UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES
AND
TOXIC SUBSTANCES

June 22, 2001

MEMORANDUM

Product Name: FRONTLINE TOP SPOT (DOGS)
EPA Reg. No.: 65331-3
DP Barcode: D275794
Case No: 060305
Submission: S599233
Chemical: 129121 Fipronil

From: Byron T. Backus, Ph.D., Toxicologist
Technical Review Branch
Registration Division (7505C)

Byron T. Backus 6/22/2001

To: Ann Sibold/Arnold Layne
Insecticide Branch
Registration Division (7505C)

Registrant. MERIAL LIMITED

ACTION REQUESTED: "Please re-review the attached tox review and determine if the eye and skin irritation studies are correctly categorized..."

BACKGROUND: This package includes a review dated July 17, 1995 from Lucy D. Markarian of the Precautionary Review Section. In this review, the product formulation was assigned to toxicity category III in terms of eye irritation and toxicity category III in terms of dermal irritation.

COMMENTS AND RECOMMENDATIONS:

1. For the purposes of this action, TRB has examined only the Markarian review dated 7/17/95. TRB has not reexamined the actual study submissions (according to the Markarian review, the eye irritation study is in MRID 43577708, and the skin irritation study is in 43577709).

2. The Markarian review indicates that 4/6 rabbit eyes were positive for corneal opacity on day 1 (and 4/6 were positive for iridial irritation and 4/6 were positive for conjunctival redness at day 1). 2/6 eyes were still positive for corneal opacity on day 3. No eyes were positive for irritation effects on day 7. These findings place the test material in toxicity category III in terms of eye irritation (in order for the test material to have been assigned to toxicity category IV by this exposure route, all eyes would have had to be clear of irritation on day 1).

3. For skin irritation, the Markarian review indicates that at 1 hr 3/6 sites scored "2" for erythema and 3/6 scored "1." At 24 and 48 hrs 2/6 scored "2" for erythema and 2/6 scored "1." At 72 hrs 1/6 scored "2" for erythema and 1/6 scored "1." As edema is not mentioned, it is assumed that all sites scored "0" for edema

   The Primary Irritation Index can be calculated then from the following:

   Total 1 hr score = (3 x 2) + (3 x 1) = 9
   Total 24 hr score = (2 x 2) + (2 x 1) = 6
   Total 48 hr score = (2 x 2) + (2 x 1) = 6
   Total 72 hr score = (1 x 2) + (1 x 1) = 3

   The summation of 9 + 6 + 6 + 3 = 24; the mean score at any of the time points is then 6; and the mean score per animal per time point is 1.0 This is the PII value.

   Usually a PII of between 0 and 2 places the test material in toxicity category IV in terms of dermal irritation (there is no reason to assign the test material to toxicity category III in this case, as individual dermal irritation scores were not that high, and there was an improvement over the observation period).

Author: Tina Levine at DCOPP12
Date: 02/29/96 09:12 AM
Priority: Normal
Receipt Requested
TO: Richard Keigwin at DCOPP2
Subject: Re: Question on a review for 65331-EUP-E
---------- Message Contents ----------

Tina--

I have a follow-up question on one of Lucy's reviews. In a 7/17/95 review, Lucy indicated that certain studies were acceptable for the EUP only and would have to be repeated prior to registration. However, as I recall, you and I had a discussion subsequently and I think we decided that the reviews would also be acceptable for full registration.

Could you please reconfirm this?

Thanks!

--Rick

Rick--

I have given this review another look to be sure, and checked with Mark and Ian, and I believe that we do not need repeat studies. You can file this message in the jacket if you need to.

