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OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: PP#6E3410 -- Chlorothalonil in/on Mushrooms. Amendment Dated 3/10/92.

DP Barcode: D176820. CBTS # 9738.
MRID # 422455-01, -02.

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12/8/92

TO: Hoyt Jamerson, PM 43
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and

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IR-4's present submission is a response to our memo of 6/22/89 (S. Willett) and a subsequent meeting held 2/27/90 (Memorandum of Conference dated 3/7/90). In the 6/22/89 memo we concluded that the nature of the residue of chlorothalonil in mushrooms was only partially understood. "Better characterization of the unidentified water soluble compounds is needed before this tolerance request for use of chlorothalonil on mushrooms can be considered." In the 3/7/90 conference, CBTS (then DEB) suggested that the proposed application rate and possibly the PHI be changed to reduce chlorothalonil levels. The need for additional plant metabolism data "could possibly be reconsidered" after such an amendment has been submitted.

IR-4's submission includes revised Sections B and F with supporting residue data. The tolerance proposed for chlorothalonil, tetrachloroisophthalonitrile, and its metabolite, 4-hydroxy-2,2,6-trichloroisophthalonitrile [SDS-3701],in/on mushrooms has been changed from 8 ppm to 2 ppm.

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Summary of Deficiencies Remaining to Be Resolved

1. Complete sample histories from the Avondale, PA and Chatham, PA field trials should be submitted. This information should include dates of analysis.

2. The petitioner should submit a revised Section F in which a tolerance of 7.0 ppm is proposed for the combined residues of chlorothalonil and its metabolite SDS-3701 in/on mushrooms.

Conclusions (relating to this memo only)

1. The nature of the residue in mushrooms is adequately understood. The residue to be regulated is chlorothalonil, per se, and its metabolite SDS-3701. Although the soil metabolism study indicates that chlorothalonil is converted principally to metabolite SDS-46851, 3-carboxy-2,5,6-trichlorobenzamide, mushroom residue analyses suggest that soil metabolism is not the principal source of residue. Chlorothalonil is the major species found.

2. Residue data from two field trials were submitted. However, the data from the Avondale, PA trial do not include sample histories, including dates of analysis. This information must be submitted if results from this trial are to be used in the determination of the anticipated residue of chlorothalonil in/on mushrooms.

3. Previous residue data do not support the proposed tolerance of 2.0 ppm. Unless the petitioner can demonstrate why previous data should not be considered, the petitioner should submit a revised Section F in which a tolerance of 7.0 ppm is proposed for residues of chlorothalonil and its metabolite SDS-3701 in/on mushrooms.

Recommendation

CBTS recommends against the proposed tolerance for reasons given in Conclusions 2 (incomplete information from PA field trial) and 3 (revised Section F needed).

Detailed Considerations

IR-4's revised Section B reads in part as follows:

Apply to casing soil surface of culture beds using

sufficient water to obtain adequate coverage, at least 20 gallons per 1,000 sq. ft. Make the first application of 2.75 fluid oz. [0.13 lb ai] per 1,000 sq. ft. at pinning and 2.75 fluid oz. per 1,000 sq. ft. between each break. Apply no more than 4 applications (11 oz.) per crop. DO NOT apply BRAVO 720 to a mature mushroom or to beds within 48 hours of when the next mushroom harvest is to be made.

We assume that a registration is being sought only for BRAVO 720, which contains 6 lb active ingredient (ai) per gallon (0.0469 lb ai/fl. oz.) The previous label also contained use directions for BRAVO 500 and BRAVO W-75. The total quantity of active ingredient applied per crop is 0.52 lb. The previous maximum use level per crop was 19.25 fl oz., or 0.90 lb ai.

Nature of the Residue

Metabolism of chlorothalonil in plants was reviewed by S. Willett in her memo of 6/22/89. New data have not been submitted since then; however, a DCI was issued 7/31/91 in which metabolism studies were required in celery and snap beans as part of the reregistration requirements for that pesticide. As requested by IR-4 in the 3/7/90 meeting, we are reconsidering metabolism requirements for use in/on mushrooms in the light of the submitted data.

