6-20-78

EEB REVIEW REVIEW

DATE: IN ____ OUT ____ IN 4/12/77 OUT ~~5/26/78~~ 6/20/78 IN ____ OUT ____

FISH & WILDLIFE ENVIRONMENTAL CHEMISTRY EFFICACY

FILE OR REG. NO. 677-313

PETITION OR EXP. PERMIT NO. 6F1749

DATE DIV. RECEIVED 4/6/77

DATE OF SUBMISSION

DATE SUBMISSION ACCEPTED 4/12/77 3C1D - No

TYPE PRODUCT(S): I, D, H, (F,) N, R, S

PRODUCT MGR. NO. 21 (E. Wilson)

PRODUCT NAME(S) Bravo 6F

COMPANY NAME Diamond Shamrock

SUBMISSION PURPOSE Amend label - add use on peaches and cherries (sour)

CHEMICAL & FORMULATION Chlorothalonil (tetrachloroisophthalonitrile)

1.0 Introduction

1.1 The registrant wants to amend the label by adding uses on peaches and sour cherries. This is a re-submission.

1.2 See previous reviews of 677-313 (6F1749, peaches and cherries and 6F1799, soybeans) dated April 27, 1976 and June 22, 1976.

1.3 Accession number 096074.

2.0 Directions for Use

2.1 Peaches

Apply 3/4 to 1 1/8 lb. ai per 100 gallons of water not to exceed 5 5/8 lb ai/A/application. Repeat as directed.

2.2 Sour cherries

Apply 9/16 to 1 1/8 lb ai per 100 gallons of water not to exceed 11 1/4 lb ai/A/application. Repeat as directed.

2.3 Do not apply within 7 days of harvest. Apply by ground equipment only. Do not graze treated areas.

2.4 Do not reuse empty container. Destroy by perforating, crushing and burying or discarding in a safe place. Do not apply where runoff or when drift may occur. Do not contaminate water with waste disposal.

2.5 This product is toxic to fish.

3.0 Discussion of Data

3.1 The registrant has responded to RD questions in the August 3, 1977 letter and has submitted the new data listed below:

1. Effect of Chlorothalonil on Populations of soil Microorganisms

2. The Effect of Chlorothalonil on Soil Microflora

3. Effect of Chlorothalonil on Soybean Nitrogen Fixation

4. Biodegradation of Daconil 2787

This data is submitted as an amendment to the original registration application of February, 1976 (PP 6F1749). See our review of 6F1749, reg. #677-313 dated April 27, 1976.

3.2 Data review

3.2.1 The registrant's responses to RD questions as posed in point 6 of the August 3, 1977 letter, satisfactorily resolve those questions.

3.2.2 Effect of Chlorothalonil of Populations of Soil Microorganisms (page J - 239)

In vitro and in vivo (soil, tomato foliage) evaluations of chlorothalonil effects on soil microbes were done.

Results

1. In Vitro Fungicide Bioassay

Fungicide	% Inhibition of Mycelial Growth (1)			
	Bc	As	Py	Rs(2)
Chlorothalonil	85	65	39	70
Captan	91	73	56	66
Cyprex	71	93	89	22
Benomyl	100	47	19	100

(1) Concentration = 100 ug active ingredient/ml medium.

(2) Assay organisms:

Bc = Botrytis cinerea; As - Alternaria solani; Py = Pythium sp.; Rs = Rhizoctonia solani

2. Soil Fungicide Bioassay
Control of Rhizoctonia solani Seedling Disease

Fungicide	% Healthy Seedlings (1) (2)			
	Dosage-Pounds Per Acre			
	64	32	16	8
Chlorothalonil	50	37	13	0
Benomyl	100	100	100	94
PCNB	94	75	47	13

(1) Host: Phaseolus aureus

(2) Uninoculated control: 100% healthy seedlings
Inoculated control: 0% healthy seedlings

3. In Vitro Bactericidal Activity of Chlorothalonil

Organism	% Inhibition Concentration -ug/ml 900	64
Erwinia amylovora	0	-
Xanthomonas phaseoli	0	-
Escherichia coli	0	0
Salmonella gallinarum	-	0
Salmonella cholera-suis	-	0
Streptococcus faecalis	-	0
Staphylococcus aureus	0	0

4. Control of Xanthomonas vericatoria on Tomato Foliage

	% Disease Control Spray Concentration - ug/ml 1000	200	100	50	25
Chlorothalonil	0	-	0	0	0
Agrimycin	-	93	70	66	63