--Tina

MEMORANDUM:

Subject: EPA File Symbol/EPA Reg. No.: 65331-EUP-E
Frontline Spot Treatment

From: Lucy D. Markarian, Biologist 4/17/95
Precautionary Review Section
Registration Support Branch
Registration Division (7505W)

To: Rick Keigwin/Ann Sibold, PM 10
Insecticide-Rodenticide Branch
Registration Division (7505C)

Applicant: Rhone Merieux, Inc.
115 Transtech Drive
Athens, Georgia 30601

FORMULATION FROM LABEL:

Active Ingredient(s):	% by wt.
5-amino-1-(2,6-dichloro-4-(trifluoromethyl) phenyl)-4-((1R,S)-(trifluoromethyl)sulfanyl)-1-H-pyrazole-3-carbonitrile	9.7 %
Inert Ingredient(s):	90.3 %
Total:	100.0 %

BACKGROUND

Rhone Merieux, Inc. has submitted six studies in support of an EUP permit for the product Frontline Spot Treatment under EPA symbol 264-EUP-E. The registrant wishes to evaluate flea and tick control in dogs and cats.

RECOMMENDATION

In the original form of the submission acute oral, inhalation, eye and skin irritation studies were found to be unacceptable, and the acute dermal study was considered upgradeable if information about the application of the test material could be provided. Following several telephone conversations between Dr. Levine, chief of PRS, and the representatives of the registrant some supplemental information was received. Based on the total information received PRS has reached the following decisions:

Acute oral, eye and dermal irritation studies are classified for use for EUP purposes only. They are assigned classifications and the registrant needs to submit acceptable tests prior to registration. The acute dermal study is considered acceptable in category IV upon the receipt of the supplemental data. Category IV toxicity is assigned for inhalation hazard potential, based on considerations as explained below. Sensitization test is acceptable for the part conducted with the subject product, but the positive control study is not acceptable. It is stipulated that a sensitization study without a valid positive control test will be rejected in the future.

The following is the rationale used for the decisions.

Acute Oral

The animals were under age. HED and RSB have concurred that the animals should be between 8 to 12 weeks of age at the beginning of the test [redacted]. All the animals were reported to be six weeks of age and weighed 131 - 179 g. The average weight was 169± 6 g for males and 139± 6 g for females.

The confidence limits for the LC_{50} values are greater than 20 % as required by the guidelines. This could possibly be the result of the erratic responses observed due to the improper age and weight of the animals and the large gap between doses. The doses were not appropriately chosen.

The vehicle control group did not serve any useful purpose, because the ultimate goal of the test is to show the toxicity of the whole formulation that includes the vehicle. Control groups are not required. In this case this was unnecessary use of animals.

The laboratory has stated in the supplemental information submission that the animals were actually 7 week old weanelings,

and that it was their practice to use younger animals for greater sensitivity, and that reproduction studies were conducted for longer periods.
PRS does not find this reason enough for using immature and underweight animals. It is well established that immature animals are not necessarily more sensitive, but, conversely can be less sensitive, because their metabolism differs from that of the adults. The acute oral study has no connection with reproduction studies, which, by necessity, are conducted over longer periods. PRS is willing to assign tox category III to the acute oral hazard potential for the EUP only, with the understanding that before the product can be fully registered an acceptable acute oral study must be submitted.

Acute Dermal

The report was not specific about the wrapping of the animals. The supplemental information has provided some details that help upgrade the study. The dermal toxicity of the subject product is placed in category III toxicity.
The treatment of animals with the vehicle did not serve any purpose, because the evaluation of the complete test material is necessary. Whether the toxicity is the result of the active ingredient or the vehicle does not make a difference in the evaluation of the total formulation. This was unnecessary use of animals.
It is recommended that future submissions specify the area of application, the size and the description of the covering and binding materials. According to the guidelines the area is a minimum of 10 cm^2 for a 2 kg animal, and the area should be adjusted according to the weight of the animal. The test material is applied directly to the skin, and if solid, it has to be moistened with water prior to application on the clipped dorsum. All effort is to be made to apply the test material at the same rate (mg/cm^2) on the animals. The gauze covering should be covered with an impermeable material to preclude ingestion and inhalation of the test material and retard evaporation.

