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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
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MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Micro Flo Co. Response to Plant Metabolism Data Requirements in the Comprehensive Zineb Data Call-In of April 21, 1987 (RD Record No. 215,793; RCB No. 3481, MRID Nos. 405236-01, -02, and -03)

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Micro Flo Co. has submitted three plant metabolism studies for zineb (radishes, oranges, tomatoes) completed in January 1988 in response to the Comprehensive Data Call-In of April 21, 1987. Protocols for the application and analytical portions of these studies were reviewed in RCB on 8/17/87 and 7/21/87, respectively. The application portion of the studies was completed by Environmental Technologies International, Inc. of Raleigh, NC and the analytical portion by EPL Bio-Analytical Services, Inc. of Decatur, IL. The submitted studies do not adequately describe the nature of the residue in plants. Detailed conclusions and additional data requirements are discussed under "RCB Conclusions and Recommendations" on pp. 8-9 of this memo.

Summary of the Submitted Studies (MRID Nos. 405236-01 [radishes], -02 [oranges], and -03 [tomatoes]):

1. Radishes: Red Globe radishes (384 plants) planted in a greenhouse at the University of FL Citrus Research and Education Center on July 22, 1987, were foliarly sprayed on 8/14/87 (3 week-old plants) and 10/5 (11 week-old plants) with 2 lb 75% WP formulation/100 gal (first application) and 240 mg 75% WP/100 ml (second application - rate approximately equivalent to first) to runoff. The first application mixture consisted of 32% "hot" (^{14}C) material and the second of 1.5% hot material (sp. activity =17.2 mCi/mmole - 94% pure). From 8/26 to 10/5, plants were removed to a growth chamber because greenhouse temperatures were

above the optimum growth range for radishes. No overhead watering was done. Samples were collected 2.5 hours and 1, 3, 7, 14,.21, and 54 days after the initial treatment. At each sampling time, 5 radish plants were removed from each of three flats, the roots rinsed in deionized water, and the roots and leaf crowns separated. Samples were bagged and frozen until shipping. Samples were shipped in dry ice to EPL Bio-Analytical Services, Inc. in Decatur, IL and arrived with 13 days after collection.

On arrival, samples were lyophilized and macerated to achieve homogeneity. An aliquot of the lyophilized sample was combusted to determine total ^{14}C-activity. An additional aliquot was extracted four times with 50% methanol plus water containing 10% (w/v) tetrasodium EDTA. A final extraction was also made without EDTA. After removal of the methanol from the combined extract (rotary evaporation supplemented by drying in a stream of nitrogen), the aqueous extracts were partitioned with hexane. Aliquots of the hexane phase were subjected to LSC and aliquots of the aqueous phase were subjected to both LSC and TLC. Silica gel plates were co-spotted with hot zineb standard and cold (unlabeled) ethylenethiourea (ETU) and ethylene (diisothiocyanto)sulfide (EBIS). Plates were developed with ethanol plus chloroform and benzene (1 +5 +10). After development and drying, plates were overlaid with X-ray film to determine radioactive areas. Areas corresponding to zineb and ETU (verified by UV light) were scraped and the scrapings subjected to LSC. Results other than for initial combustion were reported only for 21- and 54-day roots and 0-, 21- and 54-day leaves. These data are summarized below in Tables 1-3.

Table 1. Distribution of ^{14}C-residues in radish roots and leaves.

DAIT[a]	Initial Combustion (ppm)[b]	Extracts/ Washes (ppm)	Bound Residues (ppm)	% Recovery
0 - leaves only	1368	1798	133	141
	890	1112	117	138
	1054	1214	122	127
21 - roots	21	30	21	244
	11	59	42	931
	33	10	8	53
21 - leaves	160	72	24	60
	211	95	22	55
	196	65	19	43

(table continued)

54[c] - roots	12	9	9	147
	29	11	5	56
	12	7	4	92
54 - leaves	110	95	11	96
	146	160	21	124
	53	66	8	139

[a]Days after initial treatment.
[b]Expressed as zineb equivalents.
[c]Two days after second treatment.

Table 2. Distribution of zineb and ETU in radish roots and leaves.

DAIT[a]	Zineb (ppm)	ETU (ppm)[b]
0 - leaves only	1104	146
	1175	105
	373	180
21 - roots	9	1
	32	1
	4	0
21 - leaves	27	1
	23	1
	13	0
54 - roots	2	1
	2	2
	3	0
54 - leaves	19	1
	22	2
	9	3

[a]Days after initial treatment.
[b]Expressed as zineb equivalents.

Table 3. Percent characterization of ^{14}C-residues in radish roots and leaves.

DAIT[a]	% extracted	% identified of total extracted ^{14}C	% identified of total ^{14}C
0 -leaves only	93	70	65
	91	115 (100)[b]	104 (91)[b]
	91	46	41

(table continued)

21 - roots	60	33	20
	58	56	33
	59	40	24
21 - leaves	76	39	29
	81	25	21
	77	20	15
54 - roots	50	33	17
	69	36	25
	64	43	27
54 - leaves	90	21	19
	89	15	13
	91	18	16

[a]Days after initial treatment.
[b]The percent identified cannot be greater than 100% of extracted.

