were radioassayed for total 14 C residues and extracted, chromatographed, or radioassayed for determination and quantitation of metabolites.

Methodology

To determine total 14 C residues, plant tissues were oven dried at 95 C for 2-4 hours, ground, and combusted to 14 CO₂ for radioassay. Control plants were combusted (wet) to ensure that no radioactivity was lost by oven drying. Soil was air dried and combusted to measure 14 CO₂. Liquid scintillation counting (LSC) was performed to quantify radioactivity. Combustion and 14 CO₂ trapping efficiencies were determined to be 98-100%. The amount of 14 CO₂ released during the aging period was determined by trapping volatile 14 C in an NaOH trap and sampling the trap after 34, 43, 64, and 98 days of aging; 14 C was measured by LSC. Determination and quantitation of 14 C residues (extractable and unextractable) in both soil and plant tissues was conducted by solvent extraction techniques, thin-layer chromatography, and radioassay procedures as outlined in Figure 1.

Results

Table 1 presents the distribution of 14 C residues in soil after 14 weeks of aging. Less than 1% of the radioactivity applied to the soil was volatilized during the aging period, and < 3% remained as intact [14 C]chlorothalonil. Data on the quantitation and characterization of 14 C residues in lettuce, carrots, and beans are presented in Tables 2, 3, and 4, respectively. The principal [14 C]chlorothalonil-extractable metabolites found in the tissues of all three plant types were water-soluble residues. Total 14 C residues in the tissues of all plant types tested generally increased throughout the test period and were composed almost entirely of extractable residues in all cases. Metabolite I ([14 C]4-hydroxy-2,5,6-trichloroisophthalonitrile) also was found in plant

tissues (carrots and beans). This metabolite was concentrated primarily in the lower stem and leaf tissues of bean plants and did not travel as well through plant tissues as water-soluble residues.