Acute Inhalation - Unacceptable

The following are the reasons of rejection:

1. The quality assurance statement is not acceptable. 40 CFR states that the conduct of each test has to be inspected and at intervals as necessary to assure the integrity of the study. According to the statement the test was not inspected at all, and it is reason enough to reject the study. This has been referred to OECA.

2. The age of the animals has to be included in the report. All is known is when they arrived at the laboratory and when they were used. The males, as judged by their weight, do not seem to be in the correct age range. All males were over 300

4.

g, and most of them significantly over 300 g. The acceptable age frame is 8 to 12 weeks of age, and preferably 200 to 300 grams. Animals in he proper age range that are underweight should not be inducted into a study. Failure to gain weight may signify underlying disease state. By the same token animals over 300 grams should not be used, especially with pesticides that are known to accumulate in fatty tissue.

3. The nominal chamber concentration in the test group is larger than the actual measured concentration. It is hard to understand how the concentration within the chamber can be higher than what was actually introduced into the chamber, especially if one considers that some of the test material will cling to the chamber walls. While it is understood that the chamber concentration can vary, and some peaks can occur that show higher concentrations of test material than the nominal concentration, the difference should not be as great as it was continually.

4.Since the test material was nebulized, and there was apparent difficulty in measuring the chamber concentration gravimetrically, it appears that the test would have been served better if chamber concentrations had been measured by some other analytic method than gravimetric determination.

5. Equilibration time needs to be included in the report.

6. The oxygen concentration and/or number of air changes per hour was not given.

7. The vehicle control group did not serve a useful purpose. It was unnecessary use of animals. An air control group may have been better if a control group was desired.

6. In the supplement sent for the inhalation test the laboratory has given the moisture from the animals that is introduced into the chamber under normal respiration conditions as reason for the greater gravimetric concentration than the nominal concentration. This is not acceptable, because at no time the percentage of humidity was over the prescribed limit. As a matter of fact in the beginning it was under 40 %, which is under the acceptable limit. Under these circumstances the excess of moisture into the chamber from the animals is not a valid argument. Even if it were, then the gravimetric determinations should have been conducted differently. The filters should have been weighed as soon as the sampling was complete, as well as after desiccating the filters to determine the moisture content, and the actual concentration calculated accordingly.
If analytic determination is not routinely performed in the laboratory, this can be done away from the laboratory as long as samples are impinged at the time of sampling. Analytic determination is the method of choice.
The primary interest is the actual chamber concentration, regardless of how efficacious the generation system is. When the

chamber concentration exceeds the nominal concentration for whatever reason, the test is unacceptable. It is laboratory's responsibility to give an accurate chamber concentration.

To reach a conclusion, PRS has considered the following:

If accepted at face value the test is conducted at three times the concentration for the required 2 mg/l acceptable for category IV placement of the inhalation hazard.

MMAD or the SGD are not actually calculated, but it is stated that 95 % of the particles were under 3.5 um. Given the method of generation, this might be acceptable.

The inhalation toxicity of the active ingredient.

The possibility of inhalation hazard from a spot treatment product for pets.

It is concluded that the presented study, while not acceptable in itself, contributes twords making some decisions; and category IV toxicity is assigned to the inhalation hazard potential without accepting the study.
No further testing is required in this area.

Eye Irritation - unacceptable

By the request of the registrant the laboratory treated both eyes of the rabbits. According to the guidelines, this is not acceptable. The test is rejected. The contralateral eye always serves as reference to the tested eye, and cannot be used. Treatment of the contralateral eye with the vehicle alone did not serve any purpose, because the effect of the complete formulation on he eye is sought.
The vehicle also needs an untreated eye for comparison.

A new eye irritation study needs to be submitted prior to the registration of the product. For the issuance of the EUP only, the eye irritation potential can be placed in category III.

