2-28-94

MEMORANDUM:

Subject: EPA File Symbol/EPA Reg. No.:100-TLE
Primo WSB For Turf Growth Management

From: Lucy D. Markarian, Biologist LM 10/12/93
Precautionary Review Section
Registration Support Branch
Registration Division (7505W)

To: Joanne I. Miller, PM 23
Fungicide-Herbicide Branch
Registration Division (7505C)

Thru: Thomas C. Ellwanger, Section Head
Precautionary Review Section
Registration Support Branch
Registration Division (7505W)

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

FORMULATION FROM LABEL:

Active Ingredient(s):	% by wt.
4-(cyclopropyl-a-Hydroxy-methylene)-3,5-dioxo-cyclohexanecarboxylic acid ethyl ester	25 %
Inert Ingredient(s):	75 %
Total:	100 %

BACKGROUND

Ciba-Geigy agricultural division has submitted six studies to support the registration of a new product Primo WSB under EPA symbol 100-TLE. The formulation comes in water soluble bags for the ease of mixing, and is soluble in water. It is supposed to retard excessive growth of turf when it is actively growing, and decrease the need for frequent mowing, while encouraging the increase in turf density, color and quality depending on actual weather conditions.

RECOMMENDATION

The submitted studies with the exception of the sensitization assay are considered core minimum data and can support the registration.

The inhalation study is placed in category III toxicity due to failure to generate a uniform and acceptable particle size, which was minimally respirable to the test model. Although no animals died at the limit concentration, there were signs of toxicity which may have been more severe with a higher proportion of respirable particles. PRS has considered the difficulty in generating the test atmosphere in this compromise. If the registrant is not willing to accept this placement, then a new test must be submitted. If, as suggested by the label the product is soluble, it is recommended that it be dissolved and a suitable atmosphere be generated from the solution. Considering that the product is to be sprayed, this appears a logical approach.

The sensitization test is considered supplementary, but upgradeable if it can be shown that the use of 0.5 ml saline was essential to the moistening of the powdered test material. PRS encourages moistening of the solid test material with minimum of vehicle. Otherwise the test material needs to be in solution in the proper solvent.
The referenced positive control test is not acceptable, because it is conducted using the same concentration in ethanol for induction and elicitation. Any control assay must be conducted using the same procedure as the test material itself.
The ability to induce sensitization has not been demonstrated.
The rationale for the rating of the tests is given below.

Acute oral- core minimum

The report does not state why the test material had to be used at 40 % dilution. Whether there was need for this or it was arbitrarily chosen is not clear. As toxicity decreases with dilution, the test material should be intubated at the highest workable concentration.

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Acute dermal- core minimum

The test material was applied in about 1:1 dilution. The guidelines state that a solid test material should be only moistened. If there were extenuating circumstances that necessitated the 1:1 dilution, this should have been stated in the report.
Diarrhea and/or decreased defecation observed after the sixth day of observation are stated not to be product related. If this is known as a certainty, the real cause of the manifestation of these symptoms should be given. In the absence of any explanation it is assumed that possibly there was an underlying disease state and these animals should not have been on study. It is remarkable that necropsy found no abnormalities in at least the intestines of these animals.

Acute Inhalation- Core minimum

Although the chamber concentration was over 5.0 g/l the MMAD was greater than 4 microns when averaged, and the percentage of respirable particles low (about 10 % <1.1). As a result the exposure was not truly to an atmosphere where the true inhalation hazard potential could be determined. MMAD of 3.9 to 4.9 is not acceptable by current standards. However, considering the difficulty of generating a more acceptable atmosphere, PRS will accept the test in category III toxicity as core minimum data. Due to the difficulty in attaining a more acceptable MMAD, particle size analysis could have been made at closer intervals. The initial determination of 3.9 micra could possibly have been maintained, or it could have been determined that 4.9 was an exception to a more uniform and acceptable particle size distribution if more frequent determinations were made.
The label of the product states that it is dissolvable. If this is actually the case, the use of a solution would have been an acceptable form of generating the test atmosphere.

If the registrant is not willing to accept category III toxicity placement of the product for inhalation toxicity, then a new test can be submitted that shows an acceptable MMAD in a category IV concentration.

Any new submission should be explicit about sampling procedures for the determination of chamber concentrations. The specific sampling vehicle, zone, rate and volume should be included. These were missing from the submitted test.

There was too much variation in the chamber concentration. This apparently was adjusted by adjusting the air flow. The variations in the air flow were not presented as recorded, just an average air flow presented. This is not in accordance to GLP regulations.

Eye Irritation- Core minimum

Any staining with fluorescein after the instillation of the test

material is considered a corneal lesion, however mild, and must be graded accordingly. One eye stained at 24 and 48 hrs, but was not graded with opacity.
Washed eyes are not required for registration.

