3-4-92

010104

MEMORANDUM:

Subject: EPA File Symbol/EPA Reg. No.:100-TGL

From: Lucy D. Markarian, Biologist 2/27/91
Precautionary Review Section
Registration Support Branch
Registration Division (H7505C)

To: Susan Lewis/Benjamin Chambliss, PM 21
Fungicide-Herbicide Branch
Registration Division (H7505C)

Thru: Thomas C. Ellwanger, Section Head E 3/4/92
Precautionary Review Section
Registration Support Branch
Registration Division (H7505C)

Applicant: Ciba Geigy Corporation
Agricultural Division
P.O.Box 18300
Greensboro, NC 27419

FORMULATION FROM LABEL:

Active Ingredient(s):	% by wt.
Metalaxyl: N-(2,6-dimethylphenyl)-N-(methoxyacetyl)alanin methyl ester	50.0 %
Inert Ingredient(s):	50.0 %
Total:	100.0 %

BACKGROUND

Seven studies have been submitted to support the registration of Ridomil 50W Fungicide under EPA symbol 100-TGL. Two of the studies are eye irritation assays.
The product is marketed as a soluble bag packed in an outer protective container.

RECOMMENDATION

Oral toxicity _ Core minimum

Some of the animals were not at the recommended weight of 200 to 300 g range. This could account for the five fold difference in the oral toxicity between the males and the females.

Dermal Toxicity_ Supplementary- Upgradeable

The test material is reported to have been moistened with deionized water at 0.79 ml/mg. This amounts to a large quantity of water per rabbit. The dose for a 2.5 k rabbit at 2020 mg/kg is 5050 mg. If this is moistened at the rate of 0.79 ml/mg the rabbit would require 3989.5 ml of water. This probably was not the case. Stillmeadow should clarify as to what was really used to moisten the test material. The study may be upgraded upon clarification of this point.

Eye Irritation_ Core Minimum
(First Study: Dated June 4, 1991)
Whenever there is staining from fluorescein it is a sign of corneal opacity and it must be recorded as such. The use of plus sign when there is staining is more than a dullness, and should be recorded as opacity, however slight this opacity might be. This is based on the assumption that any eye that stained prior to the test was not used for the purpose. If eyes that stained at the pretest screening were used for the test,then the test is not a valid assay.

Dermal irritation_ supplementary, upgradeable

It is hard to visualize how a 500 mg aliquot can be mixed with 3ml of water and can be contained under a 2.5 x 2.5 cm patch. The need to use 3.0 ml of liquid to moisten 500 mgs is also out of the ordinary. The study may be upgraded by supplying explanations.

Dermal Sensitization_ Supplementary

The test does not define the sensitization potential of the formulation; therefore, it is graded supplementary. the reasons for the conclusion are:

The guidelines state that the performing laboratory is free to choose the method used among the many listed; however, stipulation is made under the heading of Procedures that "The procedures used are those described by the methodology chosen". PRS finds that the laboratory has made too many modifications in the Buehler method to be able to achieve the purpose.

The Buehler method has been explained in many publications under his own name or as jointly authored by his colleagues since 1965, when it was originally published. It is also discussed and the points essential to the test reiterated by Robinson et.al.[1] From all these publications the essentials of the test that PRS feels are indispensable to the integrity of the test are :

a. The proper screening for the induction and elicitation concentrations.
The emphasis is on concentrations. Buehler[2] stresses the importance of concentrations and vehicles "The choice of concentrations and vehicles used during the induction and elicitation phase of testing is very critical to the meaningfulness of the results obtained" In the presented tests there was no choice in the concentration. All prescreening was made with different quantities of 100 % test material which may have been moistened. Different quantities are not the equivalents of different concentrations. In the same article it is stated that the challenge concentration is the highest nonirritating concentration as determined using 4 guinea pigs and defined as "that concentration in the solvent used that induces responses in the four guinea pigs no more severe than two grades of 0.5 and two grades of 0". This naturally is on the Buehler scale.
At the prescreening 500 mg of test material resulted in one grade of 1 and one of 0. This might have qualified as the induction concentration if the scale for grading was the Buehler scale and a solution was used.

b. Good contact between the test material and the skin.
To achieve this Buehler chooses occlusion, restraint and the use of rubber dam. The laboratory's method was sufficiently close to the Buehler way.

c.The use of naive controls.