The means of evaluating the eyes, the source of light, if magnification or slit lamp is used, should be clearly indicated. The eyes should be evaluated using a source of white light, as close to day light as possible. PRS recommends the use of magnification or slit lamp. Hand held penlights are not acceptable.

Dermal Irritation - Unacceptable

By the request of the registrant the rabbits were treated with two different materials. This is not an acceptable practice and the study would normally be rejected without further evaluation. The comparison is between the treated area with the complete formulation and the untreated skin,

or at best untreated but patched area. The site treated with the vehicle could not serve as comparison area even if two test materials per animals were acceptable, and the second material is the vehicle for the first.
The report should clearly define the patching and wrapping method rather than specify the manufacturer of the material used. The report does not clarify how the animals were patched or wrapped.
PRS has assigned category III for the primary skin irritation potential for the issuance of the EUP. Prior to full registration an acceptable primary irritation study has to be submitted.

Sensitization

The test is accepted only because it was conducted with the undiluted test material. The following critique is offered:

1. The report does not give any details of the pre test screening. The number of animals is not specified, nor the individual scores given.

2. The age of the animals is given as 1 to 3 months old. A one month old guinea pig(28 days) is too young to be inducted in any test. The weight of the animals did not indicate any animals that were not in the proper age group.

3. The positive control test is unacceptable. Any positive test that is referenced must be conducted within six months of the date of the currently submitted test. The referenced test was conducted in January of 1994, whereas the present test is conducted between November (initiation date not given) and December 26 of 1994. This is more than six months apart.

4. The positive control test is induced at 0.05 % DNCB and elicited at 0.1 and 0.5 %. Vehicle in each of the events is not disclosed. The methodology used specifies that the induction concentration should be a slightly irritating concentration, and the elicitation concentration should be the highest nonirritating concentration. If 0.05 % was the slightly irritating concentration, higher concentrations are not expected to be less irritating, and consequently cannot qualify as the highest nonirritating concentration. There were no signs of irritation at 0.1 %, and only at 0.5 % there were positive reactions. It is not certain that the reaction induced at 0.5 % was sensitization. It probably was irritation, as even 0.1 % (generally considered an irritating concentration) did not result in irritation. The vehicle may make a difference in the resulting reaction. However, assuming that ethanol and acetone were used for induction and elicitation, respectively, the results are still questionable. 0.1 and 0.5 % DNCB in acetone are established to be irritating concentrations. 0.05 % is slightly irritating in ethanol, and may be acceptable for

induction. Buehler has been able to induce at this concentration, and elicit at 0.03 %.
The ultimate proof of sensitization is the ability to elicit responses at a concentration that is lower than the induction concentration. This concentration is described by Buehler[1] as that concentration that results in two grades of 0 and two grades of ±, when tested in four guinea pigs. This concept is applicable to any control material, including the positive control. The test did not demonstrate the laboratory's ability to induce sensitization.

5. PRS recommends and encourages the use of the scoring system designed by the author of the test to avoid confusion. A Buehler test is best evaluated on the Buehler scale.

Future submissions without a valid positive control tests will not be acceptable. It is expected that the induction and elicitation concentrations will be chosen according to the dictates of the methodology used for all test and control materials. The guidelines allow the laboratory to choose from a list of acceptable tests. However, once it is chosen, it is required that the methodology is followed closely enough as not to compromise the integrity of the assay.

LABEL

The toxicity profile of the product is:

Acute Oral	Category III assigned for EUP only
Acute Dermal	Category IV
Acute inhalation	Category IV assigned
Eye irritation	Category III assigned for EUP only
Dermal Irritation	Category III assigned for EUP only
Sensitization	Not a sensitizer

The recommended precautionary label is for the EUP only, and may have to be changed upon the submission of the requested tests.

The signal word is CAUTION.

The precautionary statement must Read:

Harmful if swallowed, causes moderate eye irritation. Avoid contact with eyes and skin. Wash thoroughly with soap and water after handling. Remove contaminated clothing and wash before reuse.