Metabolism data were reviewed for lettuce, carrots and tomatoes as well as a rotational crop study on wheat, carrots and lettuce. Data from a field rotational crop study (cold study) done on wheat, carrots, snap beans and spinach were also reviewed.

Application of chlorothalonil to lettuce foliage resulted in little metabolism of parent at 21 days. At this time 87.1% of the residue was chlorothalonil, 2.0 percent was SDS-3701 (see Attachment), and 10.9% remained unidentified.

When chlorothalonil was foliarly applied to carrots at a total rate equivalent to 4.3 lb ai/A, the total residue in one root sample at 21 days was 0.051 ppm, of which 45.3% was parent and 3.9% was SDS-3701. Characterization in foliage was inadequate. In one 21 day sample having total residue of 9.7 ppm, 26.6% was identified as parent and 9.9% was identified as SDS-3701. No attempt was made to identify the aqueous polar fraction or post extraction solids which together comprised over 50% of the total residue. No chromatograms were submitted.

Chlorothalonil was foliarly applied to tomatoes at a total rate equivalent to 6.3 lb ai/A. The total residue in fruit at 14 days averaged 0.6 ppm.; the total residue in vines averaged 14.0

ppm. At 14 days, in fruit, parent averaged 58.3% of the total residue, SDS-3701 averaged 3.0%. Polar non-extractables, i.e., aqueous solubles, averaged 31.4%. No species were identified in this fraction, but base hydrolysis followed by methylation and GC/MS showed that 50% had been converted to 5-chloro-2,4,6-trimethoxyisophthalonitrile, the trimethoxy analog of parent. The authors concluded that the actual metabolite(s) had two intact CN groups and suggested mono- or disaccharide conjugates. At 14 days, in vines, parent averaged 41.4% of the total residue; SDS-3701 averaged 7.5%. No attempts were made to characterize the aqueous or solid fractions.

Because chlorothalonil is applied to soil in the growing of mushrooms, the crop rotation study with ^{14}C-chlorothalonil (MRID # 00139550) is especially relevant:

Soil was treated with 10 ppm chlorothalonil and aerobically aged in the absence of light. Treated soil was analyzed 30 and 88 days after treatment. The soil was extracted with acetone/HCl. No attempt was made to characterize the solids ("post extraction solids", or PES). The acetone extract was rotoevaporated and the remaining aqueous solution extracted with ether. The ether extract was analyzed by HPLC. The remaining water solubles were not characterized.

Lettuce, wheat and carrots were grown in treated soil aged 30 and 88 days and harvested at maturity. Plant parts were extracted in the same manner as soil except that characterization was more extensive: The organosoluble (ether) fraction was also methylated and analyzed by GC. The water solubles remaining from ether extraction were evaporated and the residue refluxed in butanol/HCl. The resulting solution was treated in the same way as the initial acetone/HCl solution, and the resulting ether solubles were quantitated by HPLC or GC/MS. No attempt was made to characterize the post extraction solids. It was felt that fractions not solubilized by acetone/HCl would not be bioavailable.

Results of soil analyses at 30 and 88 days are given in the following table. Structures of metabolites are given in an attachment to this memo. SDS-2787 is parent chlorothalonil. In addition, soil samples taken from pots in which lettuce, wheat or carrots were grown was removed and analyzed when the crops were harvested. Post treatment times varied from 62 to 157 days. Six soil samples were analyzed -- lettuce, wheat and carrots grown in soil aged 30 days and in soil aged 88 days. The averages are given in the last row of the table. Complete results appear in Table 1 of the original report.