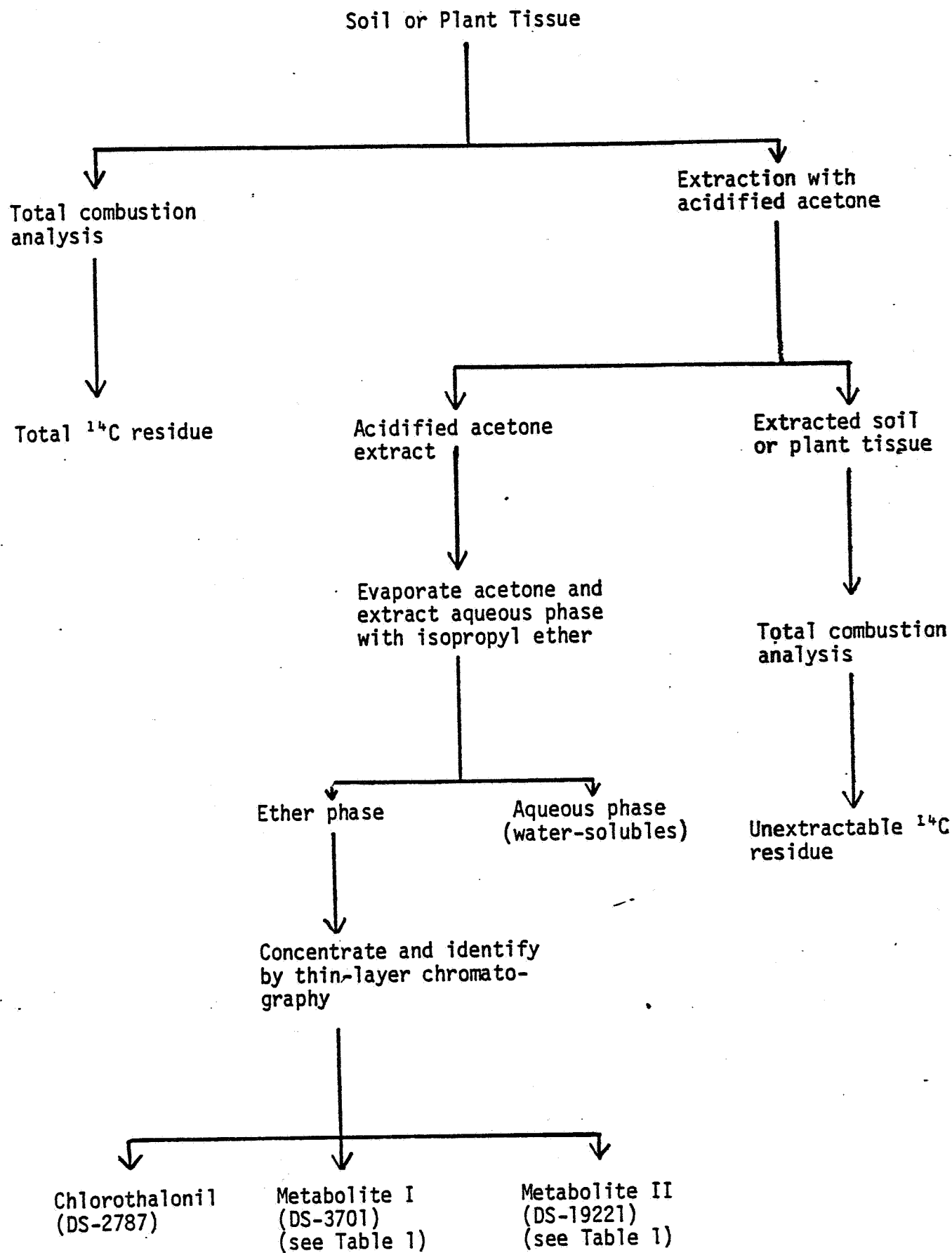


Figure 1. Scheme for characterization and quantitation of the ^{14}C residues in soil and plants.

Table 1. Distribution of ^{14}C residues in soil amended with [^{14}C]chlorothalonil and aged for 14 weeks.

Residue	Percent of applied ^{14}C
Unextractable	43.8
Water-solubles	37.8
[^{14}C]-4-Hydroxy-2,5,6-trichloro- isophthalonitrile (DS-3701) (Metabolite I)	21.6
Chlorothalonil	2.9
[^{14}C]-3-Cyano-2,4,5,6-tetrachloro- benzamide (DS-19221; Metabolite II)	<2.0

Table 2. Quantitation and characterization of ^{14}C residues in lettuce leaves grown in soil amended with $[^{14}\text{C}]\text{chlorothalonil}$ and aged for 14 weeks prior to planting.

Time (days)	$[^{14}\text{C}]\text{Chlorothalonil}$ equivalents (ppm) ^a			
	Total ^{14}C residues	Extractable ^{14}C residues	Unextractable ^{14}C residues	Water-soluble ^{14}C residues
30	0.46	0.53	<0.01	0.35
45	0.96	0.84	<0.01	0.84
63	1.19	1.06	<0.01	0.86
				Ether-soluble ^{14}C residues
				<0.18
				<0.18
				<0.18

^aValues given are the averages of duplicate tests.

Table 3. Quantitation, distribution, and characterization of the ^{14}C residues in carrot plants 90 days after planting in [^{14}C]chlorothalonil-amended soil aged for 14 weeks prior to planting.

Sample	[^{14}C]Chlorothalonil equivalents (ppm) ^a				
	Total ^{14}C residues	Extractable ^{14}C residues	Water-soluble ^{14}C residues	Intact [^{14}C]chlorothalonil	Metabolite I _b Metabolite I _c
Roots	0.40	0.34	0.28	0.02	0.07 0.06
Tops	2.20	2.34	2.11	0.05 ^d	0.11 0.09

^a Values given are the averages of duplicate tests.

^b 4-Hydroxy-2,5,6-trichloroisophthalonitrile.

^c 3-Cyano-2,4,5,6-tetrachlorobenzamide.

^d This value for one test only; no duplicate was analyzed.

Table 4. Quantitation, distribution, and characterization of the ^{14}C residues in the aerial portions of bean plants grown for 30 or 63 days in soil amended with ^{14}C chlorothalonil and aged 14 weeks prior to planting.

