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

FEB 4 1999

OFFICE OF PREVENTION, PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Registration of a Manufacturing-Use Product (MUP) **Citriodiol Insect Repellent Concentrate** (EPA Reg. Symbol 305-LO), 100.0% Oil of Eucalyptus containing 65% *p*-menthane-3,8-diol, *cis* and *trans* isomers, and Three End-Use Products: **REPEL® NATURAL Insect Repellent Lotion** (EPA Reg. Symbol 305-LA) containing 30% Oil of Eucalyptus, **REPEL® NATURAL Insect Repellent Non-Aerosol Pump** (EPA Reg. Symbol 305-LT) and **REPEL® NATURAL Insect Repellent Aerosol** (EPA Reg. Symbol 305-LI) each containing 40% Oil of Eucalyptus (Chemical Nos. 040503 and 011550; Review of Product Chemistry, Toxicity, and Efficacy Studies; MRID Nos. 446242-01 through -10, 446240-01 through -03. 446~~24~~239-01 through -03, 446241-01 through -05; Case Nos. 062646, 062648, 062647, 062650; Submissions S547945, S557954, S547950, S549547; DP Barcodes D248909, D248912, D248911, D249986).

FROM: Carol E. Frazer, Ph.D., Toxicologist Carol
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THROUGH: Robyn Rose, Entomologist Robyn Rose
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Freshteh Toghrol, Ph.D., Senior Scientist F. Toghrol
Biochemical Pesticides Branch
Biopesticides and Pollution Prevention Division (7511C)

TO: John T. Tice, Regulatory Action Leader
Biochemical Pesticides Branch
Biopesticides and Pollution Prevention Division (7511C)

ACTION REQUESTED:

Wisconsin Pharmacal Inc. requests registration of a biochemical pesticide manufacturing-use product (MUP) **Citriodiol Insect Repellent Concentrate** (EPA Reg. Symbol 305-LO) with 100.0% Oil of Eucalyptus (containing ~65% *p*-menthane-3,8-diol, *cis* [43%] and *trans* [22%] isomers) and three End-Use Products (EUPs): **REPEL® NATURAL Insect Repellent Lotion** (EPA Reg. Symbol 305-LA) containing 30% Oil of Eucalyptus, **REPEL® NATURAL Insect**

Repellent Non-Aerosol Pump (EPA Reg. Symbol 305-LT) and **REPEL® NATURAL Insect Repellent Aerosol** (EPA Reg. Symbol 305-LI) each containing 40% Oil of Eucalyptus. To support these registrations, the registrant submitted product chemistry comprised of the §151B series and §152B-10, -11, -13, -14, -15, and -17, primary eye (§81-4) and primary dermal (§81-5) irritation studies for all these products. In addition, the 305-LO package contains acute oral (§81-1) and acute dermal (§81-2) toxicities, dermal sensitization (§81-6), a reverse mutation assay (§84-2), a 21 day dermal absorption study (§85-2), post-marketing surveillance data and a waiver for a 21-day subchronic dermal toxicity study. An acute inhalation (§81-3) toxicity study was included in the 305-LI package. The foregoing information on 305-LO is subsumed in MRID Nos. 446242-01 through 10, for 305-LA, 446240-01 through 03, for 305-LT, 446239-01 through 03 and for 305-LI, 446241-01 through 04. Also submitted was a summary of efficacy (§156B) information on the active ingredient, in MRID 446241-05.

Wisconsin Pharmacal provided all chemistry data for 305-LO in MRID 446242-01 and -02, for 305-LA in MRID 446240-01, for 305-LT, MRID 446239-01 and for 305-LI in MRID 446241-01. Toxicologic studies were conducted by Safepharm Laboratories Limited for 305-LO (MRIDs 446242-03 through -08), Medical Advisory Services for Travelers Abroad, Ltd. performed the dermal absorption study, gathered the Summary of Customer Complaints, and performed the Efficacy Studies (MRIDs 446242-09, -10 and 446241-05). Tox Monitor Laboratories, Inc. conducted the toxicity studies for the end-use products, 305-LA (MRIDs 446240-02. -03), 305-LT (MRIDs 446239-02, -03), and 305-LI (MRIDs 446241-02, -03, -04).

CONCLUSIONS:

The label of the MUP gives the name Citriodiol Insect Repellent Concentrate, while the CSF states the name as Citriodiol. The CSF is the official name and the label should parallel it. The active ingredient on the label is given as 100.0% Extract of lemon eucalyptus. This should be renamed oil of eucalyptus, containing 65% *p*-menthane-3,8-diol, ~43% *cis* and 22% *trans* isomers. The CSF also needs reworking, giving the correct name of oil of eucalyptus, rather than extract, and an inclusion of the correct amounts of the *cis* and *trans* isomers of the *p*-menthane-3,8-diol for upper and lower limits as given in the preliminary analysis CFR 158.170. The isomers should also be labeled as actives in the purpose of formulation column, 15, on the CSF.

1. The label names for all the EUPs are correct, but the active ingredients will need to be changed in both the labels and the CSFs to follow that of the MUP, oil of eucalyptus containing 65% *p*-menthane-3,8-diol, 43% *cis* and 22% *trans* isomers. EUP labels all state to apply as needed. A specific limit should be stated, e.g., every two hours.

2. Each of the toxicity studies submitted to support the registration of Citriodiol, 305-LO were Supplementary, as the test material was inadequately defined. The test material was identified as PMD-07 in these studies, but no Batch or Lot number included as required by FIFRA Guidelines, nor was the material chemically described for most of the studies. This is also true of some of the efficacy studies.

BPB has tentatively assumed PMD-07 is the MUP and reviewed the 305-LO toxicity studies as such until more data is received

3. The primary eye irritation studies performed by Tox Monitor on 305-LA, 305-LT and 305-LI should have included more description on how the ocular examination was performed beyond the treatment with fluorescein for corneal problems, i.e., was an ophthalmoscope used or perhaps just a penlight?

4. Most of the efficacy studies are inappropriate for the product. Almost all of them tested similar products at a concentration approximately 25% higher in the active ingredient. Many of the studies were not well documented and the material not well presented. One *Aedes aegypti* cage-test mosquito study performed for Wisconsin Pharmacal Company is considered acceptable as it tested all the end-use products submitted for registration. In addition however, BPB would like additional mosquito studies done on two other species, an *Anopheles* and a *Culex* species. In addition to the cage test, a minimum of one field test on mosquitoes should be conducted.

   The tick tested was the sheep tick *Ixodes ricinis*, a carrier of Lyme disease in other countries, but not considered one of the main carriers of the disease in this country. The deer tick (*Ixodes scapularis*) is the primary carrier in the US, and it is not known if the deer tick will respond in the same way to this product. The method of testing is also considered inappropriate, as it did not use humans.

   If stable flies, midges and sand flies are to be kept on the label as pests the product will repel, field tests on all species are required plus a lab test on the stable fly.

   BPB will conditionally approve registration as a mosquito and tick repellent with the proviso that Wisconsin Pharmacal Company perform further mosquito studies and a deer tick test.

5. On June 24, 1998, EPA requested a 21-day dermal toxicity study be completed before registration, but expressed willingness to waive this requirement if the registrant could show low dermal absorption and could provide evidence of safety in humans. The dermal absorption studies submitted were conducted in humans and indicated extremely low absorption. The human safety data submitted was evaluated via a marketing surveillance of 1000 users of the product in which the only complaints were cosmetic (smell and feel). A review of manufacturers' logs over a 2 ½ year period indicated the most common complaints were skin irritation (burning or stinging) or rash. The complaint rate was 20 complaints from an estimated 300,000 users, or approximately 0.007%.

BPB reviewed the dermal absorption data and does not believe it is adequate for risk assessment. A 21-day dermal toxicity study must be accomplished, but BPPD will consider a conditional registration, which would become permanent after the 21-day study is completed, reviewed, and found acceptable.

BPB considers the material supplied by Wisconsin Pharmacal Company sufficient to conditionally register their new biochemical pesticide technical grade active ingredient and the end-use products, when the test material is identified and submitted and with a commitment to complete the additional studies, subchronic dermal toxicity and efficacy studies in *Anopheles* and *Culex* mosquito species and the deer tick.

Data evaluation reviews on the MUP, 305-LO and three end-use products, 305-LA, 305-LT and 305-LI are as follows:

TOXICITY PROFILE
CITRIODIOL INSECT REPELLENT (305-LO), MUP
100% Oil of Eucalyptus containing 65% *p*-menthane-3,8-diols
(43% *cis* and 22% *trans* isomers)

Acute oral toxicity	III	Supplementary	MRID 446242-03
Acute dermal toxicity	III	Supplementary	MRID 446242-04
Acute inhalation toxicity	IV	Cited	MRID 446241-04
Primary eye irritation	II	Supplementary	MRID 446242-05
Primary dermal irritation	III	Supplementary	MRID 446242-06
Dermal sensitization	No	Supplementary	MRID 446242-07
Mutagenicity (Ames test)	No	Supplementary	MRID 446242-08

LABELING: The Signal word is "Warning" because of the grade II toxicity rating for primary eye irritation.

PRECAUTIONARY STATEMENTS:

Causes substantial but temporary eye injury. Harmful if swallowed or absorbed through skin. Do not get in eyes or on clothing. Avoid contact with skin. Wear goggles or face shield. Wash hands before eating, drinking, chewing gum, using tobacco or using the toilet. Remove contaminated clothing and wash clothing before reuse.

STATEMENT OF PRACTICAL TREATMENT (SOPT):

IF SWALLOWED: Call a physician or Poison Control Center. Do not induce vomiting. Drink promptly a large quantity of milk, egg whites, gelatin solution, or if these are not available, drink large quantities of water. Avoid alcohol.

IF ON SKIN: Wash with plenty of soap and water. Get medical attention. For Category III, add "if symptoms persist."

IF IN EYES: Hold eyelids open and flush with steady, gentle stream of water for 15 minutes. Get medical attention.

Probable mucosal damage may contraindicate the use of gastric lavage.

BPB's reviews of 305-LO data are summarized below.

Study Summaries:

PRODUCT CHEMISTRY OF CITRIODIOL (305-LO) MUP

Guideline §151B-10: Product identity and disclosure of ingredients (MRID 446242-01)

Citriodiol contains 100.0 % Oil of Eucalyptus, which is further treated to obtain 65% *p*-menthane-3,8-diol, 43% *cis* and 22% *trans* isomers. This product is to be used to formulate end-use insect repellents against mosquitos and ticks.