2. Oranges: Two 4 foot (5-year old) Parson Brown orange trees in buckets in a greenhouse at the University of Florida Citrus Research and Education Center were foliarly sprayed on 8/14/87 (136 days after bloom) and 11/13 (227 days after bloom) with 2 lb 75% WP/100 gal and 240 mg 75% WP/100 ml, respectively. The first application mixture consisted of 15% "hot" material and the second of 9% hot material. No overhead watering was done. Trees were sampled at 2 hours, and 7, 14, 28, 56, and 93 days after the initial treatment (the 93 day samples were collected 2 days after the second treatment). At each sampling time, one fruit plus several leaves were collected from each tree. Samples were bagged and frozen until shipment in dry ice to EPL Bio Analytical Services, Inc. in Decatur, IL. On receipt, samples were analyzed using the same protocol as described above for radish leaves and roots. Results other than for initial combustion were reported only for 0-, 56- , and 93-day samples. These data are summarized in Tables 4-6.

Table 4. Distribution of ^{14}C-residues in orange fruit and leaves.

DAIT[a]	Initial Combustion (ppm)[b]	Extracts/ Washes (ppm)	Bound Residues (ppm)	% Recovery
0 - fruit	23	28	2	124
	22	28	1	133
	3	7	0	234
0 - leaves	671	531	36	84
	39	33	8	107
	137	107	15	89

(table continued)

56 - fruit	13	5	1	49
	37	25	15	107
	55	27	9	66
56 - leaves	90	47	22	78
	108	24	12	33
	347	144	45	54
93[c] - fruit	30	46	4	166
	9	13	2	166
	34	25	1	78
93 - leaves	59	26	12	65
	28	29	8	129
	159	147	29	110

[a]Days after initial treatment.
[b]Expressed as zineb equivalents.
[c]Two days after the second treatment.

Table 5. Distribution of zineb and ETU in orange fruit and leaves.

DAIT[a]	Zineb (ppm)	ETU (ppm)[b]
0 - fruit	27	8
	31	5
	10	0
0 - leaves	310	217
	25	31
	81	11
56 - fruit	15	6
	28	1
	30	2
56 - leaves	55	2
	50	1
	157	6
93 - fruit	33	10
	8	26
	23	11
93 - leaves	38	7
	33	31
	128	17

[a]Days after initial treatment.
[b]Expressed as zineb equivalents.

Table 6. Percent characterization of ^{14}C-residues in orange fruit and leaves.

DAIT[a]	% extracted	% identified of total extracted ^{14}C	% identified of total ^{14}C
0 - fruit	97	125 (100)[b]	121 (97)[b]
	97	129 (100)	124 (97)
	88	143 (100)	125 (88)
0 - leaves	94	99	93
	80	170 (100)	137 (80)
	88	86	75
56 - fruit	83	420 (100)	350 (83)
	63	116 (100)	73 (63)
	75	119 (100)	89 (75)
56 - leaves	67	121 (100)	81 (67)
	67	213 (100)	142 (67)
	76	113 (100)	86 (76)
93 - fruit	94	93	88
	81	262 (100)	213 (81)
	93	136 (100)	126 (93)
93 - leaves	67	173 (100)	115 (67)
	78	220 (100)	173 (78)
	84	99	82

[a]Days after initial treatment.
[b]The percent identified cannot be > 100% of extracted.

3. Tomatoes: Six 3-month old plants (var. Walters) in three pots in a greenhouse at the University of FL Citrus Research and Education Center were foliarly sprayed with a spreader sticker on 11/1/87 (7 days after fruit set) and 12/5 (42 days after fruit set) with 2 lb 75% WP/100 gal and 240 mg 75% WP/100 ml, respectively. The first application mixture consisted of 22% "hot" material and the second of 73% hot material. No overhead watering was done. Fruit was sampled (one fruit/rep) at 0, 1, 3, 6, 9, 13, and 36 days after the initial treatment (the 36-day samples were collected 2 days after the second treatment). Samples were bagged and frozen until shipment in dry ice to EPL Bio Analytical Services, Inc. in Decatur, IL. On receipt, samples were analyzed using the same protocol as described above for radish roots and leaves. Results other than for initial combustion were reported only for 0 and 36 day samples. These data are summarized in Tables 7-9.

Table 7. Distribution of ^{14}C-residues in tomato fruit.

DAIT[a]	Initial Combustion (ppm)[b]	Extracts/ Washes (ppm)	Bound Residues (ppm)	% Recovery
0	148	101	35	92
	105	237	14	238
	231	457	19	206
36[c]	34	38	8	138
	53	40	15	103
	17	24	3	156

[a]Days after initial treatment.
[b]Expressed as zineb equivalents.
[c]Two days after the second treatment.

Table 8. Distribution of zineb and ETU in tomato fruit.