Bean sample ^b	^{14}C Chlorothalonil equivalents (ppm) ^a							
	Total ^{14}C residues	Extractable ^{14}C residues	Unextractable ^{14}C residues	Water-soluble ^{14}C residues	Ether-soluble ^{14}C residues	Intact ^{14}C chlorothalonil	Metabolite ^{14}C	Metabolite ^{14}C
30 Days								
Section 1	2.21	2.31	0.07	1.32	0.77	0.04	0.47	0.02
Section 2	1.24	1.31	0.03	0.76	0.41	0.01	0.29	0.01
Section 3	1.13	1.24 ^e	0.03 ^e	0.67 ^e	0.42 ^e	---	---	---
Section 4	0.75	1.01 ^e	0.02 ^e	0.44 ^e	0.41 ^e	0.01	0.27	0.02
63 Days								
Section 1	6.78	6.68	0.14	2.60	1.75	0.02	1.69	<0.01
Section 2	3.72	3.47 ^e	0.09 ^e	2.45 ^e	0.88 ^e	<0.01 ^e	0.82 ^e	0.03 ^e
Section 3	5.40	4.92	0.09	3.56	1.02	0.02	0.96	<0.01
Section 4	7.85	5.64	0.08	3.23	0.93	<0.01 ^e	0.88	<0.01
Section 5	4.35	1.13 ^e	<0.02 ^e	---	---	---	---	---
Fruit - 5th node (1 pod & beans)	0.87	0.67	0.03	0.30	0.47	0.03	0.35	0.03

^a Values given are the averages of duplicate samples, except where otherwise noted.

^b Section 1: hypocotyl + first internode + primary leaves.
 Section 2: second internode + first trifoliate leaf.
 Section 3: third internode + second trifoliate leaf.
 Section 4: fourth internode + third trifoliate leaf.
 Section 5: fifth internode + fourth trifoliate leaf.

^c 4-Hydroxy-2,5,6-trichloroisophthalonitrile.

^d 3-Cyano-2,4,5,6-tetrachlorobenzamide.

^e Only one sample analyzed.

^f No samples analyzed.

Conclusions

Residues of aged [^{14}C]chlorothalonil are able to translocate and accumulate in rotational leafy, fruit, and root crops. Primary residues that translocate and accumulate included unidentified extractable water-soluble compounds and the metabolite 4-hydroxy-2,5,6-trichloroisophthalonitrile. Other compounds found to accumulate, but to a much lesser degree, in plant tissues include the metabolite 3-cyano-2,4,5,6-tetrachlorobenzamide, ether-soluble residues, and chlorothalonil. Water-soluble residues were found to have the highest potential for translocating of all detectable residues and were freely mobile through all tissues examined. Accumulation of all the compounds listed above increased in general throughout the 2- to 3-month test period, with no concentration plateau reached in the plant tissues. The results and conclusions of this study indicate that a field study using formulated products is merited to determine whether water-soluble residues and metabolites are leached into the soil or are absorbed and translocated into rotational crop plant tissues.

ENVIRONMENTAL FATE ANALYSIS

Chlorothalonil will be readily transformed in sandy loam soil. Less than 3% of the applied [^{14}C]chlorothalonil remains in soil 14 weeks after treatment at 10 ppm. Less than 1% is released as $^{14}\text{CO}_2$ during this period. Unextractable and unidentified water-soluble residues each account for about 40% of the applied [^{14}C]chlorothalonil. After 14 weeks, the predominant metabolite is 4-hydroxy-2,5,6-trichloroisophthalonitrile, which is present at 20% of the applied chlorothalonil. A minor metabolite (<2% of the applied chlorothalonil) is 3-cyano-2,4,5,6-tetrachlorobenzamide. A similar pattern is observed when the soil is treated with [^{14}C]chlorothalonil at 10 ppm every week for 14 weeks, except that other minor metabolites are detectable. These metabolites are 2,5,6-trichloro-3-carboxybenzamide, 3-cyano-2,5,6-trichloro-4-hydroxybenzamide, and 3-cyano-2,5,6-trichlorobenzamide.

Gross populations of bacteria and fungi will be reduced by 25-30% during the first 2 weeks after treatment of soil with a flowable formulation of chlorothalonil (Bravo 6F) at 10 ppm ai. By the 3rd week, populations recover to levels found in untreated soils. When sucrose is concomitantly added at 1% (w/w soil), fungal populations are markedly reduced (~75%) during the 1st week and bacterial populations are markedly stimulated (~350%) during the first 2 weeks after treatment, relative to population levels in sucrose-amended untreated soils. In each case, populations return to near normal levels by the 3rd week. The growth and spore germination of the fungus Trichoderma harzianum on potato-dextrose agar are reduced 50% by chlorothalonil at 12 and 15 ppm, respectively. Soil phosphatase activity is not affected by treatment of soil with Bravo 6F at 10 ppm ai.