The following table summarizes information submitted by the registrant regarding the active ingredient.

Chemical Names:	oil of eucalyptus (1) oil of lemon eucalyptus *p*-menthane-3,8-diol (2), isopulegol hydrate, 2-hydroxy-α,α,4-trimethylcyclohexanemethanol *cis* isomer (3) *trans* isomer (4)
CAS Registry Nos.:	8000-48-4 (1) 42822-86-6 (2) 198456-48-0 (3) 91739-72-9 (4) Numbers in parentheses in the CAS Registry Nos. list refer to previously numbered Chemical Names
Synonym:	PMD PMD rich oil (PMDRO) extract of lemon eucalyptus
Chemical Families:	essential oils alcohols
Source of Biochemicals:	extracted from leaves of *Eucalyptus citriodora*, one of the eucalyptus species. This oil is primarily composed of one chemical which with time breaks down into the other active ingredients, *p*-menthane-3,8-diol, *cis* and *trans* isomers. For this [redacted]
Mode of Action:	insect repellant
Molecular Formula of *p*-menthane-3,8-diol:	$C_{10}H_{20}O_2$

Manufacturing process information not included.

Quwenling, made from the waste distillate after oil is extracted from lemon eucalyptus (*Eucalyptus maculata citriodon)*, is a well-known herbal repellent in China, and this mixture has been used in Europe and Malaysia as an insect repellent for several years.

A confidential statement of formula was submitted by the registrant, which must be altered to reflect the added active ingredients.

BPB's Comment: Data submitted on the product identity satisfy the requirements of 40 CFR 158.155.

Guideline §§151B-11: Manufacturing process (MRID 446242-01)

– In Confidential Appendix

Guideline §151B-12: Discussion on the formation of unintentional ingredients (MRID 446242-01)

In Confidential Appendix

Guideline §151B-13: Analysis of samples (MRID 446242-01)

In Confidential Appendix

Guideline §151B-15: Certification of ingredient limits (MRID 446242-01)

In Confidential Appendix

Guideline §151B-16: Analytical methods for certified limits (MRID 446242-01)

In Confidential Appendix

Guideline §151B-17: Physical and Chemical Characteristics (MRID 446242-02)

The registrant submitted information on the physical and chemical characteristics of Citriodiol Insect Repellent Concentrate which are summarized below:

STUDY TYPE	CHARACTERISTIC
Color	Yellowish brown
Physical State	Liquid at 20 ± 2 °C.
Odor	Faint citrus odor similar to citronella

Stability	Product shipped in stainless steel containers and stored in high density polyethylene (HDPE) containers. Product will not come in contact with metals other than stainless steel or with metal ions during shipment or storage
Oxidation/Reduction	Product components will act neither as oxidants or reductants
Flammability	Flash point of 197 °F
Explodability	Product components will not explode
Storage Stability	Stored at ambient temperature for a period of 2 months and 1 week, the *cis* component increased from 38.2% to 46.4% and the *trans* level went correspondingly down from 24.8 to 21.9
Miscibility	In spray, stick and roll-on formulations product has been shown to remain suspended up to 2 years
Corrosion Characteristics	Over a 2-month period, visual inspection of HDPE carboys in which product was stored revealed no evidence of damage to carboy. Monitoring of carboys and storage containers will continue for at least 1 year
pH	pH of aqueous phase of 30% suspension in deionized water is typically 8.6
UV/visible Absorption	Spectrum shows weak absorbances at about 206 nm (bandwidth about 20 nm, molar absorptivity 616) and 236 nm (bandwidth about 20 nm, molar absorptivity 253)
Viscosity	230 cP at ambient temperature
Melting Point	Not applicable
Boiling Point (estimated)	Between 129 and 142 °C at 5 torr
Density	0.946 g/cm^3 at 25 °C
Dissociation Constant	Doesn't dissociate

Octanol/Water Partition Coefficient	Coefficient of active is approximately 80 and corresponding log P_{OW} is about 1.9
Solubility	In water at room temperature, 9 g/l
Vapor Pressure	Active at room temperature, is estimated to be 0.3 torr (40 Pa)

BPB's Comment: Data submitted on the physical and chemical characteristics of the MUP satisfies the requirements of 40 CFR 158.190.

PRODUCT TOXICOLOGY FOR CITRIODIOL INSECT REPELLENT CONCENTRATE

The Agency tentatively assumes that the PMD-07 test material is equivalent to the MUP until it is identified by the registrant.

Guideline §81-1: Acute Oral Toxicity Study in Rats (MRID 446242-03)

The LD_{50} of Citriodiol Insect Repellent Concentrate is 2,408 mg/kg in rats. Decreased respiratory rate and labored respiration, as well as lethargy, ataxia, ptosis and hunched posture seen in animals at all doses. Abnormalities noted in necropsy of dead animals at all doses were dark liver and kidneys, hemorrhagic lungs, gastric mucosa and intestines. Lowered weight gain at highest doses. Classification: Supplementary, test substance data incomplete; Toxicity Category III.

Guideline §81-2: Acute Dermal Toxicity Study in Rabbits (MRID 446242-04)

A single limit dose of Citriodiol Insect Repellent Concentrate was tested in male and female rabbits. The $LD_{50} > 2,000$ mg/kg. No deaths, overt toxicity or dermal irritation observed, but some indication of bodily injury, as weight gain below normal. Classification: Supplementary, test substance batch/lot number not supplied; Toxicity Category III.

Guideline §81-4: Primary Eye Irritation Study in Rabbits (MRID 446242-05)

Single (0.1 ml) dose of Citriodiol Insect Repellent Concentrate applied to male and female rabbits' eyes. This substance is substantially irritating to rabbit eyes, causing hyperemia, chemosis, discharge and corneal opacity in all animals, and, in one rabbit, the corneal effect lasted through day 7. One rabbit was getting better, but suddenly started to fail and was sacrificed at day 4. Classification: Supplementary, as test material not completely described; Toxicity Category II.

Guideline §81-5: Primary Dermal Irritation Study in Rabbits (MRID 446242-06)

Single (0.5 ml) dose of Citriodiol Insect Repellent Concentrate applied to 6 cm^2 of skin of 6 male rabbits. This substance is a moderate irritant with all animals responding with grade 1 erythema and grade 1 edema at the first and second readings, except for one rabbit with grade 2 erythema at 24 hours. The rabbit with grade 2 erythema reduced to grade 1 by 48 hours, but developed desquamation which lasted throughout the rest of the observations. Complete clearance of erythema by 7 days, but all animals developed desquamation. Classification: Supplementary as incomplete definition of test material; Toxicity Category III.

Guideline §81-6: Delayed Contact Hypersensitivity in Guinea Pigs (Buehler Technique) (MRID 446242-07)

Intradermal induction (5%), topical induction (100%) and challenged with 100% Citriodiol Insect Repellent Concentrate by Magnusson & Kligman maximization test, no positive response. Classification: Supplementary, but with the submission of test substance data, BPB will accept the data as a non-sensitizer, as the information from the consumer surveillance study (~300,000 users) indicated <0.007% negative response complaints. Classification: Supplementary; Toxicity Category Non-sensitizer.

Guideline §84-2: Reverse mutation assay "Ames test" using *Salmonella typhimurium* (MRID 446242-08)

PMD-07 does not produce bacterial mutation in a variety of *Salmonella typhimurium* cells either with or without exogenous activation. Classification: Acceptable

Guideline §85-2: Dermal Absorption of *cis* and *trans para* Menthane-3,8-Diol (§85-2) (MRID 446242-08)

Two subjects were dermally treated at approximate maximum dose with PMD, and urine collections taken at 8-hour intervals. After 3 months, the same 2 subjects were again treated, and urine and blood samples taken to measure dermal absorption and blood uptake. Levels of dermal absorption as a comparison of dose applied were low in all instances, with approximately 0.01% absorbed. Classification: Unacceptable.

Guideline §152-16: Consumer Information Concerning the Use of in Product Formulations (MRID 446242-09)

Over a 3-year period, when a PMD product was on the market in Europe, 20 complaints were received, <0.007% of ~300,000 users.

BPB's Comment: Data submitted on the product toxicity of Citriodiol Insect Repellent Concentrate does not satisfy the requirements of 40 CFR 158.690, but BPB will reconsider it when the missing information is supplied for the test material and the dermal toxicity study is completed.

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

Product Manager: 91 **Reviewer:** Carol Frazer, Ph.D.
MRID No.: 446242-03 **Report Date:** March 29, 1994
Testing Laboratory: Safepharm Laboratories Limited
Report No.: 640/1
Author(s): R. Driscoll
Species: Sprague-Dawley rat
Weight: males: 125-156 g; females: 120-140 g
Age: 5 to 8 weeks
Sex: 18 males, 18 females
Source: Harlan U.K. Ltd., Blackthorn, Bicester, Oxon U.K.
Test Material: PMD-07 (no batch or lot number given); pale brown solid block
Quality Assurance (40 CFR §160.12): Included, acceptable

Summary:

1. **LD_{50} (mg/kg):** 2,408 (95% confidence limit: 1,914 - 3,030)
2. **Toxicity Category:** III
3. **Classification:** Supplementary

Procedure: Animals acclimated at least 5 days. Test material warmed to 80°C in a water bath and prepared as required as a solution in arachis oil B.P. Final dosing at 1,414, 2,000 and 2,828 mg/kg at a dose-volume of 10 ml/kg to fasted rats (5M, 5F) as a solution in arachis oil B.P. Deaths and overt signs of toxicity recorded ½, 1, 2 and 4 hours after dosing and, subsequently, once daily for 14 days. Rats weighed prior to treatment and on days 7 and 14, or at death. Food withheld overnight before dosing and ~2 hours thereafter. Rats necropsied at death.

Results: The LD_{50} of PMD-07 is 2,408 mg/kg for all animals (1,914 - 3,030), 2,454 (1,896 - 3,175) for males and 2,636 (1,470 - 4,729) for females. Common signs of systemic toxicity noted in all dose groups were ataxia, coma, hunched posture, lethargy, ptosis, decreased respiratory rate and labored respiration with additional loss of righting reflex. An isolated incident of tiptoe gait was noted in one male treated with 1,414 mg/kg, and incidents of splayed gait noted in females at the low and mid-dose. Surviving animals recovered 3 to 5 days after dosing. Common abnormalities at necropsy were hemorrhagic lungs, dark liver and kidneys and hemorrhage and sloughing of the gastric mucosa, and possibly the large and small intestines.

