AUG-7 1995

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MEMORANDUM:

Subject: EPA File Symbol/EPA Reg. No.: 432-TOA
Aqua-Permanone

From: Lucy D. Markarian, Biologist 8/7/95
Precautionary Review Section
Registration Support Branch
Registration Division (7505W)

To: George LaRocca/Adam Hayward, PM
Insecticide-Rodenticide Branch
Registration Division (7505C)

Applicant: Roussel Uclaf Corporation
95 Chestnut Ridge Road
Montvale, NJ 07645

FORMULATION FROM LABEL:

Active Ingredient(s):	% by wt.
Permethrin	20.0 %
Piperonyl Butoxide, Technical	20.0 %
Inert Ingredient(s):	
	60.0 %
Total:	100.0 %

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BACKGROUND

Roussel Uclaf Corporation has submitted a full battery of tests in support of the registration of Aqua-Permanone under EPA symbol 432-TOA. The product is primarily intended for use against adult mosquitos as a space spray by air or ground application.

RECOMMENDATION

The submitted acute oral toxicity study is acceptable. Acute dermal, eye and skin irritation studies may be upgraded if the requested information can be provided and is found acceptable. The acute inhalation and sensitization studies are not acceptable. New acute inhalation and sensitization tests have to be submitted.
The rationale for the classification of the studies is given below.

The Quality assurance statements from the MB Research laboratories are not acceptable. All are signed by the study director as well as other officials, and by the QA officer. The QA office is supposed to operate independently of the study director and the opinion has to be unbiased. If the QA statement is signed by all officials, then the statement has not achieved its goal.
This has not been the reason for any rejections at this time, but has been referred to OECA. Future submissions with inadequate QA statements may not be considered acceptable.

Acute Dermal

The area of application is described as 10 % of the body surface. According to the guidelines the dimensions of the area must be given as well as the mg/cm^2 needs to be calculated.

Greater than 45 % in the 2000 mg/kg level and greater than 50 % at the 5000 mg/kg level appear to have been left unabsorbed after a 24 hr exposure, and at both levels the animals had not fully recovered at termination. It would seem that less than 5000 mg/kg was capable of causing death, and less than 2000 mg/kg was capable of causing toxicity that would not resolve in 14 days. It is important to ascertain that the test material was correctly applied. The test may be upgraded upon the submission of the following information:

1. The areas of application in measured units.

2. The amount of test material applied per cm^2.

3. The thickness of gauze used for covering the application site.

Acute inhalation

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1. The use of animals that have not been observed for five or more days prior to induction into a test is unacceptable. The guidelines are very clear about this.
The females in the 1.18 mg/l group were in house only one day prior to induction into the study. There were 2/5 deaths in the females in contrast with no deaths in the males. Since the animals had not been observed adequately prior to the exposure, it cannot be a certainty that the trauma of being transported and possibly fasting during shipment had no effect on the results, the opinion expressed by the laboratory notwithstanding.

2. The results of the exposures are very erratic. There is no true dose-response pattern. The deaths in the females at 1.18 and 3.24 mg l are the same as the deaths at 5.03 and 6.63 mg/l levels. This may be attributable to the different lots, the MMAD, and to the females that were not quite suitable to be inducted into the test.

3. The laboratory has not explained why the exposure at 5.03 mg/l was considered unacceptable and left out of calculations.

4. The chamber concentration at 1.18 mg/l varied from 0.751 to 1.473 mg/l. The lowest concentration was only during the first hour of the exposure, from then on the chamber concentration was almost twice that of the first hour. It cannot be concluded that the death of the animals was at 1.18 mg/l, because ,in effect it was much higher for three hours.
5. The MMAD at the two higher levels is not acceptable. The limit is 4.0 um. At both exposures the MMAD was appreciably higher. The 5,03 mg/l level was not used for calculations.It is claimed that the LC_{50}s > than 6.63 for the males. This is not true, because that level is not an acceptable test. The MMAD ranged from 4.6 to 4.9, with no deaths in the males. The absence of deaths may mean that the product was not respirable enough to cause deaths, but enough to cause toxic effects. The only thing that can be said is that the LC_{50} is > than 3.24 mg/l for the males, which would place the males in category IV, but would not change the acceptability of the test as a whole.