Dermal irritation- Core minimum

The reason for the 1:1 dilution of the test material instead of just moistening it (according to the guidelines) was not given.

Dermal sensitization- Supplementary upgradeable

1. The use of different quantities in the determination of induction and elicitation concentrations is not acceptable. It was a fortunate accident that the largest quantity resulted in no irritation at 24 hrs and only 1/2 showed grade 1 irritation at 48 hrs. What is unusual is that even this type of irritation was not observed during induction and elicitation.

2. 500 mg in 0.5 ml saline is a 50 % dilution. If the test material was only moistened (less than 0.5 ml saline) the result could have been different. The reason for using as much saline as used has not been explained. It is not known if this was necessary or merely convenient. The establishment of sensitization is concentration dependent; therefore, this point has to be clarified.

3. 500 mg in 0.5 ml of saline is stated to be the "maximum quantity of test material producing no more than minimal irritation upon initial dosing". If this was the minimally irritating concentration, what was the highest nonirritating concentration to be used for challenge? Ideally induction is at a mild to moderately irritating concentration and challenge at the highest nonirritating concentration, defined as two grades of 0 and two grades of 0.5 when tested in four guinea pigs according to the Ritz and Buehler reference in the report.

4. The dates and the results of the referenced positive control test are not given.

5. The referenced positive control test was conducted using 0.06 % DNCB in 95 % ethanol for both induction and elicitation. Any control assay has to follow the same protocol as the test material. Induction and elicitation cannot be at the same concentration, unless 100 % test material is used, provided that 100 % is shown to be completely nonirritating. The ultimate proof for the establishment of sensitization is the ability to elicit at a lower nonirritating concentration than the induction concentration. This is defined by the author of the test as the highest nonirritating concentration. The reason for this is that elicitation is also concentration dependent. It

is that elicitation is also concentration dependent. It cannot be at such a low level that is not detectable by the alerted and proliferated T- Cells. Using the same concentration for induction and elicitation is not acceptable with a test material that is used at a concentration other than 100 %.

6.The use of ethanol for induction and elicitation is not acceptable. Ethanol has shown sensitization potential in some instances. Buehler recommends that if induction is in an ethanol, elicitation should be in acetone or any other suitable vehicle.

The laboratory has not demonstrated the ability to induce sensitization.

LABELING

The toxicity profile of CGA-163935 25 WP is

Acute oral	Cat. IV
Acute dermal	Cat. III
Acute inhalation	Cat. III
Eye Irritation	Cat. III
Dermal Irritation	Cat. IV
Sensitization	Supplementary/ upgradeable

The signal word is CAUTION.

The Precautionary statement should include:

Harmful if absorbed through skin or inhaled. Causes moderate eye irritation. Avoid contact with skin, eyes, or clothing and breathing dust or spraymist. Wash thoroughly after handling. Remove contaminated clothing and wash before reuse.

The Statement of Practical treatment should include:

If on skin	Wash with plenty of soap and water. Get medical attention.
If inhaled	Remove victim to fresh air. If not breathing give artificial respiration, preferably mouth to mouth. Get medical attention.
If in eyes	Flush with plenty of water. Call physician if irritation persists.

The label may have to be revised upon the submission of the outstanding data.

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

Product Manager:23 **Reviewer:** L. Markarian
MRID No.:425578-02 **Report Date:**1/13/92
Testing Facility: Stillmeadow, Inc. **Report No.**8585-91
Author(s): Janice O. Kuhn
Species: Rat, New Zealand White
Age: Young adult
Weight: Males 181-211 g, Females 204-219 g
Source: Harlan Sprague Dawley, Houston Texas
Test Material:CGA-163935 25WP-A FL-912094 ARS-16402 Beige Powder
Quality Assurance (40 CFR §160.12): Included

Conclusion:

1. **The estimated LD_{50} is** > 5050 mg/kg
2. **Tox. Category:** IV **Classification:**Core minimum

Procedure (Deviations from §81-1):

Fasted animals were intubated with a 40 % solution in deionized water. Observations were three times on the day of intubation and daily thereafter. Body weights were recorded at initiation and on days 7 and 14. Necropsy was performed on all animals.