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

8

The statement of practical treatment must read:

If swallowed	Call physician or get medical attention. Drink one or two glasses of water and induce vomiting by placing finger in the back of the throat. If a person is unconscious do not give anything by mouth or induce vomiting.
If in eyes	Flush eyes with plenty of water. Get medical attention if irritation persists.
If on skin	Wash thoroughly with soap and water. Get medical attention if irritation persists.

PRS requests forwarding a copy of this review to the laboratory.

Tox Chem No: 129121 Fipronil Current Date: 7/17/95

Laboratory: Centre International de Toxicologie (C.I.T.), Miserey, 27005 Evreux, France
Inveresk Research International (IRI), Ltd., Tranent EH33 2NE, Scotland

STUDY	MATERIAL	MRID NO	RESULTS	TOX CAT	CORE GRADE
Acute Oral(rats) LD_{50} test 12781TAR 3/2/95 CIT	RM1601E/62 (test formulation) RM1601E/64 (vehicle control)	435777-05			Unacceptable Assigned Category III for EUP only
Acute Dermal(rats) Limit test 12008TAR 11/25/94 CIT	RM1601E/62 (test Formulation) RM1601E/64 (vehicle Control)	435777-06	LD_{50} > 5000 mg/kg	IV	Acceptable
Acute Inhalation Limit Test(rats) IRI 654387 1/31/95 IRI	RM1601E/62 (test Formulation) RM1601E/64 (vehicle Control)	435777-07			Unacceptable Assigned Category IV
Eye Irritation in Rabbits 12010TAL 11/25/94 CIT	RM1601E/62 (test Formulation) RM1601E/64 (vehicle Control)	435777-08			Unacceptable Assigned Category III for EUP only
Deremal Irritation in Rabbits 12005TAL 11/25/94 CIT	RM1601E/62 (test Formulation) RM1601E/64 (Vehicle Control)	435777-09			Unacceptable Assigned category III for EUP only

Tox Chem No: 129121 Fipronil Current Date:7/17/95

Laboratory:Centre International de Toxicologie(C.I.T.), Miserey, 27005 Evreux, France
Inveresk Research International (IRI), Ltd.,Tranent EH33 2NE, Scotland

S T U D Y	M A T E R I A L	MRID NO	R E S U L T S	TOX CAT	CORE GRADE
Sensitization in guinea pigs 12011TSG 12/26/94 CIT	RM1601E/62 (test Formulation) RM1601E/64 (vehicle Control)	435777-10	Not sensitizer	NA	Acceptable

41

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

Product Manager:10 **Reviewer: L. Markarian**
MRID No.435777-05 **Report Date:3/2/95**
Testing Facility:CIT **Report No.12781TAR**
Author(s):S. De Jouffrey
Species:Rat , Sprague Dawley
Age:six weeks
Weight:M 159 - 178 g, F 132 - 149 g
Source:Ifia Crédo, 69210 L'Arbusle, France
Test Material:RMI601E/62 (JO 700) yellowish liquid, SG 1.03
RM 1601E/64 (JO 701) light yellow Liquid,SG 0.97

Quality Assurance (40 CFR §160.12):Included, Adequate

Conclusion:

1. **LD_{50} (mg/kg):** **Males = 3208 (1730 - 8608)**
Females = 2821 (1445 - 14546)
Combined = 2995 (1996 - 5697)
2. **Tox. Category:** **Classification:Unacceptable**

Procedure (Deviations from §81-1):

Fasted animals were intubated at three levels with the test material as received. A group of ten animals were intubated with the vehicle alone. Dose volume was adjusted according to the specific gravity of each of the respective formulations. Observations were frequent on the day of intubation and daily thereafter. Body weights were recorded at initiation and on days 8 and 14. Necropsy was performed on all animals.