At the highest dose, 2,828 mg/kg, 4M died, 3 the first hour after dosing and 1 in the second hour. One female died at the second hour after dosing and 2 further deaths occurred on the day following. The survivors had no abnormalities.

There were 2 deaths at 2,000 mg/kg on the first day, one M and one F. The survivors had no abnormalities.

At the lowest dose, 1,414 mg/kg, one female died on the day following dosing. Necropsy in animals unusual in that slight hemorrhage or sloughing in the gastric mucosa was noted of 3 treated animals that survived dosing. Weight gain in most animals exhibiting gastric mucosal damage at death was lower than normal.

BPB's Comment: No information on exactly what was tested, PMD-07 is not known to the Agency, (batch/lot number), When this is provided, BPB should find this material meets the requirement for acute oral toxicity testing §81-1.

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

Product Manager: 91 **Reviewer:** Carol Frazer, Ph.D.
MRID No.: 446242-04 **Report Date:** March 29, 1994
Testing Laboratory: Safepharm Laboratories Limited
Report No.: 640/2
Author(s): R. Driscoll
Species: Sprague-Dawley rat
Weight: males: 221-237 g; females: 208-234 g
Age: 10 to 14 weeks
Sex: 5 males, 5 females
Source: Harlan U.K. Ltd., Blackthorn, Bicester, Oxon U.K.
Test Material: PMD-07 (no batch or lot number given); pale brown solid block
Quality Assurance (40 CFR §160.12): Included, acceptable

Summary:

1. **LD_{50} (mg/kg):** >2,000

2. **Toxicity Category:** III

3. **Classification:** Supplementary

Procedure: Animals acclimated at least 5 days. On day before treatment, backs and flanks of animals clipped free of hair using veterinary clippers to expose skin area of approximately 5 cm by 4 cm. Treatment dose was 2,000 mg/kg was applied uniformly to area of skin approximating 10% of total body surface using a graduated syringe. Surgical gauze measuring 7 cm x 4 cm placed over treatment area and semi-occluded with self-adhesive bandage (HYPERTIE). Bandage further secured with a piece of BLENDERM wrapped around each end. Shortly after dosing, dressings examined to ensure they were securely in place.

Overt signs of toxicity recorded ½, 1, 2 and 4 hours after dosing and subsequently once daily for 14 days. After the 24 hour exposure period the bandage was carefully removed and the treated skin and surrounding hair wiped with cotton wool moistened with distilled water to remove residual test material. Animals observed for evidence of dermal irritation following removal of dressings and daily thereafter. Rats weighed prior to treatment and on days 7 and 14, or at death. All rats necropsied.

Results: LD_{50} >2,000 mg/kg, with no deaths. No overt signs or symptoms of clinical toxicity, or any dermal irritation. There was, however, evidence of some injurious effects. Body weight gain was unusual, with 2 females losing weight (maximum of 2 lbs.) in the first week, with three in the second week gaining no or slight poundage.

BPB's Comment: No information on exactly what PMD-07 is nor was batch/lot number provided. When this information is provided, BPB should find this material meets the requirement for acute dermal toxicity testing, §81-2.

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

Product Manager: 91 **Reviewer:** Carol Frazer, Ph.D.
MRID No.: 446242-05 **Report Date:** March 29, 1994
Testing Laboratory: Safepharm Laboratories Limited
Report No.: 670/4
Author(s): R. Driscoll
Species: New Zealand White rabbit
Weight: males: 2.52 kg; females: 2.42-2.52 kg
Age: 12-20 weeks
Sex: 2 male, 4 female
Source: David Percival Lts., Moston, Sandbach, Cheshire, U.K.
Test Material: PMD-07 (no batch or lot number provided); pale brown solid block
Quality Assurance (40 CFR §160.12): Included, acceptable

Summary: 1. **Toxicity Category:** II

2. **Classification** Supplementary

Procedure: Animals acclimated 5 days. Test material warmed to ~80 °C to produce a liquid and cooled to ~ 30 °C prior to instillation. Eyes of rabbits examined with fluorescein and ultraviolet light at least 24 hours prior to dosing, and again without fluorescein immediately before dosing by an ophthalmoscope. One rabbit treated with 0.1 ml undiluted test substance instilled into the conjunctival sac of the right eye, holding the eyelids shut for about 1 second to assess for pain. Single drop of local anaesthetic instilled into both eyes of animals 1-2 minutes before treatment. In the final test, undiluted test substance (0.1 ml) instilled into conjunctival sac of right eye of the remaining five animals and eyelids held together about 1 second. Contralateral eyes served as control. Ocular responses recorded at 1, 24, 48, 72 hours and on days 4, 7 and 14 post-instillation with ophthalmoscope. Scoring system used presented in study report.

Results: This test substance is irritating to rabbit eyes. All rabbits had grade 1 iritis, which was gone in one rabbit by 72 hours, and in 4/6 by 7 days. All animals had grade 2 redness and chemosis and grade 3 discharge at the 1 hour reading, which decreased to non-significance by 7 days in 4/6. All rabbits also displayed grade 1 corneal opacity, which dissipated in 4/6 by day 7, while the 5th developed an area of vascularity, which subsequently disappeared by day 14. The 6th rabbit was sacrificed on day 4, because of increased pain responses. This rabbit had an increased corneal opacity to grade 4 and increased redness and discharge to grade 3.

BPB's Comment: Normally the ocular response in this single animal would indicate a corrosive response and yield no subsequent registration. The daily readings in this rabbit, however, indicate the responses had originally started dropping by 72 hours, only to increase drastically by day 4. The additional rabbit with vascularization however, is another reason why BPB will probably consider this study acceptable to meet §81.4 if data on the test substance is provided.

DATA EVALUATION REVIEW FOR PRIMARY DERMAL IRRITATION (§81-5)

Product Manager: 91
MRID No.: 446242-06
Reviewer: Carol Frazer, Ph.D.
Report Date: March 29, 1994
Testing Laboratory: Safepharm Laboratories, Limited
Report No.: 640/3
Author(s): R. Driscoll
Species: New Zealand White albino rabbit
Weight: male: 2.10-2.32 kg, female: 2.21-2.33 kg
Age: 12-20 weeks
Sex: 4 males, 2 females
Source: David Percival Ltd., Moston, Sandbach, Cheshire, U.K.
Test Material: PMD-07 (no batch or lot number); pale brown solid block
Quality Assurance (40 CFR §160.12): Included, acceptable

Summary:

1. **Toxicity Category:** III
2. **Classification:** Supplementary

Procedure: Animals acclimated 5 days. Test material warmed to 60 °C to liquefy and cooled to ~30 °C before use. Rabbits clipped on dorsal/flank area and skin examined for irritation on day prior to test. Test material (0.5 ml) applied to test site and covered with a 2.5 x 2.5 cm gauze patch secured with a strip of surgical adhesive tape (r~2.5 x 4 cm BLENDERM). Trunk of each rabbit wrapped in an elasticated corset (TUBIGRIP) to prevent interference with patch. After 4 hours exposure, wrappings removed and test site swabbed with cotton wool soaked in diethyl ether. Observations for erythema and edema were made at 1, 24, 48, 72 hours and 7 days following patch removal. Grading scale used for scoring presented in study report.

Results: This product is a moderate dermal irritant. Grade 1 erythema and edema observed in all rabbits at 1 hour reading. At 24 hour reading, 1/6 rabbits had grade 2 erythema, and 1 rabbit had grade 0 edema, with the rest having grade 1 erythema and edema. The rabbit with grade 2 erythema reduced to grade 1 by 48 hours, but developed desquamation which lasted throughout the rest of the observations. Erythema decreased in 4/6 rabbits at 72 hours to grade 1, with 3/6 developing desquamation. Although erythema had disappeared by 7 days, all rabbits had developed desquamation. Edema started disappearing by 24 hours in 2/6 rabbits, and was completely gone by 72 hours.

BPB's Comment: When the test material is appropriately defined with batch or lot number as required by the Guideline, the data will be accepted for §81-5..

DATA EVALUATION REVIEW FOR DERMAL SENSITIZATION (§81-6)

Product Manager: 91 Reviewer: Carol Frazer, Ph.D.
MRID No.: 446242-07 Report Date: March 29, 1994
Testing Laboratory: Safepharm Laboratories Limited
Report No.: 640/5
Author(s): R. Driscoll
Species: Dunkin-Hartley albino guinea pig
Weight: 333-424 g
Age: 8-12 weeks
Sex: 38 females
Source: David Hall Limited, Burton-on-Trent, Staffordshire, U.K.
Test Material: PMD-07 (no batch or lot number); pale brown solid block
Positive Control: α-hexylcinnamaldehyde (Technical 85%) induction: intradermal - 25% in arachis oil B.P., topical - 100%; challenge: 100% and 75% in arachis oil B.P.
Quality Assurance (40 CFR §160.12): Included, acceptable

Method: Magnusson & Kligman maximization

Summary: 1. **Rating:** Non-sensitizer

2. **Classification:** Supplementary

Procedure: Acclimation period 5 days. Test material prepared for use by warming to ~80°C to produce a liquid and prepared as follows: intradermal induction -- 5% (w/v) in arachis oil B.P., and 5% (w/v) in a mixture of Freund's Complete Adjuvant plus arachis oil B.P. (1:1); topical induction -- undiluted as supplied; and topical challenge -- undiluted as supplied and 75% (v/v) in arachid oil B.P. Range-finding of test substance: 4 animals for intradermal injection at 1%, 5%, 10%, 25% v/v in arachis oil; for topical induction, 2 previously injected pigs at undiluted and 75%, 50% and 25% in arachis oil B.P.; and for topical challenge in 2 control animals, at undiluted and 75% v/v in arachis oil B.P.