5. Reduction of Biological Activity of Chlorothalonil With Montmorillonite Clay (1)

Amendment	% Inhibition of Germination
None	0
Chlorothalonil - 1 ug/ml	90
Montmorillonite clay 1000 ug/ml	0
Chlor. 1 ug/ml + clay 1 ug/ml	84
Chlor. 1 ug/ml + clay 10 ug/ml	78
Chlor. 1 ug/ml + clay 100 ug/ml	47
Chlor. 1 ug/ml + clay 1000 ug/ml	0.5

(1) Percent inhibition of germination of conidia of Alternaria solani on potato dextrose agar amended as indicated. Incubated 4 hr. at 27° C.

Conclusions

1. Chlorothalonil becomes biologically unavailable through binding to clay particles at rates of 1 part chlorothalonil to 1000 parts clay.

2. The 7 bacteria tested consisted of 6 human pathogens not normally found in the soil

3. There was no consistency in the parameters tested and observed so we could not compare results.

4. This study does not satisfy our requirements for a study on the effects of chlorothalonil on soil microbes because:

   a. We do not know the concentration in lb ai/A represented by 100 ug ai/ml medium in the chart titled In Vitro Fungicide Bioassay.

b. The studies on the control of Rhizoctonia solani and on Xanthomonas vesicatoria employ plant pathogens which is unsuitable.

c. The chart on bactericidal activity of chlorothalonil on 7 bacteria includes 6 human pathogens which is unsuitable.

d. We do not know the equivalent lb ai/A concentration represented by 1 ug chlorothalonil/ml potato dextrose agar in the chart titled Reduction of Biolcgical Activity of Chlorothalonil With Montmorillonite Clay.

5. An example of an acceptable protocol is given in the recommendations.

3.2.3 The Effect of Chlorothalonil on Soil Microflora (page J-246)

About 50 grams of sandy loam soil (62% sand, 31% silt, 6.8% clay, 3.2% OM, pH = 6.7 and 1/3 bar moisture = 25.9) was fortified with cold chlorothalonil to 20 ppm. Distilled water was added to 90% saturation and the flasks were sealed and incubated at 28° C. Samples were taken at 45 and 90 days and the soil was analyzed for parent chemical at 55 days.

Effect of Chlorothalonil Soil Treatment on Colony Counts of Soil Microflora

Medium	Colony Forming Units Per Gram X 10^4** At 45 days Control	At 45 days + Chlorothalonil	At 90 days Control	At 90 days + Chlorothalonil
Total Bacteria				
KH*	1630(230)[a]	920(99)[a]	800(158)[c]	1090(335)[c]
TA	1440(650)[b]	710(366)[b]	1250(573)[g]	1500(784)[g]
Actinomycetes				
SCA	66(8.5)[h]	74(48)[h]	90(10.5)[d]	100(15)[d]
RBA	4.4(3.6)[h]	4.3(4.9)[h]	7.8(5.3)[e]	11(4.5)[e]
Fungi				
RBSF	7.0(3.3)[g]	5.9(2.1)[g]	4.5(2.6)[h]	4.2(2.7)[h]
MAF	17.0(7.9)[c]	10.3(4.5)[c]	12.6(2.4)[h]	12.0(8.0)[h]
V-8	14.7(5.0)[b]	7.7(4.7)[b]	17.3(11.7)[i]	12.6(6.4)[f]

* The agar media used to enumerate the soil organisms were grouped as media specific for bacteria, actinomycetes, and fungi. Accordingly, the following codes were established. For bacterial media: KH, general bacterial count medium (Kado, 1970), and TA, tryptose agar for total aerobic bacterial counts (Kolacz, 1970). For actinomycetes media: SCA, starch caseinate agar plus antibiotics, selective (Williams, 1965), and RBA, rose bengal agar plus antibiotics (Williams, 1965). For fungi media: RBSF, rose bengal agar (Martin, 1950), MAF, malt agar plus lactic acid and antibiotics (Difco manual), and V-8, tomato juice agar plus antibiotics (Papavizas, 1959).