Results:

Dosage mg/kg (concentration)	(Number Killed/Number Tested)		
	Males	Females	Combined
test 1000 (0.97 ml/kg)	0/5	1/5	1/10
2000 (1.95 ml/kg)	1/5	1/5	2/10
5000 (4.86 ml/kg)	4/5	4/5	8/10
vehicle control 5000 mg/kg (5.16 ml/kg)	0/5	0/5	0/10

Symptoms & Gross Necropsy Findings:

In the test group hypoactivity, sedation, and piloerection was observed in all groups. At 2000 mg/kg dyspnea and at 5000 mg/kg hypersalivation was observed in a few animals.

In the vehicle control group piloerection, sedation, dyspnea was observed in all animals. 2/5 males showed lateral decubitus, and 1/5 males showed hypoactivity and emaciation. All symptoms were resolved by day 8.

Necropsy of all the survivors revealed no gross pathology. The necropsy of the decedents revealed advanced autolysis.

13

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

Product Manager:10 **Reviewer: L. Markarian**
MRID No.: 435777-06 **Report Date:11/25/94**
Testing Laboratory:CIT **Report No.:12008TAR**
Author(s):S. De Jouffrey
Species:Rat, Sprague Dawley
Weight:M 242 - 277 g, F 212 - 226 g
Age: eight weeks
Source:Iffa Crédo, 69210 L'Arbusle, France
Test Material:RM1601E/62 (JO 700) yellowish liquid, SG 1.03(test material)
RM 1601E/64 (JO 701) light yellow Liquid,SG 0.97(Vehicle Control)
Quality Assurance (40 CFR §160.12):Included, Adequate

Summary:

1. The estimated LD_{50} is > 5000 mg/kg

3. Tox. Category: IV Classification:Acceptable

Procedure (Deviation From §81-2):

The test material and the vehicle control were applied to the clipped skin of ten rats each on approximately 10 % of the body surface (as calculated by Meeh's formula) as received. the sites were covered with "hydrophilic gauze patch" and secured with "hypoallergenic aerated smiocclusive dressing and a restraining bandage". The size or the thickness of the bandage is not stated, nor the restraining bandage described. At 24 hrs the wrappings were removed. The sites were not washed. No residue was observed. Observations were frequent on the day of treatment and daily thereafter. Body weights were recorded at initiation and on days 8 and 15. Necropsy was performed on all animals.

Results:

Reported Mortality

DOSAGE mg/kg	(NUMBER KILLED/NUMBER TESTED)		
	Males	Females	Combined
5000 (test)	0/5	0/5	0/10
5000 (vehicle)	0/5	0/5	0/10

Symptoms & Gross Necropsy Findings:

The only symptom of toxicity was hypoactivity, observed on the day of application.
Necropsy revealed no observable gross pathology.

18

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

Product Manager:10 **Reviewer: L. Markarian**
MRID No.: 435777-07 **Report Date:1/31/95**
Testing Laboratory:Inveresk Research **Report No.:IRI 654387**
Author(s):P.C.Kieran
Species:Rat,Sprague Dawley
Weight: test M 305-370, F 242-274 g,Vehicle M 308-372, F224-276
Age: not specified
Source:Charles River, Marston Rd., Margate, Kent, UK
Test Material:RM1601E/62 (JO 700) yellowish liquid, SG 1.03(test material)
RM 1601E/64 (JO 701) light yellow Liquid,SG 0.97(vehicle Control)
Quality Assurance (40 CFR §160.12):not acceptable

Summary:

1. **The estimated LC_{50} is**
2. **Mean Concentration:**

3. **Tox. Category:** **Classification: Unacceptable**

Procedure (Deviation From §81-3):

Nose only exposure was in a cylindrical aluminum dynamic flow chamber. The test material was introduced from the top and exhausted from the bottom via a filtered vacuum line. The chamber was placed in a hood.

Chamber air flow was measured with flow meters, temperature with a mercury thermometer within the chamber, and humidity with wet bulb dry bulb hygrometers. All the parameters were recorded at 30 minute intervals.