DAIT[a]	Zineb (ppm)	ETU (ppm)[b]
0	67	36
	150	52
	209	58
36	22	6
	22	2
	16	9

[a]Days after initial treatment.
[b]Expressed as zineb equivalents.

Table 9. Percent characterization of ^{14}C-residues in tomato fruit.

DAIT[a]	% extracted	% identified of total extracted ^{14}C	% identified of total ^{14}C
0	74	102 (100)[b]	76 (74)[b]
	95	85	81
	96	58	56
36	83	74	61
	73	60	44
	89	104 (100)	93 (89)

[a]Days after initial treatment.
[b]Percent identified cannot be > 100% of extracted.

RCB Conclusions and Recommendations:

The submitted studies did not provide any new information regarding the metabolism of zineb in plants. Basically, the studies were greenhouse residue studies for zineb and ETU, using radiolabeled material. The authors stated that "no other metabolite was found above 10% of the total [^{14}C]-Zineb applied." However, no data were submitted in support of this statement. The only separation technique used was TLC and no radioactive areas were identified in the submitted tracings other than origin material (presumed to be zineb per se) and ETU. The authors proposed a metabolic pathway in plants based on a 1977 article by Rhodes in J. Agric. Food Chem. (25:528) concerning a related compound (maneb). However, none of the metabolites identified in the published paper (ETU, ethyleneurea, Jaffe's base, 2-imazoline, hydantoin, and glycine) other than ETU were identified in the submitted studies.

The most pertinent sampling intervals for which detailed data were submitted were 21 days posttreatment for radishes, 56 days posttreatment for oranges and 36 days posttreatment for tomatoes. Although there is no PHI for radishes and a 5-day PHI for tomatoes, the 0-day data submitted for these crops are not considered to be the most useful for determination of the metabolites likely to occur in plants following treatment with zineb. Extraction efficiency of ^{14}C-residues was borderline acceptable in radish leaves 21 days after treatment (76-81%), orange fruit 56 days after treatment (63-83%), and tomatoes 36 days after initial treatment (73-89%). However, in 21-day radish roots, no more than 60% was extracted. Furthermore characterization of residues in both radish roots and leaves was poor ($\leq$56% of extracted ^{14}C, mean = 35% ; $\leq$33% of total ^{14}C).

The studies, as submitted, do not satisfy the DCI requirements for data regarding the metabolism of zineb in plants due to insufficient characterization of ^{14}C-residues and failure to use a confirmatory method in identification of zineb and ETU. It may be possible, if sufficient samples have been maintained in frozen storage, to salvage the studies with additional analytical work. The following data/information must be submitted:

1. The extraction procedure and the logic behind it must be better described. For example, it was stated that the final "wash" or extraction of the lyophilized sample using 50% methanol plus water without EDTA was necessary to remove the sodium EDTA. It is not clear why removal of the sodium EDTA from the plant residue would be necessary. Presumably, the final wash was added to the previous four extracts with EDTA prior to partitioning with hexane.

2. The ^{14}C-extraction efficiency must be improved, particularly for radish roots. Possible additional extraction techniques

include the use of alternate solvents, dilute acids, hot water, and base or enzyme hydrolysis. Also, large discrepancies and variability in the percent recovery (extracted and bound ^{14}C) of the initial combustion values must be explained (e.g., percent recovery for 21-day radish roots ranged from 53-931%).

3. Data are required to confirm that the origin material scraped and counted as zineb per se was, in fact, the unaltered parent material or its anion. [The extracted material is not likely to include zineb per se due to the removal (chelation) of the Zn moiety during the extraction procedure.]

4. From the submitted TLC tracings, it looks as if the hot zineb standard separated into origin material (zineb per se?), ETU, and two highly mobile undefined radioactive areas. The two undefined areas were apparently <u>not</u> seen in chromatograms from sample extracts even though zineb per se was presumably present at the origin. The authors must (i) address the nature of the two undefined mobile radioactive areas that occur in the chromatograms of the hot zineb standard and (ii) explain why these areas did not occur in the sample chromatograms.

5. The authors state that "unidentified components of the terminal residue are shown in tracings of the radioautogram of the TLC plates." However, the TLC tracings submitted did not clearly indicate any radioactive areas other than ETU and origin material, except the two highly mobile unidentified areas associated with the hot zineb standard. The authors must submit data showing separation of individual metabolites and quantitation of each metabolite identified. At a minimum, the sample extracts must be cochromatographed with Jaffe's base, EBIS, ethyleneurea, 2-imazoline and glycine to confirm the proposed metabolic pathway. An alternative method must be used to confirm the identity of each metabolite (e.g., HPLC with a radioactivity detector; MS). Also, ^{14}C-residues incorporated into natural constituents, such as sugars or lignin, must be identified and confirmed. Any additional characterization of residues must be accompanied by storage stability data with known standards to ensure that the metabolites found are not artifacts resulting from degradation in storage.

Unless the deficiencies described above (points 1-5) can be addressed to the Agency's satisfaction using previously generated samples, it may be necessary to <u>repeat</u> the plant metabolism studies for zineb.

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