Results:

Dosage	(Number Killed/Number Tested)		
	Males	Females	Combined
5050 mg/kg (12.6ml)	0/5	0/5	0/10

Symptoms & Gross Necropsy Findings:

There was no mortality. Piloerection was observed in 3/5 females during the day of intubation only. Necropsy revealed no abnormalities

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

Product Manager:23 **Reviewer:** L. Markarian
MRID No.: 425578-03 **Report Date:**1/9/92
Testing Laboratory: Stillmeadow, Inc. **Report No.:**8586-91
Author(s): Janice O. Kuhn
Species: Rabbit, New Zealand White
Weight:M 2.075-2.675 K, **F** 2.075-2.400 K
Source: Ray Nichols Rabbitry, Lumberton, Texas
Test Material:
Quality Assurance (40 CFR §160.12): Included

Summary:

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

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

Procedure (Deviation From §81-2):

The test material was applied moistened with 2 ml/kg of deionized water (approximately 1:1 dilution) to a 10 X 10 cm area of clipped skin. The site was covered with 2 ply gauze and the trunks of the animals were wrapped in orthopedic stockinette secured with tape. At 24 hrs the wrappings were removed and the sites washed with water and clean cloth. Observations were three times during the day of application and daily thereafter. Body weights were recorded at initiation and on days 7 and 14. Necropsy was performed on all animals. Symptoms appearing after the 6 th day of the observation period were not considered to be product related.

Results:

Reported Mortality

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

Symptoms & Gross Necropsy Findings:

Starting on day 6 and 11, respectively, two males had slight to moderate diarrhea, and 1 female showed decreased defecation. The laboratory states that these were not considered to be product related. The male that did not recover, and the female showing decreased defecation showed weight loss at termination. There is no indication if the product caused any skin irritation. Necropsy revealed no abnormalities

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

Product Manager:23 **Reviewer:** L. Markarian
MRID No.: 425578-04 **Report Date:**2/13/92
Testing Laboratory: Stillmeadow, Inc. **Report No.:**8587-91
Author(s): Mark S. Holbert
Species: Rat, Sprague Dawley
Weight:Males 251 - 293 F 199 - 237 g
Source: Harlan Sprague Dawley Inc., Houston Texas
Test Material:CGA-163935 25WP-A FL-912094 ARS-16402
Batch Code 626-13-1 Beige powder
Quality Assurance (40 CFR §160.12): Included

Summary:

1. **The estimated LC_{50} is** > 0.574 mg/L

2. **Mean Concentration:** 5.04 and 0.574 mg/L

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

Procedure (Deviation From §81-3):

Exposure was in a 500 L New York university design dynamic flow chamber for four hours. There were two exposure levels at the registrant's request one at 5.04 mg/l and one at 0.574 mg/l.

The test material was sifted through a flour sifter (2mm screen) prior to the generation of the aerosols. Then it was hammer milled twice. The first milling was not successful, therefore it was milled with dry ice a second time. This was successful in reducing the particles to the lowest possible size. The report states that further reduction would not be possible "without possibly risking product integrity".

The test atmosphere for the 0.547 mg/l level was generated using a Gem T Tost air mill that drew the test material from a motorized disc delivery system, eluteriated the created aerosol through a baffleing chamber, and after dilution with filtered air it was introduced it to the exposure chamber.

The test atmosphere for the 5.04 mg/l level was generated using a Venturi Aspirator that drew the test material from the motorized disc delivery system. The aerosol was diluted with filtered air prior to introduction into the exposure chamber.

Chamber air flow was maintained through a calibrated critical orifice and recorded at 30 minute intervals. Chamber temperature and humidity were measured with Taylor Hygrometer and recorded at 30 minute interval. The chamber humidity was not measured at 0.547mg/l level because the cistern of the hygrometer was left empty.

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Chamber concentrations were determined gravimetrically at half hour intervals using filters. The type of filter, rate of sampling or volume not provided.

Particle size analysis was twice per exposure using an Andersen Cascade impactor, by sampling from the breathing zone at the rate of 28.3 lpm for 0.5 - 3 minutes.

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

Results:

Chamber Concentration mg/l		
Gravimetric	5.043	0.547
Range	2.484-7.950	0.4831-0.8489
MMAD±SGD um		
I	3.96±2.872	4.132±2.527
II	4.91±2.884	4.253±2.580
% < 1.1 um		
I	12.2	9.16
II	8.33	9.04
Chamber		
Temperature°F	70-73	71-75
Humidity %	57-65	-----
Air flow LPM	106.2	106.2
Mortality		
Male	0/5	0/5
Female	0/5	0/5
Signs of Toxicity	decreased activity, fur coated with test material, gasping, respiratory gurgle, nasal discharge, salivation, ptosis, chromodacryorrhea, flatulence, piloerection, withdrawn testes. Normal by day 3	
Necropsy findings	none	

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

Product Manager:23
MRID No.: 425578-05
Testing Laboratory: Stillmeadow, Inc
Author(s): Janice O. Kuhn
Species: Rabbit, New Zealand White
Sex:3 M & 3 F
Weight:not specified
Source: Ray Nichols Rabbitry
Dosage:0.1 ml (36.9 mg)
Test Material:CGA-163935 25WP-A FL-912094 ARS-16402 Batch Code 573-41-1 Beige powder
Quality Assurance (40 CFR §160.12): Included

Reviewer: L. Markarian
Report Date:6/3/91
Report No.:8129-91

Summary:

1. **Toxicity Category:**III

2. **Classification:**core minimum

Procedure (Deviations From §81-4):

0.1 ml of powdered test material was instilled in the conjunctival sacs of nine pre examined eyes. Six were observed unwashed and three washed with deionized water 30 seconds after instillation.Evaluations were according to Draize. Fluorescein was used to confirm corneal findings.