Table 1

Distribution of Chlorothalonil and Metabolites in Soil Samples

	Mass Balance	Distribution of Residue (% of Recovered Radioactivity)							
		2787	46851	3701	19221	47523/4	47525	PNE[1]	PES[2]
30 Day	94.1%	11.4	22.6	4.0	6.2	3.9	2.0	18.6	26.9
88 Day	82.4%	5.2	24.9	8.8	5.2	1.3	2.1	17.0	32.7
62-157 Days	89.2 ±9.2%	2.2 ±1.3	12.7 ±3.9	7.5 ±1.8	3.0 ±0.7	2.0 ±0.9	<0.5	12.6 ±2.7	57.7 ±6.2

1. PES = Post Extraction Solids 2. PNE = Polar Nonextractable, i.e., aqueous fraction.

At harvest the plants were separated into their respective parts, ground and their residue levels determined by combustion and LSC. Average residue levels found in lettuce and carrots varied from 0.9 ppm (carrot root grown in soil aged 88 days) to 3.3 ppm (lettuce in soil aged 30 days). Except for carrot top, levels from lettuce or carrot grown in soil aged 88 days were lower than corresponding levels from soil aged 30 days. However, residue levels in wheat grain, chaff and straw were not only much higher than levels found in the other RACS but were significantly higher when grown in 88 day aged soil. Wheat grain from wheat grown in 30 day aged soil averaged 3.3 ppm; wheat grain from wheat grown in 88 day aged soil averaged 21.6 ppm. Corresponding values for straw and chaff were 51.9 and 63.8 ppm for straw and 7.8 and 43.9 ppm for chaff.

Plant matrices were subjected to the extraction scheme described above. The aqueous soluble fractions -- those remaining after the ether extraction -- were shown to consist of compounds related to SDS-46851 and SDS-3701. When the aqueous fractions (30 day wheat straw and 88 day samples from all the plant parts) were refluxed in acidic butanol for 96 hours, the free and butyl esters of SDS-46851 and SDS-3701 could be recovered in significant quantities. The summary of distribution of residues in crops as a percentage of total crop residue is given in the following table (Table 10 of the report):

Table 2

Distribution of Residues in Crops
as a Percentage of the Total Crop Residue

		Organosoluble		Aqueous Soluble		
	PES[1]	SDS-46851	SDS-3701	SDS-46851	SDS-3701	PNE[2]
30 Day Wheat Straw	11.9	47.3	<1	13.5	11.9	19.9
88 Day Wheat Straw	5.2	37.3	2.4	14.6	7.5	10.6
30 Day Wheat Grain	22.6	62.9	<1	NA[3]	NA	NA
88 Day Wheat Grain	11.1	59.2	<1	16.9	1.9	4.1
30 Day Lettuce (av.)	4.6	61.7	<1	NA	NA	NA
88 Day Lettuce	3.9	39.6	2.3	5.0	5.8	16.5
30 Day Carrot Root (av.)	4.6	63.0	<1.5	NA	NA	NA
88 Day Carrot Root	7.3	55.5	<1	9.4	5.6	2.2
30 Day Carrot Top (av.)	9.2	45.0	2.5	NA	NA	NA
88 Day Carrot Top	10.4	29.3	2.0	6.3	9.3	13.5

[1] PES = Post Extraction Solids
[2] PNE = Polar Non Extractable (aqueous soluble material after refluxing with acidic butanol.
[3] NA = Not Analyzed

CBTS Comment

Results from this radiolabeled study conclusively show that the major species resulting from uptake from soil are SDS-46851, SDS-3701 and compounds structurally related to these -- perhaps conjugates. Parent chlorothalonil was not detected. Further discussion is given below.