The number of air changes per hour, or the oxygen content of the chamber are not specified.
T_{90} or T_{99} is not given. The equilibration time is unknown

The test atmosphere was created using the test material as received, a Gilson (model 307) pump, and a Schlick atomizer. The chamber concentration was adjusted by adjusting the feed rate and the air flow to the atomizer. Pressurized filtered air was supplied to the atomizer through a Hydrovane compressor.

The chamber concentration was determined 14 times during the exposure with the formulation and 11 times during the exposure with the vehicle, gravimetrically. Sorbent tubes were sealed temporarily in a port at the breathing zone of the animals.The air was sampled at the rate of 1 lpm with sample volumes ranging from 0.8 - 2.8 liters. Nominal concentration was determined by dividing the total weight of the test material by the total air flow.
Particle size analysis was conducted twice during each exposure using a Marple cascade impactor, and sampling from the breathing

zone at 2 lpm. A sorbent tube served to collect what could not be collected on the different stages of the impactor.

The animals were observed prior to exposure, frequently during the exposure and daily thereafter.

Body weights were recorded at initiation and on days 2, 3, 4, 7, 10, and 14.
Necropsy was performed on all animals. The lungs were dissected and body lung ratios determined.

Results

	TEST	VEHICLE CONTROL
	RM I601E/62	RM 1601E/64
Chamber Concentration mg/l		
gravimetric	6.32	5.72
Nominal	4.51	6.16
MMAD + SGD um	Not determined	
% < 0.2 um		
I	95.8	94.0
II	96.0	94.9
Chamber		
Temperature ^{0} C	21-22	20
Humidity %	27-58	32- 81
Air flow lpm	18	18
Mortality		
Male	0/5	0/5
Female	0/5	0/5
Signs of Toxicity		
	During exposure	
Shallow breathing	10/10	10/10
	Post exposure, resolved by day 2	
Red facial stains	10/10	0/10
unkempt	9/10	0/10

Necropsy findings

Males, Test: slightly mottled kidneys (3/5), mottled or dark lungs(2/5) slightly dark spleen (1/50 and liver with pale center (1/5)
Females, Test: pale lungs(3/5), lightly dark spleen(1/5)
Males, control:Mottled Kidneys(3/5), pale or reddened lungs(2/5).
Females, Control:mottled kidneys(1/5), pale or mottled lungs(3/5), dark spleen(1/5)

16

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

Product Manager:10
MRID No.: 435777-08
Testing Laboratory:CIT
Author(s):S. De Jouffrey
Species:Rabbit, New Zealand white
Sex:Male
Weight:2.5± 0.1 kg
Age: not included
Source:Elevage Cunicole de Val de Selle,
80160 Prouzel, France
Dosage:0.1 ml
Test Material:RM1601E/62 (JO 700) yellowish liquid, SG 1.03(test material)
RM 1601E/64 (JO 701) light yellow Liquid,SG 0.97(vehicle Control)
Quality Assurance (40 CFR §160.12):Included, acceptable
Summary:

1. Toxicity Category:

2. Classification:Unacceptable

Reviewer: L. Markarian
Report Date:11/25/94
Report No.:12010TAL

Procedure (Deviations From §81-4):
By the request of the registrant the laboratory treated both eyes of the rabbits. Undiluted test material was instilled in the conjunctival sacs of both eyes. One eye received the test material and the other the vehicle only. Evaluations were at 1, 24, 48, 72 hrs and daily to day 7, according to Draize.
Fluorescein was used to confirm corneal findings. No auxiliary light or magnification was used.
Results:

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

* not possible to read discharge. Test material present in the eye
** Purulent material in the eye