Chlorothalonil and its metabolites can be accumulated by various vegetable crops planted in rotation cycles. When [^{14}C]chlorothalonil was aged with sandy loam soil, <3% of the applied amount (10 ppm) remained 14 weeks later when the soil was seeded with vegetables. However, chlorothalonil residues accumulated at levels of 0.02 and 0.03 ppm in 90-day-old carrot roots and 63-day-old beans, respectively. Total ^{14}C residues in 63-day-old lettuce were at 1.19 ppm, about 90% of which were extractable unidentified water-soluble metabolites. Total ^{14}C residues in 90-day-old carrot tops and roots were present at 2.2 and 0.4 ppm, respectively; 70-90% of these residues were extractable unidentified water-soluble metabolites. Two identified metabolites, each present at about 0.1 ppm (in tops) and 0.07 ppm (in roots), were 4-hydroxy-2,5,6-trichloroisophthalonitrile (metabolite I) and 3-cyano-2,4,5,6-tetrachlorobenzamide (metabolite II). Radiolabeled residues in 63-day-old beans ranged from 3.72 to 7.85 ppm in various nonfruiting sections, and residues in the pods and beans were at 0.87 ppm. Combined residues of metabolites I and II were at about 0.9-1.7 ppm in nonfruiting sections and about 0.4 ppm in pods and beans. About 70-90% of the total residues were unidentified extractable metabolites.

Chlorothalonil and its metabolites will also accumulate in bluegill sunfish to a moderate extent but are rapidly depurated. At low concentrations of [^{14}C]chlorothalonil (0.003 ppm) in water, the maximum bioconcentration factor for ^{14}C residues is about 200, occurring during 3-7 days' postexposure in a flow-through system. Depuration of ^{14}C residues has a half-life of about 10 days. Extractable polar and nonpolar residues each represent about 17% of the applied chlorothalonil. When the fish are exposed to the radiolabeled metabolite 4-hydroxy-2,5,6-trichloroisophthalonitrile at 0.005 ppm, ^{14}C residues are concentrated by a factor of about 50 during the first 3 days postexposure. Levels decline thereafter to 0.07 ppm by the 28th day of exposure, and by the 10th day of depuration they are <0.01 ppm. However, at a high level of exposure to the metabolite (0.5 ppm), about 15% of the fish die within 1 month, at which time a bioaccumulation factor of about 100 is obtained. Levels decline thereafter with a half-life of about 15-20 days, and depuration has a half-life of about 10 days. About one-third of the ^{14}C residues are extractable, and the majority of these are polar.

4. RECOMMENDATIONS

- 4.1 The fate of chlorothalonil in the environment has not been sufficiently described to support the proposed soybean use. Only the following data requirements have been satisfied:

- 4.1.1 Hydrolysis
- 4.1.2 Photolysis
- 4.1.3 Aerobic soil metabolism
- 4.1.4 Leaching
- 4.1.5 Effects of microbes on the pesticide
- 4.1.6 Bluegill fish accumulation study

- 4.2 The following data requirements have not been fully satisfied:

- 4.2.1 Anaerobic soil metabolism - See the recommendations of our review of 677 - 313, 6F 1799 (soybeans) dated June 22, 1976, section 4.3.5 This requirement has not been addressed.
- 4.2.2 Field dissipation - Such data were previously submitted and reviewed in our evaluation of PP 1F 1024 dated July 15, 1971. Field dissipation of the parent compound was shown but analysis was for parent compound only and soil profiles were not provided. Field dissipation data are needed as described in the July 10, 1978 Proposed Guidelines, sections 163.62-10(a) and 163.62-10(b)(1).
- 4.2.3 Catfish accumulation study - According to the July 10, 1978 Proposed Guidelines, sections 163.62-11(a) and 163.62-11(d), these data are needed.
- 4.2.4 Effects of the pesticide on microbes - The submitted data satisfied this requirement in part. Refer to our evaluation of 677-313, 6F1749 dated May 26, 1978, section 5.2.1 (5), pages 16-17, for other microbes and microbe functions to be tested.
- 4.2.5 With regard to the soil adsorption/desorption study, we note the soil was sieved at 60 mesh which removed large sand particles. We would expect this to result in higher K values than expected if such fine sieving had not been done. How do you anticipate the sieving done in this study affected the K values derived compared to the K values that would be derived if sieving were not done?
- 4.3 The laboratory rotational crop data show the need for an 18 month crop rotational restriction in that ^{14}C residues are present in the rotational crop at the tested rotational interval. To remove the 18 month crop rotation restriction, rotational crop data acquired under actual field use conditions are needed. These data must show no detectable residues at the desired rotational interval. These field data must be compared to the lab ^{14}C data. Refer to the Proposed Guidelines, sections 163.62-11(a), and 163.62-11(b).