Field Rotational Crop Study (MRID # 00139550)

Chlorothalonil (BRAVO® 500) was applied at three locations (GA, TX and CA) at eight weekly intervals to soil. Spinach, snap beans, carrots and wheat were planted in the treated soil and in untreated plots at intervals of 14, 30, 60, 90 days and approximately 1 year after the last application to soil. The crops were grown to normal maturity and harvested. Crops were only analyzed for SDS-3701 and SDS-46851. The highest residues were found in the CA trials. Levels of SDS-46851 generally increased with the interval between soil treatment and planting. For example, levels in wheat grain grown in CA were 0.17 ppm from wheat planted 14 days after the last treatment and 0.68 ppm from wheat planted 60 days after the last treatment. Thereafter, residue levels declined. It should be noted that soil from CA sampled 60 days after 8 applications of BRAVO, showed chlorothalonil, *per se*, as the major component, followed by SDS-

3701 and SDS-19221. SDS-46851 was not detected. SDS-3701 was the major component of treated TX soil. In general, concentrations of SDS-46851 exceeded those of SDS-3701 in plant matrices, even though SDS-46851 was not found at high concentrations in soil. Samples were not analyzed for chlorothalonil itself, even though it was the major component in the CA soil.

CBTS Comment

Growing of mushrooms in compost treated with chlorothalonil is not exactly analogous to growing crops in soil previously treated with the pesticide. Compost contains more organic matter than soil and the typical pH is about 7. After the full mushroom crop has been harvested, the spent compost is discarded. Nevertheless, it is clear from the metabolism studies and the radiolabeled crop rotation study that the major metabolites have been identified. Metabolites found in the aqueous fraction from the crop rotational study could only be released by acidic butanol reflux. It does not seem likely that these aqueous metabolites would be bioavailable to mushrooms, but even these released metabolites are structurally related to SDS-3701 or SDS-46851.

The two metabolism studies required by the DCI are not likely to produce additional information regarding mushroom metabolism. The crop rotation studies (radiolabeled and field studies) are more useful in this regard. We conclude that the nature of the residue in mushrooms is adequately understood. Chlorothalonil, per se, SDS-3701 and/or SDS-46851 may be the principal constituents of the residue. Available residue data indicate that parent is the major constituent.

Analytical Method

The analytical method is described in SDS Biotech's Report No. 3136-88-0138-MD-001: "General Analytical Procedure for the Determination of Residues of Tetrachloroisophthalonitrile (Chlorothalonil, SDS-2787), SDS-3701, SDS-46851, HCB and PCBN on Selected Crops". The method is similar to Report # 702-3CR-84-0074-001, described in N. Dodd's 11/12/86 memo, except that the earlier method did not include analysis for SDS-46851.

Mushrooms are chopped and extracted with acetone/10 N H_2SO_4 (19/1 v/v). The acetone is evaporated from the filtered extract, and sodium bicarbonate is added to adjust the pH to 4.5. The solution is then extracted with petroleum ether, which removes chlorothalonil, hexachlorobenzene (HCB) and pentachloro-benzonitrile (PCBN). These latter species are separated on a Florisil column and separately quantitated by GC with an electron capture detector. Hazleton Laboratories, performing laboratory for one of the residue studies, omitted the Florisil cleanup "due

to inconsistent recoveries".

The aqueous phase remaining after the petroleum ether partition is adjusted to pH <2 and NaCl added. The resulting solution is extracted with diethyl ether and the ether evaporated. SDS-3701 is converted to its methyl ether derivative and SDS-46851 to its methyl ester derivative using diazomethane. The two derivatives are cleaned up and separately eluted on an alumina column. Quantitation is by GC with an electron capture detector.

The IR-4 California Laboratory used a Hall Electrolytic Conductivity detector in its GC analyses.

Percent recoveries are given in the following table.

Table 3

Percent Recoveries for Chlorothalonil, Impurities and Metabolites

	Hazleton Laboratories		IR-4 California Laboratories	
Compound	Fortification Range (ppm)	Average Recovery	Fortification Range (ppm)	Average Recovery
Chlorothalonil	0.03-5.0	91.8±13.7%	0.02-2.0	94.2±8.4%
HCB	0.01-0.05	96.8±8.8%	0.006-0.3	95.5±4.2%
PCBN	0.02-0.10	81.8±13.5%	0.10-0.50	98.9±4.0%
SDS-3701	0.03-0.50	77.9±17.7%	0.02-0.10	99.0±14.4%
SDS-46851	0.05-0.50	78.3±5.3%	0.06-0.30	82.8±10.1%

Percent recoveries are acceptable.