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[1]Robinson,M.K., Nusair,T.L., Fletcher,E.R., and Ritz, H.L., AReview of the Buehler Guinea Pig Skin Sensitization Test and Its Use in Assessment Process for Human Skin, Toxicology, 61 (1990) 91-107, Elsevier Scientific Publishers Ireland Ltd.

[2]Ritz, H.L. and Buehler, E.V., Planning Conduct, and Interpretation of Guinea Pig Sensitization Patch Tests, Current Concepts in Cutaneous Toxicity, 1980, Academic Press

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Buehler[3] states that " The significance of reactions in the experimental group is based on intensity and incidence relative to the two control groups". By two control groups he means a naive control and a vehicle control, if a vehicle other than water is used. Positive controls only reiterate the capacity of the laboratory to induce sensitization and are not base for comparison. The test material is generally induced at a higher concentration than challenge. This is based on the premise that if sensitization is achieved a nonirritating lower concentration would elicit a reaction at a remote area from the induction site. Inducing and eliciting at the same concentration does not show that this has happened. Buehler finds the inclusion of naive controls so important that he advocates the use of fresh naive controls, ones never used before, at each rechallenge. No naive controls were used.

d. The use of the Buehler scale for evaluations.
The Buehler approach to the results is quantal .It is there or it is not, regardless of presence of edema. The only times when gradations have significance in a Buehler test is if there is irritation in the naive controls or if it is important to decide whether the test material is a strong or weak sensitizer.The Draize Scale is primarily for dermal irritation, and is graded in approach. Grade 1 reactions are nonremarkable. It is doubtful if a grade 1 erythema accompanied with grade 1 edema could remain nonremarkable. This type of grading only adds up to confusion. The presented scale does not indicate that grade 1 erythema is nonremarkable. The presence of grade 1 erythema at 48 hours after challenge , in a guinea pig that showed no irritation before, indicates the possibility of sensitization. If this kind of reaction had happened in a naive control as the test animal,then the sensitization potential would have been better defined.

The scoring of the reactions are not according to the presented scale.
According to the presented scale slight eschar is to be graded with an erythema score of 4. Yet time after time erythema score of 3 with eschar is recorded. This is in violation of good laboratory practices. If it is indicated that a certain method is to be used and that method is specified (the scoring system is included in the report) then it is expected that the method is followed. Eschar reported at the tenth induction application indicates only grade 2 erythema in the test group. 9/10 sites after the 10th application in the positive control group are also not graded correctly to give examples. These are not the only incidences. The general impression is that the tendency is to minimize the

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[3]Buehler, E.V. and Griffin,J.F., Experimental Skin Sensitization in the Guinea Pig and Man, Animal Models in Dermatology (H.I.Maibach,ed),Churchill Livingstone, Edinburg,London, New York, 1975

irritation. This is contrary to the aim of any test. Furthermore, the [redacted] of the quality assurance is questioned. If the quality assurance states that the data has been reviewed and "is confident that the protocol described in the final report was followed throughout the course of the study...." then confidence in that statement is not possible, because a grading system is advocated but not followed.
A new sensitization test must be submitted.

The inhalation study is accepted as guideline data.

The eye irritation test number 8061-91 is guideline data, however cannot change the eyeirritaion toxicity category. PRS will always consider the worst possibility in labeling a product. Submitting a second eye study that showed no opacity after seven days does not signify that the occurrence of irreversible opacity (present at 21 days) is not possible or probable at yet another assay.

LABELING

The signal word is "DANGER" based on the eye study number 8060-91.