6. The females appear to be the more sensitive sex. The calculated LC_{50} shows limits of 0.215 - 23.104 mg/l. The confidence limits, according to the guidelines, should not be more than 20 % of the median value. The limits in this case far exceeds the acceptable range. Additionally, since the exposure at the highest and lowest levels are not acceptable tests, there is not enough data to allow the calculation of LC_{50}. The conclusion is that based on the acceptable portion of the data (3.24 mg/l exposure) the LC_{50} of the females cannot be determined. If the females are more sensitive, then the LC_{50} of the product is dependent on the

females. Based on the results of the test, the LC_{50} of the product cannot be determined.

A new inhalation test needs to be submitted.

Eye Irritation

The source of light, if auxiliary light was used ,or if magnification or any other device was used for the evaluations has to be reported. No such information was included in the report.
The study may be upgraded if the outstanding information is provided

Dermal Irritation

The test may be upgraded if the following information can be provided:

1. The size and description of the patch including the thickness of the patching material.
2. Confirmation of the size of the application site.
3. If the test material was applied to the skin directly.
4. A description of the semiocclusive dressing.

Sensitization

1.The screening showed that 50 % was the more correct concentration for induction. 25 % was demonstrated to be nonirritating, but was not established as the highest nonirritating concentration. The concentrations were arbitrarily chosen. The test cannot be successful when the induction and elicitation concentrations are the same.

2.The positive control was induced and elicited at the same irritating concentration. Although the numerical evaluations do not show the need after the initial induction, the induction site was moved at this and after each subsequent induction where no more than grade 2 erythema was observed. Apparently the need was present, but the numerical values do not reflect this. 0.1 % DNCB in ethanol has been shown in publications to be moderately irritating and acceptable for induction. 0.1 % in diethyl ether is also irritating. Therefore, the rationale for using the same concentration for both induction and elicitation is not apparent. The ultimate proof of sensitization is to be able to elicit at a lower concentration than used for induction.
The laboratory has not demonstrated the ability to induce sensitization, or show the sensitivity of the animals.

3. There were no naive controls. In a Buehler test the basis for comparison is the naive control group. The test group results are evaluated relative to the naive controls. The results from the initial induction cannot be used for

comparison, because the protocol calls for induction at a somewhat irritating concentration for induction and the highest nonirritating concentration for elicitation. This is defined by Buehler[1] as that concentration that when tested in four guinea pigs would result in two grades of 0 and two grades of ±. By definition the two cannot be the same. The only exception is when the undiluted test material is shown to be completely nonirritating and is used for both purposes. This was not the case.
Fresh naive controls are needed for rechallenge.

A new sensitization test has to be submitted.

LABEL

The label will be reviewed when the toxicity profile is fully defined.

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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

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

Product Manager: 13
MRID No. 432237-04
Testing Facility: MB Research
Author(s): Daniel R Cerven
Species: Rat, Wistar
Age: young adult
Weight: M209 - 253 g, F 200 - 264 g
Source: Ace Animals
Test Material: Permanone insect Killer R7, FNB 90-1-115B Beige Liquid, pH 5.1

Reviewer: L. Markarian
Report Date: 11/7/91
Report No. MB 91-764 A

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

Conclusion:

1. LD_{50} (mg/kg): **Males =** 1200 (1000 - 1400)
Females = 800 (600 - 1100)
Combined = 1000 (850 - 1200)

2. **Tox. Category:** III **Classification:** Acceptable

Procedure (Deviations from §81-1):

Fasted animals were intubated at five dose levels with the test material as received. Observations were frequent on the day of treatment and daily thereafter. 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 (concentration)	(Number Killed/Number Tested)		
	Males	Females	Combined
800	0/5	2/5	2/10
1000	2/5	4/5	6/10
1250	2/5	4/5	6/10
1560	4/5	5/5	9/10
5000	5/5	5/5	10/10

Symptoms & Gross Necropsy Findings:

At 800 mg/kg all males showed tachypnea, and 4/5 had tremors. The females showed tremors and hypoactivity. In addition 2/3 survivors showed chromodocryorrhea, tachypnea,and staining and soiling of the body surfaces.1/3 survivors showed chromodorhynorrhea.
At 1000 mg/kg all animals showed tremors and hypoactivity. The

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survivors also showed chromodocyorrhea, chromodorhynorrhea, Piloerection, soiled/stained body areas, some showed lethargy and ptosis.
At the three higher doses tremors and hypoactivity were noted prior to death. In addition, the survivors at 1250 mg/kg also showed flaccid muscle tone, ataxia, diarrhea, and hyperactivity . Abscesses were observed in 1/5 females at 1560 mg/kg.