** Values shown are mean and standard deviation (in parenthesis) calculated from agar dish counts as described.

Treatment versus control mean values for each specified period (days) and growth medium are significantly different at or greater than the following confidence limits calculated according to t-test for sample mean of unpaired variates:

a = 99%	e = 70%
b = 95%	f = 60%
c = 90%	g = 50%
d = 80%	h = not significantly different

Conclusions

1. This study is unacceptable for the following reasons:

   a. The soil samples were incubated in sealed jars for 45 days before the first sample was taken. During this time conditions most probably changed from aerobic to anaerobic with a resultant change in microfloral populations present.

   b. Sampling should be done several times during the first week of the experiment and weekly thereafter in order to see any initial population suppression and recovery.

   c. The experiment should be run so the identity of the microbes affected are known, such as testing effects on pure or mixed culture populations of representative soil microorganisms.

   d. What antibiotics were added to the growth media?

2. An example of an acceptable protocol is given in the recommendations.

3.2.4 Effect of Chlorothalonil on Soybean Nitrogen Fixation (page J-253)

Soybean plants, grown from seed innoculated with Rhizobium japonicum, were subject to an acetylene reduction assay at the mid pod filling stage. At this stage the aerial portion of the plant was removed and the soil and root mass treated with Chlorothalonil by soil drench to 0, 2, 4, 20 or 40 ppm. Assays were run 1 day prion to treatment and 1 and 8 days after treatment.

A parallel test was run in which soybeans were planted in soil fortified at 0, 2 and 5 ppm chlorothalonil, and then subjected to an acetylene reduction assay at the early mid pod filling stage.

Results

Effect of Chlorothalonil on Soybean Nitrogen Fixation Activity (Acetylene Reduction Activity)*

(Soil drench at soybean mid-pod stage)

u mole C_2H_4/Hour/Plant

A	B			C	
One Day Prior to Treatment	Chlorothalonil Treatment ppm	One Day After Treatment	Ratio B/A	8 Days After Treatment	Ratio C/A
19.34 ± 5.54	0	20.78 ± 7.19	1.07	20.24 ± 3.66	1.05
14.02 ± 3.68	2	19.09 ± 6.02	1.36	15.73 ± 3.71	1.12
13.62 ± 7.52	4	16.27 ± 5.97	1.19	15.61 ± 2.80	1.15
15.96 ± 9.38	20	21.09 ± 7.09	1.32	14.75 ± 3.10	0.92
15.42 ± 5.81	40	18.46 ± 3.53	1.19	14.12 ± 1.03	0.92

* Data are presented as u mole C_2H_4/hour/plant.

To estimate ug N2/hour/plant, use C_2H_4:N_2 ratio of 3.0 (Hardy, et al, 1968).
For example, in column A, 19.34 u mole C_2H_4/hour/plant = 180.51 ug N_2/hour/plant.

2. Effect of Chlorothalonil on Soybean Nitrogen Fixation Activity (Acetylene Reduction Activity)*

(Soil incorporation prior to planting)

Chlorothalonil (ppm)	u mole C_2H_4/Hour/Plant	Estimated** ug N_2/Hour/Plant
0	61.12 ± 15.17	570.43 ± 141.58
2	99.79 ± 38.45	931.34 ± 358.85
5	85.78 ± 34.36	800.58 ± 58

320.63

* Data are presented as Umole C_2H_4/hour/plant.

Assay was carried out when soybean plants were at early mid-pod stage.

** N_2 was estimated by theoretical conversion factor of 3 moles C_2H_4:1 mole N_2 (Hardy, et al, 1968)

Conclusions

1. This was not a study on a free-living nitrogen fixing bacteria but on the symbiotic relationship of Rhizobium japonicum and the soybean roots.

2. We need an assay of the acetylene used in this acetylene reduction assay since some impurities are lethal to soil microbes.

3. How old were the plants during the experiment? What percent growth have the roots attained at this point?

4. What were the results of the soil assay (described on page J-257) substantiating the theoretical chlorothalonil soil concentrations?

5. An example of an acceptable protocol is given in the recommendations.

3.2.5 Biodegradation of DACONIL 2787 (page J-264)

Our evaluation of 677-313 (6F1749 dated April 27, 1976 (p. 15) states that this study was reviewed and accepted August 6, 1971. We will not review or validate this study per Dr. Rogoff's memo of August 12, 1977.

4.0 Conclusions

4.1 In response to RD letter of August 3, 1977, (6F1749, reg. no. 677-313, peaches and cherries), the registrant has submitted new data, previously reviewed data, and has referenced previously reviewed data in responding to our questions.

4.2 The evaluation of the new data (the 3 microbe studies reviewed in 3.2.2, 3.2.3 and 3.2.4 above) allows us to conclude that the studies are unacceptable. See the recommendations below.