Storage Stability

Samples from the California field trial were obtained from 3/3/91 to 3/28/91. Analyses for all species occurred from 18 days to one month 20 days. Samples were stored at -20°C until analyses.

Samples from the two Pennsylvania trials were obtained 5/15/87 or 5/28/87. Treatment after sampling is not mentioned, nor are dates of analyses. The study report is dated 1/8/90.

According to the interim chlorothalonil Residue Chemistry Chapter, 9/15/89, data are available to demonstrate the stability of chlorothalonil and SDS-3701 in passion fruit peel and pulp stored at -15°C for up to 6 months and in mint hay stored at -20°C for up to 14 months. These data are inadequate to support the residue analyses on mushrooms. Fermenta Plant Protection Company (now ISK Biotech Corporation) has agreed to conduct storage stability studies on ten representative crops (D.

Edwards, memo of 4/11/89). We assume that these analyses will include analyses for SDS-46851.

Magnitude of Residue

As noted in the previous section, two magnitude of residue studies have been submitted. The first is a report of two field trials held during 1987:

> "Determination of Residues of Chlorothalonil (SDS-2787), SDS-3701, SDS-46851, HCB and PCBN in Crops: Mushrooms," D.C. MacGregor, 2/7/90, Document No. HLA 6012-241D, Hazleton Laboratories America, Inc., Madison, WI. (MRID # 422455-02)

BRAVO 720 was applied at a rate of 5.5 oz per 1,000 sq ft at casing or at casing and after scratching. Four or five additional applications were made at pinning and after harvest of each break. Treatment at Avondale, PA consisted of 1 application at 5.5 oz per 1,000 sq ft and 4 applications at 2.75 oz per 1,000 sq ft. The PHI was 8 days. Treatment at Chatham, PA consisted of 2 applications at 5.5 oz per 1,000 sq ft and 5 applications at 2.75 oz per 1,000 sq ft. The PHI was 4 days. Applications were made either prior to emergence or to only very small mushrooms (pea size or smaller). Results are given in Table 4.

Table 4

Residue Values (ppm) from Field Trials on Mushrooms Held in 1987

Location	Appln. Rate	PHI (Days)	Chloro-thalonil	SDS-3701	SDS-46851	HCB	PCBN
Avondale, PA	1x5.5 oz/1000 ft^2 + 4x 2.75 oz/1000 ft^2	8	<0.010	<0.010	<0.030	<0.003	<0.005
Chatham, PA	2x5.5 oz/1000 ft^2 + 5x2.75 oz/1000 ft^2	4	<0.010	0.227, 0.298	<0.030	<0.003	<0.005

These data cannot be accepted until complete sample histories, including dates of analyses are submitted.

Results from one field trial held in 1990 have been submitted in the following report:

> "Chlorothalonil: Magnitude of Residues In/On Mushrooms In Response To EPA's 25 Jul 89 Letter On PPNO. 6E3410;" G. M. Markle; 2/27/92; Lab Project ID IR-4 PR No. 237; Performing Laboratory: ITEH/IR-4 Lab, UC Davis. (MRID # 422455-01)

BRAVO 720 was applied to a plot in Watsonville, CA at a rate of 4 x 2.75 oz per 1000 sq. ft. Mushrooms were harvested 3 days after the first application and 2 days after each of the other applications. Chlorothalonil residues rose from 0.12 ppm after

the first application to 1.40/1.94 ppm after the last application. HCB, SDS-3701 and SDS-46851 were not detected at respective detection levels of 0.003 ppm, 0.01 ppm and 0.03 ppm. PCBN levels after the final treatment were 0.008 ppm and 0.012 ppm.

Earlier Residue Data

Data from five field trials were reviewed in N. Dodd's memo of 11/12/86. In three trials from Pennsylvania, BRAVO 500 was applied at casing, at pinning, and after harvest of each break. The application rate in two of the trials was 0.26 lb ai/1000 sq. ft. + (1-4) x 0.13 lb ai/1000 sq. ft. In the third trial a fifth application at 0.13 lb ai/1000 sq. ft. was made. PHI's for the harvest after the final application were 48-72 hrs.