At necropsy the survivors showed no gross pathology. Among the decedents the most prevalent observation were darker than normal lungs, darker than normal livers with pale areas, red stomachs some with pale areas, distention of stomach with gas and/or mucus, and yellow or red coloration of intestinal areas.

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

Product Manager: 13
MRID No.: 432237-05
Testing Laboratory: MB Research
Author(s): Daniel R. Cerven
Species: Rabbit, New Zealand White
Weight: 2.0 - 2.4 kg
Age: young adult
Source: Ace Animals
Test Material: Permanone insect Killer R7, FNB 90-1-115B Beige Liquid, pH 5.1
Quality Assurance (40 CFR §160.12): Included, unacceptable

Reviewer: L. Markarian
Report Date: 1/15/92
Report No.: MB 91-764 B

Summary:

1. **The estimated LD_{50} is**

3. **Tox. Category:** **Classification:** Supplementary

Procedure (Deviation From §81-2):

Two dose levels were used: 5 g/kg and 2 g/kg.
The test material as received was applied to 10 % of the body surface (area of application not specified, mg/cm not calculated) and covered with gauze dressing(thickness not indicated) secured with tape. The trunks of the animals were wrapped in plastic. At 24 hrs the wrappings were removed and the residue was washed off with water. The amount of residue was visually estimated to be 50 - 75 % of test material in the 5000 mg/kg group , and 45 - 60 % of the test material in the 2000 mg/kg group.
Observations were frequent on the day of application, and daily thereafter. Body weights were recorded at initiation and on days 7 and 14, 21 and 28 as well as at death. The observation period was extended to 28 days for the 5000 mg/kg group to show the reversibility of the effects. One male was sacrificed on day 18 due to moribund condition Necropsy was performed on all animals.

Results:

Reported Mortality

DOSAGE	(NUMBER KILLED/NUMBER TESTED)		
	Males	Females	Combined
5000 mg/kg	2/5	0/5	2/10
2000 mg/kg	0/5	0/5	0/10

Symptoms & Gross Necropsy Findings:

At 5000 mg/kg diarrhea, few feces, lethargy, emaciation, and soiling of body surfaces were observed in the majority of the animals. Marked weight loss was observed in the animals that died, and most survivors either failed to gain weight or lost weight by day 14. The weight gains at 28 days were marginal.

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Emaciation and observation of few feces persisted to day 28 in few of the animals.
The necropsy of the survivors revealed no gross pathology in the females and 2/5 males. one male showed signs of emaciation, and pale areas on the kidneys, as did two decedents. In addition the animal that was sacrificed showed pale areas on the liver, and distention of the intestines with fluid.

At 2000 mg/kg Diarrhea and soiled body surfaces were the major observations and persisted to day 14 in 6/10. At necropsy 4/10 showed no abnormalities, 4/10 had dermal abnormalities, 2/10 showed red areas on the intestines, one of which also showed distention of the intestines with fluid.
Dermal irritation was observed at both levels as

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

Product Manager: 13
MRID No.: 432237-06
Testing Laboratory: Stillmedow
Author(s): Mark S. Holbert
Species: Rat, Sprague Dawley
Weight: 214 - 283 g, F 188 - 224 g
Age: unspecified
Source: Harlan Sprague Dawley, Houston, TX
Test Material: Permanone insect Killer R7,
13599 lot FNB 92-1-67 (5.03 mg/l) received 1/25/93
13704 lot FNB 92-1-67 (6.68 mg/l) received 3/29/93
13770 lot FNB 92-1-92 (1.1 & 3.24 mg/l) received 5/13/93
Beige Liquid, pH 5.1
Quality Assurance (40 CFR §160.12): Included, Acceptable

Reviewer: L. Markarian
Report Date: 9879-93
Report No.: 7/27/93

Summary:

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

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

Procedure (Deviation From §81-3):

There were ten animals, five male and five female, in each of the four exposure groups. The males in the 1.18 mg/l group were acclimated only one day. The others are reported to have been acclimated at least five days, but the exact time is not stated. The test was conducted with three different shipments of the test material, two of which had the same lot number and expiration date and were used for the two higher levels(one each), but the last shipment had a different lot number and had a later expiration date than the others. This shipment was used for the two lower exposure levels.