Twenty test and 10 control guinea pigs weighed at the start and completion of the study. Hair removed from 40 mm x 60 mm on the shoulder region of each animal with clippers. A row of three injections (0.1 ml each) made on each side of mid-line: Freund's Complete Adjuvant plus distilled water (1:1); 5% (w/v) test material in arachis oil B.P.; 5% (w/v) test material in a 1:1 preparation with Freund's Complete Adjuvant plus arachis oil B.P. One week later, the same area clipped again and treated topically with undiluted test material (0.2-0.3 ml) on 40 mm x 20 mm filter paper held in place by surgical adhesive tape and covered with overlapping length of aluminum foil, and further secured with more elastic adhesive bandage wound around animal kept in place for 48 hours. Skin reactions quantified 1 and 24 hours following removal of patches using Draize scale. Control animals treated identically with test animals, except for the lack of test material in injections and topical treatment.

Shortly before treatment on day 21, 50 mm x 70 mm on both flanks of each animal clipped. A quantity of undiluted test material (0.1-0.2 ml) applied to shorn right flank on filter paper held in place by surgical adhesive tape, with a similar amount of 75% (v/v).test material applied to another site on same flank. Vehicle alone applied to site on left flank. Patches occluded with aluminum foil and secured by elastic adhesive bandage as for induction. After 24 hours, dressing completely removed and challenge sites swabbed with cotton soaked in diethyl ether. Before 24 hours, clippers used on flanks again. Sites were scored at 24 and 48 hours. No positive control study performed.

The positive control study was performed in the laboratory approximately 4 months before this one.

Results: No dermal sensitization exhibited in this study on test animals or controls. Slight erythema and edema observed in more than half the study animals following topical induction, but nothing seen at challenge. All study animals gained weight and none exhibited toxic reactions. The positive control yielded 70% sensitization.

BPB's Comment: The batch/lot number or other defining data was not provided for this test material, however, and until that is forthcoming, this will be considered Supplementary.

If only the batch/lot numbers are provided as required in the guideline, however, BPB will consider this study indicates the compound a non-sensitizer, however, as data from the consumer surveillance study signifies the low likelihood of sensitization, with <0.007% complaints after 3 years on the market (~300,000 uses).

DATA EVALUATION REVIEW FOR REVERSE MUTATION ASSAY "AMES TEST" USING SALMONELLA TYPHIMURIUM (§84-2)

Product Manager: 91 **Reviewer:** Carol Frazer, Ph.D.
MRID No.: 446242-08 **Report Date:** 27 January 1994
Testing Laboratory: Safepharm Laboratories Limited
Report No.: 640/8
Author(s): P.W. Thompson H.N.C.
Test Material: PMD-07 (no batch or lot number); pale brown solid block
Cell Source: British Industrial Biological Research Association
Solvent Used: dimethyl sulphoxide
Control Materials: Negative: solvent; Positive: Nonactivation -- N-ethyl-N'-nitro-N-nitrodoguanidine, 9-aminoacridine, *4*-nitro-*o*-phenylenediamine, 4-nitroquinoline-1-oxide; Activation -- 2-aminoanthracene, benzo[a]pyrene
Activation: S9 derived from Aroclor 1254 induced male Sprague-Dawley rat liver received from the British Industrial \Biological research Association
Media: Overnight culture: Nutrient broth inoculated by subculture and incubated at 37°C for 10 hours.
Plating: Top agar prepared using Difco Bacto agar and sodium chloride. Base agar plates prepared with Oxoid agar with Vogel-Bonner Medium E and d-glucose.
Strains: *Salmonella typhimurium*: TA 98, TA 100, TA 1535, TA 1537, TA 1538
Quality Assurance (40 CFR §160.12): Included, acceptable

Summary: 1. **Rating:** Non-mutagenic

2. **Classification:** Supplementary

Procedure: Test material weighed and dissolved in solvent in dilutions made on day of each experiment. Range-finding assay on duplicates of TA 100 (0.1 ml), without activation, tested solvent and 5 doses of test material (312.5, 625, 1,250, 2,500 and 5,000 µg/plate). Final test series doses, with and without activation, were assayed in triplicate against each tester strain using direct plate incorporation in 2 experiments. Plates incubated at 37°C for approximately 48 hours and revertant colonies counted. Experiment 1 doses included 0, 8.0, 40, 200, 1000 and 5,000 test material µg/plate; in experiment 2, using fresh bacterial cultures, doses were 0, 312.5, 625, 1,250, 2,500 and 5,000 (no explanation given for reason for differences in doses between experiments). Positive controls were strain-specific, and the test decision criteria encompassed in the study was presented in the study report.

Results: None of the experiments using the test material or negative/solvent controls showed any significant increase in bacterial mutants. All positive controls, however, were strongly mutagenic.

BPB's Comment: This study meets the requirements of §84-2, except for lack of defining information, i.e., lot/batch number, and indicates the test material does not produce bacterial mutation in a variety of *Salmonella typhimurium* cells either with or without exogenous activation. As this study is not required for this product, however, the categorization information is not of importance.

DATA EVALUATION REVIEW FOR DERMAL ABSORPTION OF
cis AND *trans para* MENTHANE-3,8-DIOL (§85-2)

Product Manager: 91 **Reviewer:** Carol Frazer, Ph.D.
MRID No.: 446242-09 **Report Date:** 6/6/97
Testing Laboratory: Medical Advisory Services for Travelers Abroad, Ltd.
Report No.: not applicable
Author(s): P.D. Clarke, P.J. Barrett, C. Cooksey, P.J. Street, R.J. Flanagan
Test Material: PMD [*cis* and *trans*-2-(2-hydroxypropyl)-5-methylcyclohexanol]
Quality Assurance (40 CFR §160.12): not applicable

Classification: Unacceptable

Procedure: Two healthy adult volunteers abstained from alcoholic drinks and medicines for 12 hours prior to and during study. A 50 ml urine sample was collected at 0 hour and bladder emptied prior to application of 50% (w/w) PMD (*cis*-PMD:*trans*-PMD ratio 2.4:1) in industrial methylated spirits to arms, legs and trunk in maximum likely quantity. PMD container was weighed before and after PMD application. Urine samples collected every 8 hours over 2 days. After measuring total volumes, 50 ml portions of each collection were stored in labeled bottles at -20°C prior to analysis. Total PMD doses in each subject were 95.7 and 98.5 mg/kg, respectively.

After a 3-month wash-out period, the 2 subjects again abstained from alcoholic drinks and medicines for 12 hours prior to and during study. One arm was shielded and used for 10 ml blood collection via the antecubital vein at 0 hours; urine collected and bladders emptied. Fifty per-cent PMD (w/w) in industrial methylated spirit spray was applied to the unshielded arm, legs and trunk in the maximum likely quantity. Application material weighed. Ten mls blood collected at 2, 4, 6, 8, 12 and 24 hours, separated by centrifugation. Plasma stored at -20 °C until analyzed. Urine samples collected at same time; volume measured and 50 ml portions stored at -20°C. PMD doses in subjects were 101.1 and 142.3 mg/kg.

Test ingredients extracted from biological samples in ethyl acetate, vortexed 30 sec and centrifuged before measured with a 30 m x 0.32 (i.d.) mm fused silica capillary gas chromatography with flame ionization detection. Sample analyses performed in duplicate, and assay calibration standards included in report.

Results: No adverse effects reported by either subject in either study. Levels of dermal absorption compared to dose applied were low in all instances, with approximately 0.01% in both subjects in both studies. In the first study, terpenes were detected in one subject up to 24 hours, and in the other, for 32 hours. Both subjects excreted slightly more of the *trans* isomer (175.0 and 157.4 µg) than *cis* (131.5 and 133.6 µg, respectively), even though the ratio of *cis* to *trans* was about 2:1. This was not true of the second study, in which both plasma and urine concentrations were more in line with actual levels of the isomers in the test material. In the second study, both subjects showed plasma peaks at 6 and 12 hours, with levels of *cis* and *trans*

ranging up to 526 and 359 µg/L, respectively. Plasma PMD concentrations were very low in relation to the dose applied. The excretion in urine of different ratios of the isomers in the two studies may have been due to the use of PMD formulations containing differeing amounts of impurities.

- BPB's Comment: The report covers dermal absorption and urinary excretion in 2 subjects in the first study; the second study is a repeat of the urinary excretion but adds a blood plasma sampling component in the same 2 subjects. This is an inadequate number of subjects. Furthermore, air, sweat and fecal excretion are not evaluated, nor are metabolism or subsequent degradation products. This does not meet BPB's requirement.

This report was submitted in response to a BPB recommendation that a 21-day dermal toxicity study should be performed. The registrant submitted this dermal absorption study to indicate dermal absorption is so low as not to be a problem. This study, however, indicates dermal absorption occurs, but is not useful in determining a dermal absorption rate. Therefore a toxicity endpoint is needed for use in risk characterization. A 21-day dermal toxicity study conducted at a limit dose of 1,000 mg/kg/day is necessary; no systemic effects in this study would indicate no potential hazard from repeated dermal applications.

DATA EVALUATION REVIEW OF POST MARKETING SURVEILLANCE STUDY

Product Manager: 91 **Reviewer:** Carol Frazer, Ph.D.
MRID No.: 446242-10 **Report Date:** September 7, 1997
Testing Laboratory: Medical Advisory Services for Travelers Abroad, Ltd.
Report No.: not applicable
Author(s): Sarala Nicholas, Mostafa Hosseini, Libby Greaves
Test Material: PMD [*cis* and *trans*-2-(2-hydroxypropyl)-5-methylcyclohexanol]
Quality Assurance (40 CFR §160.12): not applicable

Mosi-Guard Natural (containing PMD) is an insect repellant sold outside the United States in several formulations. Over 7 million doses have been sold and a post-marketing survey of 1093 recent users of the product was conducted. Fifty percent of the users had used the spray, 12% the roll-on gel, 11% a stick formulation and 26% a combination of 2 or more formulations. Eighty-nine percent of the subjects reported it to be highly effective, and only 4% reported it ineffective. Sixty-seven of the users used the product for more than 7 days. Nearly half (44%) used the product 8-14 days.

Four percent of the users experienced some rash, and a slightly higher proportion (p=0.04) of rashes reported among children 0-9 years old than among older users. Thirty-seven (3%) users reported some skin sensitivity (i.e., stinging) with the product. Other complaints centered around smell (19%), greasiness (18%), and clothing stains (1.4%).