The precautionary statement must include:

Corrosive. Causes irreversible eye damage. Harmful if swallowed, absorbed through skin, or inhaled. Do not get in eyes, avoid contact with skin or clothing, or inhaling dust or spray mist. Wear goggles, face shield,or safety glasses. Wash thoroughly with soap and water after handling.

The statement of practical treatment must include:

If in eyes	Hold eyelids open and flush with a gentle steady stream of water for 15 minutes.
If swallowed	Drink promptly a large quantity of milk, egg white, or gelatin mixture, or if these are not available large quantities of water. Avoid alcohol.
[redacted]	E
If inhaled	Remove victim to fresh air. If not breathing give artificial respiration, preferably mouth to mouth. Get medical attention.

Note to Physician:
Probable mucosal damage may contraindicate gastric lavage.

The precautionary label may have to be changed upon the submission of the requested data.

Note to the PM:
With the placement of eye irritation in category I toxicity the product is candidate for restricted use. PM should decide if alternative labeling language is sufficient to offset the hazard and the need for restricted use classification.

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DATA REVIEW FOR ACUTE ORAL TOXICITY TESTING (§ 81-1)

Product Manager:21 **Reviewer:** L. Markarian
MRID No.:420520-03 **Report Date:**12/5/91
Testing Facility: Stillmeadow Inc. **Report No.**8057-91
Author(s): Janice O. Kuhn
Species: Rat, Sprague Dawley
Age:not specified
Weight: Males 181 -268 g, Females 177-225 g
Source: Harlan Sprague Dawley,Inc., Houston,Texas
Test Material: Metalaxyl 50WP FL-910510. ARS 13910 Batch GP-910305 off white powder
Quality Assurance (40 CFR §160.12): Included

Conclusion:

1. LD_{50} **(mg/kg):** **Males** = 3870 (3050-4920) mg/kg
Females = 695 (600-783) mg/kg
Combined =not calculated

3. **Tox. Category:**III **Classification:** core minimum

Procedure (Deviations from §81-1):

Fasted animals were intubated with a 40 % w/v solution in deionized water at 3 levels in the males and at six levels in the females.The animals were observed three times on the day of intubation and daily for the rest of the 14 day observation period. Body weights were recorded at initiation and on days 7 and 14, and at death.Necropsy was performed on all animals.

Results:

Dosage mg/kg	(Number Killed/Number Tested)		
	Males	Females	Combined
250	-	0/5	0/5
500	-	0/5	0/5
750	-	4/5	4/5
1000	-	5/5	5/5
2000	0/5	5/5	5/10
4000	3/5	-	3/5
5050	4/5	5/5	9/10

Symptoms & Gross Necropsy Findings:

In the females at 250 mg/kg level no symptoms of toxicity or signs of gross pathology were observed .
At the 500 mg and higher levels and at all levels in the males signs of toxicity included decreased activity, Piloerection, and salivation. Additionally at doses of 750 mg/kg and above tremors, ataxia and convulsions were noted. At the doses where all animals succumbed to treatment red nasal and oral discharge and polyuria was present. Symptoms of toxicity were not present in the survivors after day 1.
The survivors at the higher dose levels showed no signs of gross pathology at necropsy. The necropsy of the decedents at all levels revealed stomachs distended with gas and yellow fluid, yellow contents of small intestines, mottled lungs with red spots, mottled black lungs, and external signs of salivation, lacrimation and discharge from mouth.

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DATA REVIEW FOR ACUTE DERMAL TOXICITY TESTING (§81-2)

Product Manager:21 **Reviewer:** L. Markarian
MRID No.: 420520-04 **Report Date:**12/5/91
Testing Laboratory: Stillmeadow, Inc. **Report No.:**8058-91
Author(s): Janice O. Kuhn
Species: Rabbit, New Zealand White
Weight: Males 2.325-2.775 K, Females 2.00-2.675 K
Source: Ray Nichols Rabbitry , Lumberton Texas
Test Material: Metalaxyl 50WP FL-910510 ARS-13910,Batch GP-910305
Quality Assurance (40 CFR §160.12): Included