To enable review of the field rotational crop data, the following questions must be answered: What is the nature of the water soluble ¹⁴C residues found in the lab ¹⁴C rotational crop study? How many compounds are in this water soluble fraction? Is the benzene ring intact in these compound(s)? Would or would not these residues be expected to be found in crops grown in non-radio-labeled chlorothalonil fortified soil and why?

- 4.4 In response to our call to the PM for actual data collected under protocol RM-78-0018, RM-79-0003, RM-78-0032, 078-3EI-79-0030-000, RM-78-0017, 268-3EI-79-0031-000, 326-3EI-79-0009-000, 329-3EI-79-0012-000, 327-3EI-79-0010-000, and 328-3EI-79-0011-000, we received no data but 3 more copies of the same protocol under a cover letter from Diamond Shamrock dated June 26, 1980. Since no data were received, we assume the data have not been generated. Protocol were not reviewed per the RD/HED Submission Review Record. (See attached).

Samuel M. Creeger Oct 20, 1980
Samuel M. Creeger
October 16, 1980
EFB/HED



Diamond Shamrock

June 26, 1980

Mr. Henry M. Jacoby
Product Manager (21)
Fungicide-Herbicide Branch
Registration Division (TS-767)
U.S. Environmental Protection Agency
Waterside Mall - East Tower
401 M Street, S.W.
Washington, D.C. 20460

Subject: Bravo 500 (EPA Reg. No. 677-313)
Amended Soybean Petition No. 6F1799
Environmental Chemistry Section J
Accession No. 099248

Dear Mr. Jacoby:

Please find enclosed three (3) copies of studies from Section J that were missing. The studies involved are; beginning with the following page numbers, 235, 255, 287, 307, 365, 385, 414, 440, 467 & 496.

These were not studies but protocol.
SMC

Thank you for your cooperation and we are sorry for any inconvenience that this may have caused.

Very truly yours,

Diamond Shamrock Corporation

Ralph P. Burton

Ralph P. Burton, Supervisor
Product Registrations

RPB:ctd

need in response to our
call for the data generated
under these protocol
SMC
EFB/HED

SUBMISSION REVIEW RECORD

Chemical Name <u>Chlorothalonil</u>		Date Received (EPA) <u>2/19/80</u>
Identifying No. (Reg.No./File Symbol/Petition No./EUP No./ SLN No./Etc.)	Action Code	Identifying No.
		Action Code
		Date Sent to HED <u>2/25/80</u>
		Statutory Due Date <u>5/25/80</u>
		(45 days) (90 days) (120 days)
<u>651799</u>	<u>332</u>	Projected Return Date <u>3/6/80</u>
<u>677313</u>	<u>332</u>	Product Mgr. <u>Freely</u>
		PM Team No. <u>21</u>

INSTRUCTIONS TO HED REVIEWER

☒ Incremental Risk Assessment [§3(c)(7)]. ☐ Total Assessment [§3(c)(5)].

Description: petition for tolerance - addition of new uses
to label - submission of application for tolerance
in registered tolerance.

§3(c)(1)(D):

☒ Use any or all available information. ☐ Use only attached data.
☐ Use only the attached data for formulation and any or all available information on the technical or manufacturing chemical.

Review Req.	Type of Review	Number of Actions						
		Regis.	Petition	EUP	SLN	§ 18		
☒	Toxicology							
☒	Ecological Effects							
☒	Residue Chemistry							
☒	Environmental Fate							

☒ Representative Labels Showing Accepted Uses Attached.

Date Returned to RD (to be completed by HED) _____