4.3 The registrant's responses to RD questions as posed in point 6 of the August 3, 1977 letter satisfactorily resolve those questions.

4.4 We note the data gap of a fish accumulation study.

4.5 Below are the conclusions of several past reviews of chlorothalonil. Note that per Dr. Rogoff's memo to Mr. Campt dated August 12, 1977, we did not validate the data from which the conclusions were drawn.

4.5.1 July 15, 1971 evaluation

1. Laboratory Test

The petitioner showed that Daconil 2787 degrades rapidly in contact with soil to DAC 3701 and unextractables. However, in many instances the identity of the soils were not given or their composition. Therefore, the major factors that influence DAC 2787 dissipation could not be totally assessed. Although it was demonstrated

that temperature, organic content and moisture content effect the rate of degradation of DAC 2787 no conclusive relationship could be established to show which had the greatest effect on degradation in different type soils. Also, analysis of DAC 3701 was not made. The soil pH had no apparent effect on the degradation of DAC 2787 in Lismore silty clay loam. Since this data is limited to one type soil, it would be difficult to justify an extrapolation of this conclusion to other type soils.

Additional data are needed on other soils to establish conclusive or a reasonable relationship that pH does not effect the degradation of DAC-2787 in soil

2. Field Test

The degradation rate of DAC 2787 was evaluated at 3 separate locations under field conditions. It was determined that DAC 2787 degrades rapidly in soil; leaches poorly and lacks mobility through the soil tested. These tests did not include DAC 3701. The degradation rate of DAC 2787 in field test correlates well with laboratory test. However, inadequate soil descriptions in terms of physical and chemical characteristics and the lack of identification of soils preclude the establishment of meaningful relationships and interpretation of the results.

3. The soil sterilization study indicated that sterile soil prolong the persistence of DAC 2787 in soil.

4. The test on volatilization effectively demonstrated that DAC 2787 was not lost by volatilization.

5. Refer to this evaluation for additional conclusion on animal metabolism, plant metabolism, etc.

4.5.2 August 6, 1971

On 7/28/71 they responded to our questions pertaining to leaching of DAC 3701.

An evaluation of their 7/28/71 reply from Dr. Eisler did not allow chemistry to conclude that "residues of DAC 3701 cannot reach the water table."

Field data submitted in the 7/28/71 respond was for muck soil only. Leaching is minimal in this type of soil since it has a high exchange capacity.

Degradation of 3701 in clay and loam soils is slow enough to allow significant leaching in these types. Also, these data were obtained under drastic conditions (incubation at 35-37° C).

Therefore, we must assume from data supplied that Daconil rapidly breaks down to 3701 which rapidly leaches but slowly degrades.

We request a reevaluation by Dr. Cueto of this petition on the basis of possible low level residues resulting in shallow well water supplies.

If the possibility of low level in water does not represent an impediment to registration, chemistry is in a position to discuss test protocols aimed at evaluating the rates of leaching and magnitude, if any, or ground water contamination.

It is not anticipated that well water levels would exceed 0.1 ppm. It should be noted that tolerance of up to 15 ppm have been allowed in crop material. This tolerance includes DAC 3071.

4.5.3 April 27, 1976 evaluation

Daconil does not leach; it has a half life of less than 30 days. Therefore, no problems are anticipated with regard to the persistance of parent Daconil. However, the principle degradation product DAC 3701 is

extremely persistent (no dissipation of this product was seen within 90 days). DAC 3701 leaches significantly in many types of soil. Both Daconil and DAC 3701 are stable to hydrolysis. Daconil is stable to photodegradation in solution and on surfaces. DAC 3701 is stable to photolysis on surfaces.

The half life of total 14_C in soil is quite long 90 days. The limited studies submitted seem to show that the 14_C is in the form of extractable residues. Clearly, for other applications we will need rotational crop data to assess this apparent hazard.

No rotational crop data was submitted as it is not germane to orchard crop uses.

Daconil shows a plateau bioconcentration of 200 x edible and 3000 x in visceral tissue; 50% is eliminated after two weeks exposure to clean water. DAC 3701 showed fish mortality (18% at .6 ppm; at lower concentrations bioconcentration plateaued at 50 x edible, 250 x nonedible.

4.6 Products formed from the parent

DAC 3701 -(4-hydroxy-2,5,6-trichloroisophthalonitrile) is formed in soil, cow milk and tissues, under pH 5, 7 and 9 (major) hydrolysis and under photolysis.