Summary:

1. **The estimated LD_{50} is** > 2020 mg/kg

3. **Tox. Category:** III **Classification:**supplementary, Upgradeable

Procedure (Deviation From §81-2):

The test material was applied to the shaved skin of the rabbits on an approximately 10 % area of the skin surface moistened with deionized water (0.79 ml/mg). The area was covered with surgical Gauze 10 x 10 cm held in place with tape. The trunks of the animals were wrapped in orthopedic stockinette. At 24 hours the wrappings were removed and the sites washed with tap water and cloth. Observations were at 1/2, 3, and 6 hrs after application and daily thereafter. Body weights were recorded at initiation and on days 7 and 14. Necropsy was performed on all animals.

Results:

Reported Mortality

DOSAGE mg/kg	(NUMBER KILLED/NUMBER TESTED)		
	Males	Females	Combined
2020	0/5	0/5	0/10

Symptoms & Gross Necropsy Findings:

There was no mortality. The only observed abnormality was decreased defecation in one female on days 6 and 7. Necropsy revealed no gross pathology.

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DATA REVIEW FOR ACUTE INHALATION TOXICITY TESTING (§81-3)

Product Manager:21
MRID No.:420520-05
Testing Laboratory: Stillmeadow, Inc.
Author(s): Mark S. Holbert
Species: Rat, Sprague Dawley
Weight: Males 241-268 g, Females 191-231 g
Source: Harlan Sprague Dawley,Inc., Houston Texas
Test Material: Metalaxyl 50WP FL-910642 ARS-13910, Batch GP-910312
Quality Assurance (40 CFR §160.12): Included

Reviewer: L. Markarian
Report Date:12/5/91
Report No.:8059-91

Summary:

1. **The estimated LC_{50} is** > 1.76 mg/L

2 **Mean Concentration:**1.76 mg/L

3. **Tox. Category:** III **Classification:**Guideline

Procedure (Deviation From §81-3):

Four hour , whole body exposure was in a 500 L dynamic flow NY University design chamber.The aerosol was generated by a Gem Trost Air Mill that aspirated the test material from a motorized revolving disc delivery system coupled to the mill. Air flow was maintained through the use of a calibrated critical orifice, and recorded at 30 minute intervals as were temperature and humidity.

The test atmosphere concentration was determined gravimetrically once per hour by sampling from the breathing zone using filters and measured volumes of test atmosphere.

Particle size determination was made twice during the exposure using an Andersen Cascade Impactor at the sampling rate of 28.3 lpm for 0.5 minutes.

Observations were frequent during exposure, at the end of the exposure, at 6 hrs and daily thereafter.
Body weights were recorded at initiation and on days 7 and 14.
Necropsy was performed on all animals.

Results:

Reported Mortality

Exposure Concentration	(NUMBER KILLED/NUMBER TESTED)		
	Males	Females	Combined
1.76 mg/L	0/5	0/5	0/10

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Chamber Concentration:
Average 1.76 mg/L Range 1.51 - 1.93 mg/L

Particle size analysis

	I	II
MMAD	**2.149 u**	**2.117 u**
SD	**2.678**	**3.593**
Particles under 3.3 u	**41.03 %**	**41.98 %**
Particles under 1.1 u	**25.29 %**	**25.00 %**

Air Flow	**Average 86.68**	**Range 77.87 - 87.78**
T-99	**26.2 minutes**	
Temperature	**Average 74° F**	**Range 73 - 75**
Humidity	**Average 95 %**	**Range 91 - 95 %**

Symptoms & Gross Necropsy Findings:

Symptoms of toxicity included decreased activity, nasal discharge, piloerection, polyuria, ptosis, respiratory gurgle, and salivation. All animals were normal on day 3. All animals showed normal gains in body weight. Necropsy revealed no gross pathology.