In one field trial held in Connecticut, BRAVO 500 was applied at a maximum rate of 2 x 0.14 lb ai/1000 sq. ft. + 2 x 0.28 lb ai/1000 sq. ft. The final harvest of mushrooms -- after the total maximum application had been applied -- was made 3 days after the final application.

In Oregon, BRAVO W-75 was applied at a rate of 0.23 lb ai/1000 sq. ft. + (1-5) x 0.12 lb ai/1000 sq. ft. The PHI's varied from 36-48 hrs.

Samples were analyzed for chlorothalonil, SDS-3701, HCB and PCBN. Chlorothalonil, per se, was invariably the principal component of the residue. The highest residue found was 7.38 ppm, after the third application in the OR trial (PHI = 36 hrs). SDS-3701 levels varied from non-detected to 0.11 ppm, with typical levels below 0.05 ppm. The maximum HCB concentration found was 0.003 ppm; the maximum PCBN concentration was 0.03 ppm. In general, residue levels increased with total application rate and decreased with increasing PHI.

The new proposed maximum use rate is 4 x 0.13 lb ai/1000 sq. ft. with a PHI of 48 hours. Data from the earlier field trials which fall within the new use limits are given in the following table.

Table 5

Chlorothalonil Residue Levels from Field Trials Held 1982-83 Consistent with Proposed Maximum Use Rates

Location	Use Rate (lbs ai/1000 ft^2	PHI	Residue Range (ppm)
Winfield, PA	0.26 + 0.13 + 0.13 + 0.13	48 hrs	1.57-1.91
Avondale, PA	0.26 + 0.13	48 hrs	1.31-1.94
	0.26 + 0.13 + 0.13	48 hrs	1.86-2.55
State College, PA	0.26 + 0.13	48 hrs	0.21-0.44
	0.26 + 0.13 + 0.13	48 hrs	0.26-0.71
OR	0.23 + 0.12	48 hrs	3.64-6.20
	0.23 + 0.12 + 0.12	48 hrs	2.98-3.22

Residue data from the Connecticut field trial were obtained at a minimum PHI of 3 days and are not relevant.

Given the limited number of field trials, we cannot eliminate the Oregon results as outliers. The available data do not allow us to conclude that an initial application of 0.23 lb ai/1000 sq. ft. followed by one application of 0.12 lb ai/1000 sq. ft. should give higher residues than four applications of 0.13 lb ai/1000 sq. ft., the proposed new maximum use rate. Therefore, the petitioner should submit a revised Section F in which a tolerance of 7.0 ppm is proposed for residues of chlorothalonil and its metabolite SDS-3701.

For risk assessment calculations, the anticipated residue for chlorothalonil in/on mushrooms will be calculated. The anticipated residue is the average residue calculated from all field trials conforming to the maximum use conditions. Unless the requested information on the more recent Avondale, PA and Chatham, PA field trials is submitted, the residue data from this trial will not be used in the calculation of an average residue.

The fact that chlorothalonil, per se, is the major species found in/on mushrooms suggests that little metabolism occurs in soil over the mushroom growing period. At this time we do not see a need for additional residue data on SDS-46851.

Meat, Milk, Poultry and Eggs

As noted previously, mushrooms are not fed to animals. Therefore, there will be no animal residues of chlorothalonil or its metabolites from the proposed use.

Attachment: Chlorothalonil and its metabolites in plants and soil.

cc: SF, RF, Circu., PP#6E3410, Reg. Std. File, Mike Flood, E. Haeberer.
H7509C:CBTS:Reviewer(MTF):CM#2:Rm800A:305-6362:typist(mtf):12/8/92
RDI:SectionHead:ETHaeberer:12/7/92:BranchSeniorScientist:RALoranger: 12/8/92.