During the period from July 1994 to August 1997, Mosi-Guard International received 20 complaints from users of their insect repellant products containing PMD as an active ingredient. Of these 20 complaints, 9 (45%) involved burning sensation or irritation, 5 (25%) reported a rash, 4 (20%) involved a respiratory effect (e.g., wheezing, sinus congestion), and 2 (10%) reported either an unspecified allergic response or contact dermatitis. This represents <0.007% of the estimated ~300,000 users.

BPB's reviews of 305-LA data are summarized below.

REPEL® NATURAL Insect Repellent Lotion (305-LA)

TOXICITY PROFILE

Acute oral toxicity	III	Cited	MRID 446242-03
Acute dermal toxicity	III	Cited	MRID 446242-04
Acute inhalation toxicity	IV	Cited	MRID 446241-04
Primary eye irritation	II	Acceptable	MRID 446240-02
Primary dermal irritation	IV	Acceptable	MRID 446240-03
Dermal sensitization	No	Cited	MRID 446242-07

LABELING: The Signal word is "Warning," because of Toxicity Rating of II on primary eye irritation.

RESTRICTED USE CLASSIFICATION RECOMMENDED:

Due to eye irritation toxicity category.

The PM Team should decide if restricted use classification is necessary or if alternative labeling will allay the requirement for restricted use classification.

CHILD RESISTANT PACKAGING REQUIRED

SIGNAL WORD: WARNING

PRECAUTIONARY STATEMENTS:

Causes substantial but temporary eye injury. Harmful if swallowed or absorbed through skin. Do not get in eyes or on clothing. Avoid contact with skin. Wear goggles or face shield. Wash hands before eating, drinking, chewing gum, using tobacco or using the toilet. Remove contaminated clothing and wash clothing before reuse.

STATEMENT OF PRACTICAL TREATMENT (SOPT):

IF SWALLOWED: Call a physician or Poison Control Center. Do not induce vomiting. Drink promptly a large quantity of milk, egg whites, gelatin solution, or if these are not available, drink large quantities of water. Avoid alcohol.

IF ON SKIN: Wash with plenty of soap and water. Get medical attention.

IF IN EYES: Hold eyelids open and flush with steady, gentle stream of water for 15 minutes. Get medical attention.

Probable mucosal damage may contraindicate the use of gastric lavage.

BPB's reviews of data are summarized below.

Study Summaries:

PRODUCT CHEMISTRY OF REPEL® NATURAL INSECT REPELLENT LOTION (305-LA)

Guideline §151B-10: Product identity and disclosure of ingredients (Label and MRID 446242-01)

REPEL® NATURAL Insect Repellent Lotion contains 30.0 % Oil of Eucalyptus. This product repels mosquitos and ticks.

An acceptable confidential statement of formula was submitted by the registrant.

BPB's Comment: Data submitted on the product identity satisfy the requirements of 40 CFR 158.155.

Guideline §§151B-11: Manufacturing process (MRID 446240-01)

In Confidential Appendix

Guideline §151B-12: Discussion on the formation of unintentional ingredients (MRID 446240-01)

In Confidential Appendix

Guideline §151B-13: Analysis of samples (MRID 446240-01)

In Confidential Appendix

Guideline §151B-15: Certification of ingredient limits (MRID 446240-01)

In Confidential Appendix

Guideline §151B-16: Analytical methods for certified limits (MRID 446240-01)

In Confidential Appendix

Guideline §151B-17: Physical and Chemical Characteristics (MRID 446240-01)

The registrant submitted information on the physical and chemical characteristics of the formulated end-use product, REPEL® NATURAL Insect Repellent Lotion, which are summarized below:

STUDY TYPE	CHARACTERISTIC
Color	White cream
Physical State	Cream
Odor	Lemon citronella
Melting Point	Not applicable
Boiling Point	Not applicable
Specific Gravity/Density	0.962 g/ml (8.03 lb./gal.)
Solubility	Not required
Vapor Pressure	Not required
pH	5.78 (1:10 dilution)
Stability	Not required
Flammability	Flash point >200°F
Storage Stability	Not required
Viscosity	26,000 centipoise @ 25°C
Miscibility	Not to be diluted w/petroleum solvents
Corrosion Characteristics	Non-corrosive
Octanol/Water Partition Coefficient	Not required

BPB's Comment: Data submitted on the physical and chemical characteristics of REPEL® NATURAL Insect Repellent Lotion satisfy the requirements of 40 CFR 158.190.

PRODUCT TOXICOLOGY FOR REPEL® NATURAL INSECT REPELLENT LOTION

Guideline §81-4: Primary Eye Irritation Study in Rabbits (MRID 442640-02)

Single (0.5 ml) dose of REPEL® NATURAL Insect Repellent Lotion applied to eyes of 6 male rabbits. This substance is substantially irritating to rabbit eyes, causing corneal opacity, iritis, hyperemia, chemosis, and discharge in all animals. The corneal effect lasted until day 10 in one rabbit, but all rabbit eyes completely cleared by day 14. Classification: Acceptable; Toxicity Category II. BPB would however, prefer more detailed information on the method of ocular examination beyond the corneal aspect

Guideline §81-5: Primary Dermal Irritation Study in Rabbits (MRID 442640-03)

Single (0.5 ml) dose of REPEL® NATURAL Insect Repellent Lotion applied to 6 cm^2 of skin of 6 male rabbits. This substance is a mild irritant with only 3/6 animals responding with grade 1 erythema at the first reading, with complete clearance at 24 hours. Classification: Acceptable; Toxicity Category IV..

BPB's Comment: Data submitted on the product toxicity of REPEL® NATURAL Insect Repellent Lotion satisfies the requirements of 40 CFR 158.690.

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

Product Manager: 91
MRID No.: 446240-02
Testing Laboratory: Tox Monitor Laboratories
Report No.: 98-0203-1
Author(s): Michael Kukulinski, B.S., L.A.T.G.
Species: New Zealand White rabbit
Weight: 2.02-2.20 kg
Age: 8-10 weeks
Sex: 6 males
Source: Kuiper Rabbitry, Gary, Indiana
Test Material: Repel Natural Lotion, I.D. 0625984; white cream
Quality Assurance (40 CFR §160.12): Included, acceptable

Reviewer: Carol Frazer, Ph.D.
Report Date: July 21, 1998

Summary:

1. **Toxicity Category:** II

2. **Classification** Acceptable

Procedure: Animals acclimated 5 days. Eyes of rabbits examined at least 24 hours prior to dosing. All rabbits treated with 0.1 ml instilled directly into the conjunctival sac of one eye; lids kept closed for 1 second. Contralateral eyes served as controls. Ocular responses recorded at 1, 24, 48, 72, 168, 240 and 336 hours post-instillation. Protocol states additional examinations will be performed up to a maximum of 21 days if persistent corneal involvement or other ocular irritation is present. Two percent sodium fluorescein and ultraviolet light provided via a Spectroline, Model Q-12, Long Wave UV-365 nm, 10X magnifier employed to reveal possible corneal injury commencing with the 24 hour observation. Scoring system used presented in study report.

Results: This test substance is irritating to rabbit eyes. Mild corneal effects observed in all rabbits, grade 1 opacity lasting up to 10 days in one animal and to 7 days in 4/5 remaining.. All animals exhibited grade 1 iritis starting at the first reading. This lasted 7 days in 3 rabbits. All rabbits also demonstrated severe conjunctival irritation. One rabbit had a grade 4 chemosis reading at one hour which diminished to grade 1 by day 7. All rabbits had a grade 2 or higher reading for all conjunctival readings at the first hour, and, in 3 rabbits, this continued until the 3rd day. On the 7th day, 5/6 rabbits still showed grade 2 redness. By day 10, however, conjunctival readings were clear of significant responses. Eyes of all animals completely cleared by day 14.

BPB's Comment: BPB will consider this study acceptable to meet §81-4, but would prefer more detailed information on the method of ocular examination beyond the corneal aspect.

DATA EVALUATION REVIEW FOR PRIMARY DERMAL IRRITATION (§81-5)

Product Manager: 91 **Reviewer:** Carol Frazer, Ph.D.
MRID No.: 446240-03 **Report Date:** July 9, 1998
Testing Laboratory: Tox Monitor laboratories, Inc.
Report No.: 98-0203-2
Author(s): Michael Kukulinski, B.S., L.A.T.G.
Species: New Zealand White albino rabbit
Weight: 2.06-2.11 kg
Age: 8-10 weeks
Sex: 6 males
Source: Kuiper Rabbitry, Gary, Indiana
Test Material: Repel Natural Lotion, I.D. 0628984; white cream
Quality Assurance (40 CFR §160.12): Included, acceptable

Summary:

1. **Toxicity Category:** IV

2. **Classification:** Acceptable

Procedure: Animals acclimated 5 days. Rabbits clipped on left side of trunk from the midline of back to the abdomen. Test material (0.5 ml) applied to 6 square centimeter test site and covered with a 2-layer gauze patch held in place with non-irritating porous tape and then covered with a semi-occlusive plastic overwrap secured in place. After 4 hours exposure, wrappings removed and excess material removed from site. Observations for erythema and edema made at ½, 24, 48 and 72 hours following patch removal. Grading scale used for scoring presented in study report.

Results: This product is an extremely mild dermal irritant. Grade 1 erythema noted in 3/6 rabbits at the first reading, but was completely clear by 24 hours.

BPB's Comment: Acceptable to BPB to complete the requirements for §81-5.

BPB's reviews of 305-LT data are summarized below.

REPEL® NATURAL Insect Repellent Non-Aerosol Pump (305-LT)

TOXICITY PROFILE

Acute oral toxicity	III	Cited	MRID 446242-03
Acute dermal toxicity	III	Cited	MRID 446242-04
Acute inhalation toxicity	IV	Cited	MRID 446241-04
Primary eye irritation	II	Acceptable	MRID 446239-02
Primary dermal irritation	IV	Acceptable	MRID 446239-03
Dermal sensitization	No	Cited	MRID 446242-07

LABELING: The Signal word is "Caution," because of Toxicity Category III on acute oral and dermal toxicity and primary eye irritation . The following labeling language is acceptable:

CHILD RESISTANT PACKAGING REQUIRED

SIGNAL WORD: CAUTION

PRECAUTIONARY STATEMENTS:

Harmful if swallowed or absorbed through skin. Causes moderate eye irritation. Avoid contact with eyes, skin or clothing. Wash hands before eating, drinking, chewing gum, using tobacco or using the toilet.