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DATA REVIEW FOR ACUTE EYE IRRITATION TESTING (§81-4)

Product Manager:21
MRID No.: 420520-06
Testing Laboratory: Stillmeadow, Inc.
Author(s): Janice O. Kuhn
Species: Rabbit, New Zealand White
Sex: males 6, Females 3
Source: Ray Nichols Rabbitry, Lumberton, Texas
Dosage:0.1 ml, equivalent of 35.23 mg powdered test material
Test Material: Metalaxyl 50WP FL-910510 ARS-13910 Batch GP-910305
Quality Assurance (40 CFR §160.12): Included

Reviewer: L. Markarian
Report Date:12/5/91
Report No.:8060-91

Summary:

1. **Toxicity Category:** I

2. **Classification:** Core Minimum

Procedure (Deviations From §81-4):
The test material was instilled in the conjunctival sacs of nine preexamined eyes. Three were washed with deionized water thirty seconds after instillation for one minute. Observations were at 1, 24, 48, 72 hours and on days 4, 7, 10, 14, 17, and 21. At 24 hrs fluorescein was used to confirm corneal findings. Evaluations were according to Draize.
One animal was found dead on day 18. This was not significant, because the eye of that rabbit had cleared prior to death.
Results:

	HOURS				DAYS					
	1	24	48	72	4	7	10	14	17	21
Corneal opacity	0/6	6/6	5/6	2/6	2/6	2/6	0/6	1/6	1/6	1/6
Iritis	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6
Conjunctivae										
Redness	6/6	5/6	2/6	2/6	2/6	0/6	0/6	0/6	0/6	0/6
Chemosis	2/6	2/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6
Discharge	6/6	5/6	3/6	1/6	0/6	0/6	0/6	0/6	0/6	0/6

Unremarkable (grade 1) redness persisted in the rabbit with opacity at 21 days to termination. The same animal showed vasculation of cornea from day 14 through day 21.
Washed eyes are not required for registration

DATA REVIEW FOR ACUTE EYE IRRITATION TESTING (§81-4)

Product Manager:21
MRID No.: 420520-07
Testing Laboratory: Stillmeadow, Inc.
Author(s): Janice O. Kuhn
Species: Rabbit, New Zealand White
Sex: 3 male and 3 female
Source: Ray Nichols Rabbitry, Lumberton, Texas
Dosage:0.1 ml, equivalent to 35.23 mg of powdered test material
Test Material: Metalaxyl 50WP FL-910510 ARS-13910, Batch FP-910305
Quality Assurance (40 CFR §160.12): Included

Reviewer: L. Markarian
Report Date:12/4/91
Report No.:8263-91

Summary:

1. **Toxicity Category:**III

2. **Classification:** Guideline

Procedure (Deviations From §81 4):
The test material was instilled in the conjunctival sacs of nine preexamined eyes. Three were washed 30 seconds after instillation with deionized water for one minute. Evaluations were at 1, 24, 48, 72 hrs, and on days 4 and 7. Scoring was according to Draize.

Results:

Observations	(number "positive"/number tested)							
	Hour	Days						
	1	1	2	3	4	7	14	21
Cornea Opacity	0/6	5/6	5/6	2/6	0/6	0/6		
Iris	0/6	0/6	1/6	0/6	0/6	0/6		
Conjunctivae								
Redness	6/6	4/6	2/6	0/6	0/6	0/6		
Chemosis	2/6	3/6	0/6	0/6	0/6	0/6		
Discharge	6/6	4/6	1/6	0/6	0/6	0/6		

Comments:

Washed eyes are not required for registration.