Results: Unwashed Eyes

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

Comments:
Any corneal staining after treatment is considered a lesion, however mild, and must be graded accordingly.
Washed eyes are not required for registration.

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

Product Manager:23
MRID No.: 425578-06
Testing Laboratory: Stillmeadow, Inc.
Author(s): Janice O. Kuhn
Species: Rabbit, New Zealand White
Age:3-6 months old
Sex:3 male & 3 female
Weight:Not specified
Dosage:0.5 g
Test Material:CGA-163935 25WP-A FL-912094 ARS-16402 Batch Code 626-13-1
Quality Assurance (40 CFR §160.12): Included

Reviewer: L. Markarian
Report Date:3/25/92
Report No.:8588-91

Summary:

1. **The Primary Irritation Index** =0
2. **Toxicity Category:**IV
3. **Classification:**Core minimum

Procedure (Deviations From §81-5):

0.5g of test material moistened with 0.5 ml of deionized water (1:1 dilution) was applied to the intact clipped skin of the rabbits beneath 2.5 X 2.5 cm gauze patch secured with adhesive tape. The trunks of the animals were wrapped with orthopedic stockinette. At 4 hrs the wrappings were removed and sites washed with wet cloth and tap water. The sites were evaluated at 3/4, 24, 48, and 72 hrs according to Draize.

Results:
No reaction was observed at any site at any interval

Special Comments:

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

Product Manager:23
MRID No.: 425578-07
Testing Laboratory: Stillmeadow, Inc
Author(s): Janice O.Kuhn
Species: Guinea Pig, Hartley
Weight:
Source:
Test Material:
Positive Control Material:
Quality Assurance (40 CFR §160.12):

Reviewer: L. Markarian
Report Date:1/23/92
Report No.:8589-91

Method:

Summary:

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

2. **Classification:**supplementary

Procedure (Deviation From §81-6):

A pre test screening was made using four guinea pigs and four quantities of test material all moistened with 0.5 ml of saline. Each guinea pig received two patches only. The quantities used were 500, 250, 100, and 50 mg of test material. It is not stated if any of the amounts dissolved in the vehicle. At 500 mg in 0.5 ml saline two grades of 0.5 were observed at 24 hrs. One grade of 0 and one grade of 1 was present at 48 hrs. Induction and elicitation were made with 500 mg of test material in saline.

There were two groups of ten animals: one for test material and one as naive control. The test group received three six hour induction applications one week apart for three weeks at the same site. The applications were made under Coverlet adhesive dressing(1.6 X 2.8 cm gauze patch backed with 3.8 X 5 cm adhesive). The trunks of the animals were wrapped in polyethylene film. The animals were restrained.

Two weeks after the last induction challenge was made at a virgin site in the same manner as the inductions. The naive controls were challenged at this time in similar fashion.

Evaluations were at 24 and 48 hrs after the first induction, 24 hrs after the second and third inductions and 24 and 48 hrs after challenge according to Buehler.

The laboratory states that positive control animals are tested periodically using 0.06 % DNCB in 95 % ethanol for induction and elicitation. However the date and the results of the positive control test are not included.

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Results:

No positive reactions are recorded in either the test or the naive control group at any interval.

Tox chem No : 112602 Trinexapac-Ethyl Current date 10/12/93
Laboratory: Stillmeadow, Inc., 12852 Park One Drive, Sugar Land, Texas

STUDY	MATERIAL	MRID NO	RESULTS	TOX CAT	CORE GRADE
Acute oral Limit Test (Rats) 8585-91 1/13/92	CGA-163935 25 WP-A FL-912094	425578-02	LD_{50} > 5050 mg/kg	IV	Minimum
Acute Dermal Limit Test(Rabbits) 8586-91 1/9/92	" " " "	425578-03	LD_{50} > 2020 mg/kg	III	Minimum
Acute Inhalation Limit test(Rats) 8587-91 2/23/92	" " " "	425578-04	LD_{50} > 0.574 mg/kg	III	Minimum
Eye Irritation In Rabbits 8129-91 6/16/91	CGA-163935 26WP-A FL-910887	425578-05	Clear within 7 days	III	Minimum
Dermal Irritation In Rabbits 8388-91 3/25/92	CGA-163935 25 WP-A FL-912094	425578-06	Nonirritating	IV	Minimum
Sensitization in Guinea Pigs 8389-91 1/23/91	" " " "	425578-07		Supplementary	upgradeable