STATEMENT OF PRACTICAL TREATMENT (SOPT):

IF SWALLOWED: Call a physician or Poison Control Center. Drink 1 or 2 glasses of water and induce vomiting by touching back of throat with finger. If person is unconscious, do not give anything by mouth and do not induce vomiting.

OR

IF SWALLOWED: Call a physician or Poison Control Center. Drink 1 or 2 glasses of water and induce vomiting by touching back of throat with finger, or if available by administering syrup of ipecac. If person is unconscious, do not give anything by mouth and do not induce vomiting.

IF ON SKIN: Wash with plenty of soap and water. Get medical attention. For Category III, add "if symptoms persist."

IF IN EYES: Flush eyes with plenty of water. Call a physician if irritation persists.

BPB's reviews of data are summarized below.

Study Summaries:

PRODUCT CHEMISTRY OF REPEL® NATURAL
INSECT REPELLENT NON-AEROSOL PUMP

Guideline §151B-10: Product identity and disclosure of ingredients (Label and MRID 446239-01)

REPEL® NATURAL Insect Repellent Non-Aerosol Pump contains 40.0 % Oil of Eucalyptus. This product repels mosquitos and ticks.

An acceptable confidential statement of formula was submitted by the registrant.

BPB's Comment: Data submitted on the product identity satisfy the requirements of 40 CFR 158.155.

Guideline §§151B-11: Manufacturing process (MRID 446239-01)

In Confidential Appendix

Guideline §151B-12: Discussion on the formation of unintentional ingredients (MRID 446239-01)

In Confidential Appendix

Guideline §151B-13: Analysis of samples (MRID 446239-01)

In Confidential Appendix

Guideline §151B-15: Certification of ingredient limits (MRID 446239-01)

In Confidential Appendix

Guideline §151B-16: Analytical methods for certified limits (MRID 446239-01)

In Confidential Appendix

Guideline §151B-17: Physical and Chemical Characteristics (MRID 446239-01)

The registrant submitted information (MRID 446239-01) on the physical and chemical characteristics of the formulated end-use product, REPEL® NATURAL Insect Repellent Non-Aerosol Pump which are summarized below:

STUDY TYPE	CHARACTERISTIC
Color	Amber tea
Physical State	Liquid
Odor	Lemon citronella
Melting Point	Not applicable
Boiling Point	189°F/87°C
Specific Gravity/Density	0.838 g/ml (6.993 lb./gal.)
Solubility	Not required
Vapor Pressure	Not required
pH	7.19 (1:10 dilution)
Stability	Not required
Flammability	Flash point 66°F/19°C (TCC)
Storage Stability	Not required
Viscosity	10 centipoise @ 25°C
Miscibility	Not to be diluted w/petroleum solvents
Corrosion Characteristics	Non-corrosive
Octanol/Water Partition Coefficient	Not required

BPB's Comment: Data submitted on the physical and chemical characteristics of REPEL® NATURAL Insect Repellent Non-Aerosol Pump satisfy the requirements of 40 CFR 158.190.

PRODUCT TOXICOLOGY OF REPEL® NATURAL INSECT REPELLENT NON-AEROSOL PUMP

Guideline §81-4: Primary Eye Irritation Study in Rabbits (MRID 442639-02)

Single (0.5 ml) dose of REPEL® NATURAL Insect Repellent Non-Aerosol Pump applied to eyes of 6 male rabbits. This substance is moderately irritating to the animals' eyes, causing corneal opacity in 5/6 rabbits up to 72 hours in one, and iritis, hyperemia, chemosis, discharge in all animals. All eyes cleared by 168 hours. BPB would, however, prefer more detailed information on the method of ocular examination beyond the corneal aspect. Classification: Acceptable; Toxicity Category III.

Guideline §81-5: Primary Dermal Irritation Study in Rabbits (MRID 442639-03)

Single (0.5 ml) dose of REPEL® NATURAL Insect Repellent Non-Aerosol Pump applied to 6 cm^2 rabbit skin on 6 animals. This substance is a minor irritant, with grade 1 erythema in 4/6 rabbits at first reading, and again at 24 hours. By 48 hours, only 1 grade 1 erythema observed, and, by 72 hours, no further irritation. Only 1 grade 1 edema observed at the first reading, with no further observations. Classification: Acceptable; Toxicity Category IV.

BPB's Comment: Data submitted on the toxicity of REPEL® NATURAL Insect Repellent Non-Aerosol Pump satisfies the requirements of 40 CFR 158.690.

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

Product Manager: 91
MRID No.: 446239-02
Reviewer: Carol Frazer, Ph.D.
Report Date: July 15, 1998
Testing Laboratory: Tox Monitor Laboratories
Report No.: 98-0201-1
Author(s): Michael Kukulinski, B.S., L.A.T.G.
Species: New Zealand White rabbit
Weight: 2.02-2.22 kg
Age: 8-10 weeks
Sex: 6 males
Source: Kuiper Rabbitry, Gary, Indiana
Test Material: Repel Natural Pump, I.D. 0625982; pale yellow liquid
Quality Assurance (40 CFR §160.12): Included, acceptable

Summary:

1. **Toxicity Category:** III

2. **Classification** Acceptable

Procedure: Animals acclimated 5 days. Eyes of rabbits examined at least 24 hours prior to dosing. All rabbits treated with 0.1 ml instilled directly into the conjunctival sac of one eye and both lids kept closed for 1 second. Contralateral eyes served as controls. Ocular responses recorded at 1, 24, 48, 72 and 168 hours post-instillation. 2% sodium fluorescein and ultraviolet light provided via a Spectroline, Model Q-12, Long Wave UV-365 nm, 10X magnifier employed to reveal possible corneal injury commencing with the 24 hour observation. Scoring system used presented in study report.

Results: This test substance is moderately irritating to rabbit eyes. Mild corneal effects observed in 5/6 rabbits, grade 1 opacity lasting up to 72 hours in one animal. All animals exhibited grade 1 iritis starting at the first reading, lasting until 48 hours in one rabbit. All rabbits also demonstrated severe conjunctival irritation. All rabbits had grade 3 discharge readings at one hour which diminished over time. One rabbit continued with grade 3 for the 24 hour reading, grade 2 at 48 hours and then not positive. One animal continued with grade 2 through 72 hours, but was completely clear by 168 hours. Chemosis, 3 grade 4 and 2 grade 3, were observed at the first reading, reducing to 0 or 1 by 72 hours. Five of six rabbits had grade 2 redness at the first hour, and one had grade 3. Significant redness continued through 72 hours in one animal, but was clear by 168 hours.

BPB's Comment: BPB will consider this study acceptable to meet §81.4. BPB would, however, prefer more detailed information on the method of ocular examination beyond the corneal aspect.

DATA EVALUATION REVIEW FOR PRIMARY DERMAL IRRITATION (§81-5)

Product Manager: 91 **Reviewer:** Carol Frazer, Ph.D.
MRID No.: 446239-03 **Report Date:** July 9, 1998
Testing Laboratory: Tox Monitor Laboratories, Inc.
Report No.: 98-0201-2
Author(s): Michael Kukulinski, B.S., L.A.T.G.
Species: New Zealand White albino rabbit
Weight: 2.04-2.34 kg
Age: 8-10 weeks
Sex: 6 males
Source: Kuiper Rabbitry, Gary, Indiana
Test Material: Repel Natural Pump, I.D. 0628982; pale yellow liquid
Quality Assurance (40 CFR §160.12): Included, acceptable

Summary:

1. **Toxicity Category:** IV

2. **Classification:** Acceptable

Procedure: Animals acclimated 5 days. Rabbits clipped on left side of trunk from the midline of back to the abdomen. Test material (0.5 ml) applied to 6 square centimeters test site and covered with a 2-layer gauze patch held in place with non-irritating porous tape and then covered with a semi-occlusive plastic overwrap secured in place. After 4 hours exposure, wrappings removed and excess material removed from site. Observations for erythema and edema were made at 1/2, 24, 48 and 72 hours following patch removal. Grading scale used for scoring presented in study report.

Results: This product is a mild dermal irritant. Grade 1 erythema noted in 4/6 rabbits at the first reading, with 4/6 having grade 1 erythema at 24 hours (not the same rabbits). At 48 hours, one rabbit had grade 1 erythema which cleared by 72 hours. Edema observed in 1/6 rabbits at the 1/2 hour reading, with no further observations.

BPB's Comment: Acceptable to BPB to complete the requirements for §81-5.

PC 011550 P-MENTHANE-3,8-DIOL

Page _____ is not included in this copy.

Pages 37 through 44 are not included in this copy.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

X Information about a pending registration action.

_____ FIFRA registration data.

_____ The document is a duplicate of page(s) _____.

_____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

DATA EVALUATION REVIEW OF EFFICACY STUDIES (§156b-2)

Product Manager: 91
MRID No.: 446241-05
Reviewer: Carol Frazer, Ph.D.
Report Date: August 4, 1998
Testing Laboratory: Medical Advisory Services for Travelers Abroad, Ltd. (MASTA)
Report No.: 080598
Author(s): RJ Dillon, Chris Curtis, Jane K. Trigg, Paul Clarke, Mary Wundrock
Test Material: Extract of Lemon Eucalyptus

This is a review of efficacy studies on Oil of Eucalyptus. It includes summaries of each study (whether or not published) and the final report if available. The studies and our review are identified with letters A through L, following.

A. Cage Testing with Anopheles-Stephensii, Aedes Aegypti and Sandfly: Preliminary laboratory tests of 50% concentration of extract of the lemon eucalyptus oil in ethanol: Conducted in London, by MASTA, 1993.

A 50% solution of extract of lemon eucalyptus oil in ethanol was tested against 2 different strains of mosquitoes (*Anopheles stephensii* and *Aedes aegypti*) noted for their different biting patterns, and against the sandfly. 100% repellency against all three biting insects for at least 6 hours and in some cases 8 hours was achieved (no data included).

B. Cage Testing with Aedes Aegypti: Preliminary laboratory tests of repellent of cis and trans isomers of p-menthane 3,8 diol (PMD) tested separately against hungry mosquitoes: Conducted in London, by MASTA, 1993.