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DATA REVIEW FOR SKIN IRRITATION TESTING (§81-5)

Product Manager:21
MRID No.: 420520-08
Testing Laboratory: Stillmeadow,Inc.
Author(s): Janice O. Kuhn
Species: Rabbit, New Zealand White
Age: Young adult (3-6 months of age)
Sex:3 male , 3 female
Source: Ray Nichols Rabbitry, Lumberton, Texas
Dosage:500 mg moistened with 3.0 ml of water
Test Material: Metalaxyl 50WP FL-910510 ARS-13910, Batch GP-910350
Quality Assurance (40 CFR §160.12):included

Reviewer: L. Markarian
Report Date:11/4/91
Report No.:8061-91

Summary:

1. **The Primary Irritation Index** =0.40
2. **Toxicity Category:**
3. **Classification:**Supplementary, Upgradeable

Procedure (Deviations From §81-5):
The test material was introduced to the shaved skin under 2.5 x 2.5 cm gauze patch and secured in place with non-irritating tape. The trunks of the animals were wrapped in surgical stockinette. At 4 hrs the wrappings were removed and the sites wiped with tap water and cloth. Evaluations were at 3/4, 24, 48, and 72 hrs after the removal of the patches. Draize scoring system was used.

Results:

Five of the animals showed grade 1 erythema at 3/4 and 24 hours. All sites appeared normal at all other observation intervals.

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DATA REVIEW FOR SKIN SENSITIZATION TESTING (§81-6)

Product Manager:21
MRID No.: 420520-09
Testing Laboratory: Stillmeadow, Inc.
Author(s): Janice O. Kuhn
Species: Guinea Pig, Hartley
Weight:330 - 400 g
Sex: Males
Source: Harlan Sprague Dawley, Inc. Houston, Texas
Test Material: Metalaxyl 50WP FL-910510 ARS-13910, Batch GP-910305
Positive Control Material:DNCB
Quality Assurance (40 CFR §160.12): Included

Reviewer: L. Markarian
Report Date:12/4/91
Report No.:8062-91

Method: Apparent Buehler, Not specified

Summary:

1. **This Product is / is not a dermal sensitizer.**

2. **Classification:** Supplementary

Procedure (Deviation From §81-6):
A pretest screening was made using different quantities of test material instead of different concentrations and two guinea pigs. It is not stated if these quantities were moistened at application or how applied, and for how long. It is stated that from this prescreening it was decided to test the formulation at 100 % moistened with deionized water. The pretest results showed that 1/2 guinea pigs had grade 1 erythema . Other quantities (250, 50, and 5 mg)showed no reaction.

Two groups of animals were used. Ten as positive controls that received applications of 0.06% DNCB in ETOH (concentration unspecified) and ten animals for the test group receiving appli- tion of 500 mg of the test material moistened with 0.5 ml of deio.. .ed water. The respective test materials were applied beneath 1.6 x 2.8 cm gauze pad secured with a 3.8 x 5 cm piece of adhesive tape on shaved skin. The trunks of the animals were wrapped in polyethylene film. The animals were restrained during the six hour exposure. At the end of the exposure the wrappings were removed and the sites were evaluated at 24 hours after applications at all exposures, and also at 48 hours after the first and last exposures. There were ten exposures . Two weeks after the last induction challenge was made at a naive site and at the induction site using the same concentrations and application method. The challenge sites were evaluated at 24 and 48 hours after applications. Scoring was according to a scale similar to Draize skin irritation scoring scale. This scale does not indicate that grade 1 erythema is not remarkable.
There were no naive controls.

Results:

After the initial induction application 1/10 showed grade 1 erythema at 24 and 48 hours. By the fifth application all sites showed grade 1 erythema and the irritation increased progressively so that after the last application there was erythema and edema (recorded as grade 1 and 2) and in one case Eschar. At challenge at naive sites the animal that had shown grade 1 irritation after the first application showed grade 1 erythema at 24 and 48 hours, but another animal that had not shown irritation after the first application showed grade 1 erythema at 48 hours. 8/10 of the induction sites showed grade 1 erythema and 3 of these showed grade 1 edema.

All the positive control animals showed grade 3 and 4 erythema and edema at the end of the induction period and all had eschar.
At challenge at the naive sites 4/10 showed grade 2 erythema and grade 1 edema, 2/10 showed grade 1 erythema without edema , and all the rest showed grade 1 erythema with grade 1 edema. All the induction sites showed 2 or 4 grade erythema and marked edema with 7/10 showing eschar.

The laboratory has concluded that the test material is not a sensitizer.