Exposure was in a 500 l New York University type dynamic flow chamber. Chamber air flow, temperature and humidity were measured through calibrated critical orifice and Taylor wet bulb/dry bulb hygrometer within the chamber, respectively, and recorded at 30 minute intervals.

The test atmosphere was created by pumping the test material(FMI pump) into a pressure operated air atomizer(Spraying Systems 1/4 JSS) and introducing the created aerosol directly into the chamber.Air flow was sufficient to maintain the oxygen level to at least 19 %. T_{99} was 21 minutes. It is not stated if the exposure was extended to compensate for the equilibration period,or if the chamber was purged prior to the removal of the animals.

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Chamber concentrations were determined every hour, analytically,

by sampling from the breathing zone at the rate of 0.602 lpm for 30 minutes and impinging in tandem impingers. Extraction was in 85 % methanol and 15 % water. Analysis was through HPLC (Beckman System gold with autoinjector, Verspack column, at 254 nm).

Particle size analysis was conducted twice per exposure using an Andersen cascade impactor by sampling from the breathing zone at 28.3 lpm for 1 to 5 minutes.

Observations were frequent during the day of exposure and daily thereafter. The animals were washed upon removal from the chamber

Results

Chamber Concentration mg/l

Analytic	1.18	3.24	5.03	6.68
Range	0.751-1.473	3.026-3.465	4.776-5.396	6.315-7.122
MMAD ± SGD um				
I	3.469± 1.762	3.29± 2.275	4.431± 2.027	4.913± 2.237
II	2.824± 2.217	3.331± 2.077	4.527± 2.512	4.629± 2.465
Chamber				
Temperature ^{0} F	69-70	69-70	70	70-71
Humidity %	81-86	95-100	100	100
Air flow lpm	109	109	110	109
Mortality				
Male	0/5	0/5	1/5	0/5
Female	2/5	3/5	1/5	3/5

Signs of Toxicity

Major signs of toxicity included decreased activity, diarrhea, muscle and body tremors, piloerection, lacrimation, nasal discharge, gaping, respiratory gurgle, polyuria, ptosis, exopthalmos, catatonia, and staggered gait.

Necropsy findings

Survivors showed no gross pathology. In the decedents mottled discolored or mottled lungs, swollen lungs,and gaseous distention of the gastrointestinal tract were observed.

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

Product Manager: 13
MRID No.: 432237-07
Testing Laboratory: MB Research
Author(s): Daniel R. Cerven
Species: Rabbit, New Zealand White
Sex: Female
Weight: 2.4 - 2.8 K
Age: Young adult
Source: Ace Animals
Dosage: 0.1 ml
Test Material: Permanone Insect Killer R7, FNB 90-1-115B Beige Liquid, pH 5.1
Quality Assurance (40 CFR §160.12): Included, Unacceptable

Reviewer: L. Markarian
Report Date: 10/03/91
Report No.: MB 91-764 D

Summary:

1. **Toxicity Category:**

2. **Classification:** Supplementary

Procedure (Deviations From §81-4):

The test material was instilled in the conjunctival sacs of nine pre examined eyes as received. Six animals were observed unwashed and three were observed following irrigation 30 seconds after instillation with water for 30 seconds. Observations were at 1, 24, 48, and 72 hrs according to Draize. Fluorescein was used at 24 hrs 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	0/6	0/6	0/6				
Iris	0/6	0/6	0/6	0/6				
Conjunctivae								
Redness	0/6	0/6	0/6	0/6				
Chemosis	0/6	0/6	0/6	0/6				
Discharge	0/6	0/6	0/6	0/6				

Comments:
Washed eyes are not required for registration, and were not reviewed.

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

Product Manager:13
MRID No.: 432237-08
Testing Laboratory:MB Research
Author(s): Daniel R. Cerven
Species: Rabbit, New Zealand White
Age: Young adult
Sex: Female
Weight: 2.0 - 2.3
Source: Ace Animals
Dosage: 0.5 ml

Reviewer: L. Markarian
Report Date:10/3/91
Report No.:MB 91-764 C

Test Material: Permanone insect Killer R7, FNB 90-1-115B Beige Liquid, pH 5.1
Quality Assurance (40 CFR §160.12): Included, not acceptable

Summary:

1. **The Primary Irritation Index** = 1.54

2. **Toxicity Category:**

3. **Classification:**Supplementary

Procedure (Deviations From §81-5):

The test material was applied to the clipped skin on a 10 cm^2 area. The patches or the semiocclusive wrapping of the animals has not been defined. The wrappings were removed at 4 hrs and the sites were washed with water. Observations were at 1, 24, 48 and 72 hrs according to Draize.