The primary active components, p-menthane 3,8 diol isomers, were tested individually to determine their repellent properties. Both right and left forearms of human volunteers were used. The right arm was used as control and 0.13 g of cis or trans PMD in ethanol (minimum effective dose) was applied to the left arm. The control arm was introduced into a cage of hungry, mainly female mosquitoes, and the number of mosquitoes probing within 45 seconds was counted. The test arm was then introduced and the procedure repeated. The test arm was evaluated every hour for the test period, and the control was retested at the end of the period to confirm the biting drive remained high.

Repellency from both cis (91.3) and trans (97.1) were high to start with, although cis (56.5%) outlasted trans (29.7%) in longevity of action (4 hours). Control bites were 30-34 initially and 38-39 at 4 hours.

No further data, e.g., volunteer numbers tested.

C. Cage Testing with Sandflies: Insect repellent trial with sandflies: Conducted by RJ Dillon, April 23, 1993.

Female *Phlebotomus papatasi*, 4-5 days old, starved prior to experiment. Control insects allowed access to left forearm for 10 minutes (23°C). Hand was covered with double layer of disposable gloves. Mosi-guard (50% AI) non-aerosol pump spray sprayed on right forearm, and a second batch of insects allowed access to the arm 2 hours after application. Additional batches of insects tested after 4, 6, 8 and 12 hours. Another group tested against the control arm after 8 hours. Blood engorged animals were defined as fed.

Mosi-guard successfully prevented probing and feeding of sandflies for at least 8 hours post-application. Some indication of lessening repellency after 12 hours. Control bites went from 95% fed at start to 100% fed at 8 hours.

No further data, e.g., volunteer numbers tested.

D. Field Testing with Aedes Aegypti: Open air repellent test: Mosi-guard Natural and Autan spray (20% DEET): Conducted in Epping Forest, UK, by Chris Curtis, London School of Hygiene and Tropical Medicine, May 19, 1993.

Two sites selected in Epping forest, close to a pond by the 'Robin Hood' roundabout where *Aedes* mosquitoes were plentiful. Two teams of two subjects each selected. Each individual exposed forearms, face and neck. Controls were performed by each team spending 15 minutes at each site and collecting any mosquitoes that settled on bare skin. Collected mosquitoes were then released. Those from site one were released at site two and *vice versa*. One team then applied Mosi-guard (50% AI) and the other Autan spray and the experiment repeated. Repellent was applied to forearms, face and neck in amounts estimated to be equivalent to those which would be used in practice.

Control collections taken from 1855-1925 hours, and treatment collections from 1930-2000 hours. Total repellency noted from both treatments, results in Table below:

It is interesting that the summary presented by the registrant states that "... Mosi-guard and DEET Spray showed 100% repellency over the 1 hour period of the trial" when the study summary mentioned only 15 minutes exposure after treatment.

E. Cage Test with Culex Quinquefasciatus: Report on repellency effects of Mosi-guard Natural against *Culex quinquefasciatus*: Conducted by the London School of Hygiene and Tropical Medicine, June 1993.

Mosi-guard Natural spray applied evenly to forearm and allowed to dry for 10 minutes. The forearm then placed on top of a 12" cube cage with 150 hungry female *Culex quinquefasciatus* for 10 minutes and number of bites counted. The arm then thoroughly washed with soap and warm water to remove all traces of repellent, and dried. Forearm then rested again on top of the cage for 10 minutes and numbers of bites counted. This procedure repeated two more cycles over the next hour. The room kept in darkness at 27°C and relative humidity of 85%.

No bites received during any of the three repellency trials, compared with a total of 45 bites during the control periods. Note: Most of the bites were on the margins of the cleaned skin, indicating a possible residual effect of the repellent from earlier sprayings.

[An additional study on chicks with the same repellent included in this summary. One chick was sprayed with 0.51 gm of spray and placed in one cage with 35 hungry female *Culex quinquefasciatus*, while a second chick was just placed in another cage with no treatment. The unsprayed chick got fewer bites (70% feeding success) than did the sprayed chick (74% feeding success).]

F. Field Testing with Blackfly: Brief assessment of Mosi-guard Natural Spray formulation as compared to DEET spray formulation against blackfly (*Simulium woodi*): Conducted in Eastern Usambara Hills, Tanzania, Nov. 27-29, 1993.

A three-day trial carried out in the Eastern Usambara Hills in Tanzania at two sites close to fast-flowing streams where *Simulium woodi* breed. Six experienced insect collectors participated, two wearing Mosi-guard Natural spray, two 50% DEET and two as controls. Treatments were rotated so each individual experienced each treatment once.

Catchers sat 10 meters apart close to a fast-flowing stream. Insects were caught in individual glass tubes. Repellent applied shortly before the start of each day's trial, which lasted eight hours, from 6-11 AM and from 3-6 PM. (A late start one day due to heavy rain.) Number of bites and time until first bite recorded for each collector and treatment.

Over the six twelve-hour days, only 28 *Simulium woodi*, 1 *Aedes aegypti* and 2 *Chrysops bicolor*, a blood feeding insect, common in the region, were caught by the Controls. One (only) of the Mosi-guard Natural treated insect collectors was bitten, this while collecting 1 *Simulium woodi* and 4 *Chrysops bicolor*; the first bite at 3 hours and 50 minutes.

A page entitled Tests for M.A.S.T.A. of "Mosiquit" repellent against mosquitoes at Muheza and Simulium woodi at Amani, Tanzania was attached. It is unclear as to whether this "Mosiquit" repellent is in any way related to Mosi-guard Natural, or why it was included at all.

This study included more data than others, but these were still limited.

G. Field Testing with Mosquitoes: A field assessment of the efficacy and longevity of Mosi-guard Natural mosquito repellent as compared with DEET: Conducted in Tanzania, by Jane K. Trigg, January 1994.

I. Field Testing with Mosquitoes: A field assessment of the efficacy and longevity of Mosi-guard Natural mosquito repellent as compared with DEET: Conducted in Tanzania, by Jane K. Trigg, January 1994.

Spray, stick and roll-on formulations of Mosi-guard Natural (50% A.I.) were tested in the field on six experienced insect collectors. Each trial ran for six nights, plus a single "long catch" for each formulation. On any one night, there two Controls (no treatment), two with Mosi-guard and two with DEET. A preliminary test showed Mosi-guard Natural spray gave complete protection over a 2½ hour period. Mosi-guard Natural roll-on was evaluated over an 8-hour period, while Mosi-guard Natural stick and spray were evaluated over a 9-hour period. Single long catch trials continued until all collectors received at least one bite, over a period of up to 11 hours.

For each formulation, a standard amount of repellent was applied to the legs and feet, from the knees downward: Spray: 0.8 g repellent; roll-on: 2.3 g repellent; stick: 1.3 g repellent. The six-day period was split so the first three nights were conducted at Teule Hospital where *Culex quinquefasciatus* was the predominant species, and the last three at Mkuzi village where Anopheline mosquitoes were predominant. This was to determine if there was variation in repellency toward different mosquito species.

Collectors sat separately about 10 meters apart and collected mosquitoes which had clearly probed in a paper cup. Cups were collected hourly, kept in a refrigerator overnight, and the catch was counted and identified as to species the following morning. All formulations effective in repelling *Culex quinquefasciatus, Anopheles gambiae* and *Anopheles funestus*. All repellents gave around 7 ½ hours protection from mosquito bites. The Mosi-guard Natural roll-on formulation gave an average protection time of 10 hours (n= 2). Number of bites and time until first bite recorded for each collector and treatment. A great deal of statistical data incorporated in this study, primarily about the relative effectiveness of Mosi-guard Natural and Deet. The 14-page printed report of this study included in this section.

II Evaluation of a Eucalyptus-based repellent against Anopheles Spp. In Tanzania, J.K. Trigg, Journal of the American Mosquito Control Association 12(2): 243-246, 1996.

This is the published version of G, I, above

A. Cage Testing with Mosquitoes: Insect repellent test report Mosi-guard Natural: Conducted at the London School of Hygiene and Tropical Medicine, March 1994.

I. Cage Testing with Mosquitoes: Insect repellent test report Mosi-guard Natural: Conducted at the London School of Hygiene and Tropical Medicine, March 1994.

Batches of 20 hungry female mosquitoes placed in netting cages. Experimenter's bare forearm placed in the cage, hand protected by latex glove. Number of bites recorded at 30 seconds, then shaken off before taking blood, and arm removed from cage. This procedure repeated using 2nd and 3rd test cage. Control run followed by application of a small measured dose of test product (50% roll-on, 50% pump spray, 50% stick) or comparison products (DEET, citronella) to the test forearm. Arm reintroduced to test cage

and numbers biting for 30 seconds recorded. More repellent added between each test run until either all mosquito biting stopped or the applied dose became uncomfortably high. At the end of the test, a further control was performed, and two control runs averaged per cage to remove any possible effects caused by reduction in hunger of females during the experiment.

About 0.35 ml (0.33-0.38) of Mosi-guard Natural (spray, stick, roll-on) gave 90% repellent efficacy. This was approximately the same as with the Gurkha (citronella, 0.38 ml), but much better than the straight 50% citronella (0.69 ml). It was however, not as good as 20% DEET (0.24 ml). A longevity study tested the length of time the repellent activity lasted for these repellents (1, 2, 3, 4, 5 hours), and the results indicated that DEET performed best, maintaining 50% repellency at 5 hours, with the straight citronella the worst, with only about 5% repellency at 3 hours. The Mosi-guard Natural varied at 5 hours from a little over 40% repellency for the roll-on and stick to about 25% for the spray.

In a further test, participants put on as much repellent as they would normally use under field conditions. These participants were not experienced with field testing, but the results were interesting. Test lasted 6 hours; the stick repellent was most efficacious, with approximately 87% repellency at 6 hours. The repellency for the spray and roll-on at 6 hours was about 50%.

A free-flying test with 20 mosquitoes in a sealed test room (13 m^3) and an observer with bare lower legs and feet recording number of feeding mosquitoes. The procedure repeated with new mosquitoes after treatment corresponding to the 90% protection dose found earlier. Following this experiment, Mosi-guard Natural protected 92-100%, with the spray and roll-on requiring application rates lower than the stick, but conversely, the stick seems to last longer.

This report does not give the species of mosquito tested or the number of testers. The MASTA published report of this study included after the submitted document, however, provides the species of mosquito, *Anopheles gambiae*. There seems a problem with this published report. The information about the way the test was performed matches the data from the earlier section, but the paper includes one additional Mosi-guard Natural test product, and does not report on one comparison test product (Gurkha, containing citronella) included in the earlier section.