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

Product Manager:10 **Reviewer: L. Markarian**
MRID No.: 435777-09 **Report Date:11/25/94**
Testing Laboratory:CIT **Report No.:12009TAL**
Author(s):S. De Jouffrey
Species:Rabbit, New Zealand White
Age:not specified
Sex:unspecified
Weight: 2.5 ± 0.2 kg
Source:Elevage Cunicole de Val de Selle, 80160 Prouzel, France
Dosage: 0.5 ml
Test Material:Test Material:RMI601E/62 (JO 700) yellowish liquid, SG 1.03(test material)
RM 1601E/64 (JO 701) light yellow Liquid,SG 0.97(vehicle Control)
Quality Assurance (40 CFR §160.12):Included, acceptable
Summary:

1. **The Primary Irritation Index =**
2. **Toxicity Category:**
3. **Classification:Unacceptable**

Procedure (Deviations From §81-5):

Two test materials were applied to the same animal at the same time. This is not an acceptable practice according to the EPA guidelines. The test material was applied to 6 cm^2 piece of "hydrophilic" gauze (thickness unspecified) and then applied to clipped skin. The patch was covered with hypoallergenic tape and the animals were put in restraining bandages (unclear if animals were restrained) At 4 hrs the patches were removed. Sites were not washed. Evaluation were at 1, 24, 48, and 72 hrs and days 5 and 6, according to Draize.

Results: Test material with AI

1 hr	3/6 grade 2 erythema, 3/6 grade 1 erythema
24 hr	2/6 grade 2 erythema, 2/6 grade 1 erythema
48 hr	2/6 grade 2 erythema, 2/6 grade 1 erythema, 1/6 dry skin
72 hr	1/6 grade 2 erythema, 1/6 grade 1 erythema, 2/6 dry skin
day 4	1/6 grade 2 erythema, 1/6 grade 1 erythema, 3/6 dry skin
day 5	1/5 dry skin
day 6	no irritation

Special Comments:

18

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

Product Manager: 10 **Reviewer:** L. Markarian
MRID No.: 435777-10 **Report Date:** 12/26/94
Testing Laboratory: CIT **Report No.:** 12011TSG
Author(s): S. De Jouffrey
Species: Guinea Pig, Hartley
Weight: 310 - 380 g
Age: 1 - 3 months old
Sex: 20 males and 20 females
Source: Centre Elevage Lebeau, 78950 Gambais, France
Test Material: Test Material; RM1601E/62 (JO 700) yellowish liquid, SG 1.03 (test material)
RM 1601E/64 (JO 701) light yellow Liquid, SG 0.97 (vehicle Control)
Positive Control Material: DNCB
Quality Assurance (40 CFR §160.12): Included, acceptable

Method: Buehler
Summary:

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

2. **Classification:** Acceptable

Procedure (Deviation From §81-6):

A pretest irritation assay was conducted using the test material as received. The number of animals used or the results of the test are not included. The main test was conducted using the test material at 100 % for induction and elicitation.

There were forty animals in the test. Ten were used as controls induced with water; twenty were used for the test material and induced with 100 % test material, and ten were used induction with the vehicle.
There were three inductions on clipped skin using 0.5 ml of each of the materials applied on 4 cm^2 gauze pad. The trunks of the animals were wrapped in hypoallergenic waterproof tape. The animals were not restrained. At six hours the patches were removed. The sites were not washed.
Challenge was two weeks after the last induction at virgin sites. Each animal was challenged with water, the test material and the vehicle for the test material in six hour exposures as described for induction. The sites were evaluated at 24 hrs after each induction and challenge. There was a 48 hr evaluation after challenge. The Draize scoring system was used.

Reference is given to a positive control study conducted with DNCB January of 1994 induced at 0.05 % and challenged at 0.5 and 0.1 % DNCB. The vehicle is not specified.

Results:

During induction there were no remarkable reactions. Occasional

grade 1 erythema (± on the Buehler scale) was observed with the test material .

At challenge all reactions were negative on all animals in all groups.

The included results of the positive control test show no reaction with 0.1 %, but positive reaction with 0.5 % DNCB. Induction scores are not included.