Results:

1 hr	Erythema 4/6 grade 2, 2/6 grade 1 Edema 2/6 grade 2, 4/6 grade 1
24 hrs	Erythema 1/6 grade 2, 5/6 grade 1 Edema 2/6 grade 1
48 hrs	Erythema 1/6 grade 2, 4/6 grade 1 Edema 2/6 grade 1
72 hrs	Erythema 2/6 grade 1 Edema 1/6 grade 1

Special Comments:

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

Product Manager:13
MRID No.: 432237-09
Testing Laboratory:MB Research
Author(s): Daniel R. Cerven
Species: Guinea Pig, Hartley
Weight: 255-283 g
Age: Not specified
Source: Ace animals, received in three shipments
Test Material: Permanone insect Killer R7, FNB 90-1-115B Beige Liquid, pH 5.1
Positive Control Material:DNCB
Quality Assurance (40 CFR §160.12): Included unacceptable

Reviewer: L. Markarian
Report Date:11/7/91
Report No.:MB 91-764 F

Method: Buehler

Summary:

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

2. **Classification:**Unacceptable

Procedure (Deviation From §81-6):

A screening was made to define the induction and elicitation concentrations. Four guinea pigs and four concentrations in distilled water were used. each animal was tested with two concentrations only. 50 and 25 % were tested in the same animals and 10 and 1 % were tested in another two animals. Grade 1 erythema was observed in 2/2 sites at 100 %, but no reaction was observed at any other site with any concentration. Induction and elicitation were at 25 % in distilled water.
The concurrent positive control study was conducted with 0.1 % DNCB in alcohol for induction and in ethyl ether for elicitation.

The test and the positive control groups consisted of ten animals each. There were no naive controls.

There were nine inductions made three times a week for three weeks. Challenge was two weeks after the last induction.

Each application was for six hours made in 0.5 ml aliquots in Hill Top chambers. The cambers were secured to the clipped skin with 2 mil thick plastic and adhesive tape. One of the sites treated with the test material was moved after the eighth induction because of eschar. All the sites in the positive control test were moved after each induction.
challenge was at the site of induction as well as at a virgin site. There was a rechallenge forty eight hrs after the primary challenge at the same sites as the primary challenge. All applications were evaluated at 6, 24, and 48 hrs according to Draize erythema scores.

Results:

In both the test and positive control groups responses to inductions became progressively more pronounced in both intensity and incidence. Following the last induction the test group showed grade 2 erythema at all but one site. The last site had been moved due to eschar after the eighth induction. The positive control group showed grade 2 or 3 erythema, but all sites had been moved.
At challenge in the test group there were two readings of grade 1 erythema at 6 hrs and one reading of grade 1 erythema at 24 hrs at the virgin site. All positive control sites showed irritation.
At rechallenge there were three grade 1 erythema readings at the primary challenge (originally virgin) site at 6 and 24 hrs.
All positive control sites showed irritation.

Tox Chem No: 109701 Permethrin(ANSI) **Current Date:** 8/17/95

Laboratory: MB Research, P.O.Box 178, Steinburg and Wentz Roads, Spinnerstown, PA 18968
STillmeadow, Inc.,12858 Park One Drive, Sugar Land, Texas 77478

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
Acute Oral LD_{50} study(Rats) MB 91-764 A 11/7/91 MB Research	Permanone Insect Killer R7	432237-04	LD_{50} mg/kg M 1200 (1000-1400) F 800 (600-1100) C 1000 (850-1200)	III	Acceptable
Acute Dermal Limit Test(rabbits) MB 91-764 B 1/15/92 MB Research	" " " "	432237-05			Supplementary Upgradeable
Acute Inhalation LD_{50} test(Rats) 9879-93 7/27/93 Stillmeadow	" " " "	432237-06			Unacceptable
Eye Irritation in Rabbits MB 91-764 D 10/3/91 MB Research	" " " "	432237-07			Supplementary Upgradeable
Dermal Irritation in Rabbits MB 91-764 C 10/3/91 MB Research	" " " "	432237-08			Supplementary Upgradeable
Sensitization in Guinea Pigs MB 91764 F MB Research	" " " "	432237-09			Unacceptable