DS-19221-(3-cyano-2,4,5,6-tetrachlorobenzamide) is formed in soil and under pH 5, 7 and 9 (major) hydrolysis.

5.0 Recommendations

5.1 We do not concur with the proposed new uses.

5.2 The 3 new microbe studies, submitted in response to point 5 of the August 3, 1977 letter, do not satisfy our requirements as follows:

5.2.1 The study titled "Effect of Chlorothalonil on Populations of Soil Microorganisms" is not adequate since

1. We do not know the concentration in lb ai/A represented by 100 ug ai/ml medium in the chart titled In Vitro Fungicide Bioassay.

2. The studies on the control of Rhizoctonia solani and on Xanthomonas vesicatoria employ plant pathogens which is unsuitable.

3. The chart on bactericidal activity of chlorothalonil on 7 bacteria includes 6 human pathogens which is unsuitable.

4. We do not know the equivalent lb ai/A concentration represented by 1 ug chlorothalonil/ml potato dextrose agar in the chart titled Reduction of Biological Activity of Chlorothalonil With Montmorillonite Clay.

5. An example of an acceptable protocol is below.

Effects of Pesticides on Microbes

Data on Effects of pesticides on microbes are obtained from studies of effects on microbial functions or microbial populations. Studies of effects on microbial function constitute a more direct approach, and are preferred to studies of effects on populations. Some effects cannot be measured directly and populations studies may be the only recourse. When the functional approach is chosen, the effects on nitrogen fixation, nitrification, cellulose, starch and protein degradation are required. When the population approach is chosen, effects on pure or mixed culture populations of representative microorganisms from soil or obtained from culture collections are required. Appropriate organisms include free-living nitrogen-fixing bacteria and blue-green algae such as Azotobacter, Colostridium and

Nostoc, and nitrifers such as Nitrosomonas and Nitrobacter. For cellulose, starch, protein and similar degradation include at least one each of soil bacteria, actinomycetes, and molds such as Bacillus, Pseudomonas, Arthrobacter, Cellulomas, Cytopaga, Streptomyces, Penicillium, Flavobacterium, Trichoderma, Aspergillus, Chaetomium, and Fusarium.

Animal or plant pathogens and indicators of fecal pollution are unsuitable.

Information on organism identity and media must be supplied. Organisms used as indicator must be identified by Linnaean name as well as common name. Cultures of microorganisms obtained from collections must also be identified by collection code numbers; other sources of microorganisms must be described. Photographic evidence for claimed pure cultures not derived from collections must be submitted. Standard maintenance and test media must be identified and other media identified and described.

6. Since the study most probably cannot be made acceptable, it should be repeated according to the above protocol.

5.2.2 The study titled "The Effect of Chlorothalonil on Soil Microflora" is lacking since

1. The soil samples were incubated in sealed jars for 45 days before the first sample was taken. During this time conditions most probably changed from aerobic to anaerobic with a resultant change in microfloral populations present.

2. Sampling should be done several times during the first week of the experiment and weekly thereafter in order to record any initial population suppression and recovery.

3. The experiment should be run so the identity of the microbes affected are known, such as testing effects on pure or mixed culture populations of representative soil microorganisms. (See the sample protocol).

4. What antibiotics were added to the growth media?

5. Since the study most probably cannot be made acceptable, it should be repeated according to the above protocol in 5.2.1 paragraph 5.

5.2.3 The study titled "Effect of Chlorothalonil on Soybean Nitrogen Fixation" is not acceptable since

1. This was not a study on a free living nitrogen fixing bacteria but on the symbiotic relationship of Rhizobium japonicum and the soybean roots.

2. We need an assay of the acetylene used in this acetylene reduction assay since some impurities are lethal to soil microbes.

3. How old were the plants during the experiment? What percent growth have the roots attained at this point?

4. What were the results of the soil assay (described on page J-257) substantiating the theoretical chlorothalonil soil concentrations?

5. Since the study most probably cannot be made acceptable, it should be repeated according to the above protocol in section 5 2.1 paragraph 5.

5.3 The registrant's responses to RD questions as po in point 6 of the August 3, 1977 letter, satisfacto resolve those questions.

5.4 We note the data gap of fish accumulation studies.

5.5 Other uses may require additional environmental chemistry data.

5.6 Per Dr. Rogoff's memo of August 12, 1977, previously reviewed data is not being rereviewed or validated at this time.

Samual M. Creeger
Environmental Chemistry Section
EEB
May 23, 1978