II. Laboratory evaluation of an Eucalyptus-based repellent against four biting athropods, J.K. Trigg and N. Hill, Phytotherapy Research, vol. 10, 313-316, 1996.

The 4 species of biting arthropods tested in the paper include: the mosquito *Anopheles gambiae*, the biting midge *Cullicoides variipennis* Coquillett, the deer tick *Ixodes ricinis* and the stable fly *Stomoxys calcitrans*.

Mosquito (*Anopheles gambiae)*

The mosquito test was the same as described in H, I.

Fly (*Stomoxys calcitrans*)

The stable fly test, using laboratory reared flies, was similar to the mosquito test. However, there were 40 flies in each cage (rather than 20); the arm remained in the cage for 1 minute (rather than 30 seconds); and a measured amount of repellent (0.35 ml) equivalent to the amount yielding 90% repellency for the mosquito, was applied at the first test, and increased to 0.5 ml for the second (rather than increasing the dose gradually). The flies were 15 days old and had been fed daily on blood since emerging as adults, but not fed on day of trial.

Mosi-guard was highly effective at repelling stable flies over the test period and still afforded 86% protection after 5 hours post-application of the lower dose of 0.35 ml and 94% at the 0.5 ml dose.

Midge (*Cullicoides variipennis* Coquillette)

Six pots with biting orifices containing 10 midges each were covered with netting and placed on a forearm for 3 minutes each. This was the basis for evaluating the repellency of the test substance. For each test, midges were added to the pot through an aspirator and allowed to settle for 10 minutes. Three pots sequentially held firmly against the arm at intervals along the treated arm (0.32 ml spread evenly over the 90 cm^2 area at a dose rate of 0.36 uL/cm^2). Number of bites recorded and process repeated with the remaining 3 pots on the untreated arm.

Testing commenced immediately after repellent dried and continued for up to 6 hours post-application. Because repellency lasted so long, additional tests were done starting at 5 hours post-application to 9 hours, or alternatively, repellent was used at a lower dose of 0.016 ml and tests lasted for 6 hours.

Hundred percent protection from midges was accomplished even after 6 hours at 0.36 ul/cm, and reducing to 70% after 9 hours. When the original dose was halved, protection remained high with only 1 bite received in 6 hours testing.

Tick (*Ixodes ricinis*)

Deer tick repellency not measured on humans. Ears of 6 laboratory rabbits (3 treated and 3 control) used instead. Repellent (0.32 ml mixed with alcohol for spreading) applied to each ear of 3 rabbits shaved 24 hours earlier. For each rabbit, a cotton earbag was fitted over each ear and attached securely to the base of the ear with surgical tape. Twenty nymphs inserted in each bag before folding the open end and sealing it. Earbags taped

together to prevent removal by grooming. For repellent treated animals, earbags were attached and ticks introduced 45 minutes after repellent application. Rabbits were checked twice over next 36 hour period to ensure that they were comfortable and earbags still attached.

Ticks usually take 24 hours to attach, remain feeding for about 7 days and then drop off. Each rabbit inspected after 43 hours; earbags opened and a count made of ticks attached, feeding or fed. Dead ticks recorded and removed from the earbag. This process repeated daily until all ticks, alive or dead were removed.

Efficacious when applied to rabbit ears, with repellent reducing attachment and feeding of nymphs. An average of 65% of nymphs fed on untreated ears, compared with just 10% on treated ears. A much higher percentage died on the treated ears than the untreated, but reason is unknown, as a study measuring repellency using filter paper crossing indicated no acaricide properties.

B. Cage Testing with Ixodes Ricinis: Laboratory tests to assess efficacy of Mosi-guard Natural against ticks: Conducted at Central Veterinary Laboratory, Addlestone, Surrey, England, by Jane K. Trigg, August 1994.

Two studies performed, one with filter paper treated with repellent, the other with live rabbits. The live rabbit study apparently the same as referred to in the Phytotherpy Research paper, H, II. The filter paper repellent study involved having 10 nymphs cross circles of filter paper. The circles were either untreated, treated with repellent (+ solvent for spreading) or treated with solvent. After treatment, papers allowed to dry and placed on untreated paper.

Most nymphs crossed to the edge of the outer circle on control papers within 5 minutes. Dispersal tended to be rapid and largely unidirectional. On repellent treated paper, there was a marked difference in nymph movement; nymphs tended to make many changes in direction with frequent stops. Some started over the treated section then turned around and came back. An average of 5.4 nymphs crossed the treated paper, while 9.1 crossed the control. The first test at 1 hour gave 40% repellency, after 3 hours, only 24.3% repellency and after 6 hours, -2.43%.

The published report on this study was included in this section.

C. Cage Testing with Triatomine: Laboratory test to assess efficacy of Mosi-guard Natural against triatomine bugs: Conducted by Jane K. Trigg, August 1994.

Rhodnius prolixus (triatomine) bugs were checked to determine if Mosi-guard Natural repelled them. The repellent was tested on an area of skin on the ventral surface of the experimenter's forearm. The arm was held resting against the netting of 5 pots each containing 1 bug until a probe of at least 10 seconds was received. Repellent applied in the amount of 3.564^{-4}ml/cm^2 and the method repeated. At this dosage the repellent appeared ineffective, therefore the dose was doubled and the test repeated. The tests were repeated after 1 hour. The

same test taken with DEET was similarly ineffective. The published report from MASTA was included with this section of the summary.

D. Cage and Field Testing with Biting Midges: Laboratory and field trials to assess the efficacy and longevity of Mosi-Guard Natural in protection against midge biting: Conducted at Purbright Laboratory, Woking, England and at Ormsary Estate, Argyllshire, Scotland, by Jane K. Trigg, Department of Medical Parasitology, London School of Tropical Medicine, July-August 1994.

I. Cage and Field Testing with Biting Midges: Laboratory and field trials to assess the efficacy and longevity of Mosi-Guard Natural in protection against midge biting: Conducted at Purbright Laboratory, Woking, England and at Ormsary Estate, Argyllshire, Scotland, by Jane K. Trigg, Department of Medical Parasitology, London School of Tropical Medicine, July-August 1994.

Laboratory studies over 4 days on the experimenter's forearms, one arm treated with 0.32 ml repellent, the other an untreated control. This report is the basis for the midge section in the publication in Phytotherapy Research as discussed in H II.

II Evaluation of a Eucalyptus-based repellent against *Culicoides impunctatus* (Diptera: Ceratopogonidae) in Scotland, J.K. Trigg, Department of Medical Parasitology, Journal of the American Mosquito Control Association, 12(2): 329-330, 1996.

Publication of an additional study on *Culicoides*, but *impunctatus* species, rather than *variipennis* Coquillett. This was a field study undertaken on the Ormsary Estate, Argyllshire, Scotland, in July. The area is dominated by damp, acidic peat-based soil, rushes, grass and moss, supporting several species of *Culicoides*, though most notably *impunctatus*.

Because earlier tests had suggested the repellent was efficacious for 5 hours, the dose (0.5 ml spread evenly from elbow to fingertips) was applied to 1 forearm of 1 subject. Another subject was treated with DEET and a third was the control. The treatments were rotated so every subject had every treatment. The repellent was allowed to dry for 5 hours before test commenced. Arms exposed to midges hourly for 10 minutes over a 3-hour period. Subjects stood 3 meters apart with their treated arm extended but all other skin covered. The test arms were monitored closely over the exposure time and all midges biting collected by an aspirator and blown into a tube of alcohol to be counted and identified later.

To maintain uniform control biting, it was necessary to have two sites: in the early evening in the wood, and in the later evening outside the laboratory with a carbon dioxide supply switched to attract the midges. Subjects stayed 1.5 m from carbon dioxide supply. Both Mosi-guard and DEET gave complete protection for 6-7 hours, and, at 8 hours, still maintained 98% protection.

An additional 3-day trial was performed to test further protection, with the repellents applied 8 hours before exposure to the midges and continuing until 10 hours after application. Even at these late stages, protection was high for Mosi-guard (99.5%) and DEET (97%).

L. Cage Testing with Aedes Aegypti: Determination of relative efficacy of a lemon eucalyptus based natural insect repellent using *Aedes aegypti* in a cage test: Conducted at Jackson, WI, USA, by Wisconsin Pharmacal Co., Inc., May-July 1998.

Detailed study report of mosquito efficacy of Wisconsin Pharmacal Inc. products. Test subjects had forearms exposed in cage with approximately 200-400 *Aedes aegypti* mosquitoes at ½ hour intervals for 5 minutes at each test interval until the first confirmed bite or 6-8 hours.

Test subjects washed their forearms with hand soap. They then placed one forearm into their respective test cage to determine a mosquito landing number. At least 3 landings within 30 seconds were required to qualify as a subject. Qualified volunteers rolled up their sleeves to the elbow and the skin above and below the target area (approximately 250 cm^2) was protected with cotton wrist bands. Hands protected with gloves. Test repellents were coded, and each arm labeled with a code corresponding to the repellent applied to that forearm. Test samples were applied by weight, and spread over the target area of the subjects's forearm with a gloved finger and allowed to air dry for approximately 30 minutes prior to first exposure. 0.4g/250 cm^2 test substance applied by weight; aerosol samples applied as close to 0.4 g/cm^2 as possible and noted in the report sheets. Test subjects were allowed to perform normal light-duty work between test periods.

The first test series was performed using lemon eucalyptus and alcohol blends with percentages of active varying between 10-60%. The lotion formula was tested at 20, 30 and 40% active. All products demonstrated efficacy on test subjects, but 10 and 20% pump sprays showed more erratic results with some failures occurring within 1 hour of application. The 30 and 40% pump sprays were effective on all test subjects (n=5) and had no bites for at least 4 hours. The 50 and 60% pump sprays were effective on all test subjects (n=3) and showed no bites over 4-6 hours. The lotions were effective on all subjects (n=4 for 20 and 30% and n=5 for 40%) for a minimum of 3 hours.

The second test series tested 2 formulations of a 50% non-aerosol pump, a 40% aerosol pump, a 50% aerosol pump and a 30% lotion. The pumps were effective on all subjects with few bites after 4 hours. The 30% lotion was slightly less effective (n=2 or 3 for each formulation tested).

The third series tested a 40% aerosol pump (n=7), 2 formulations of a non-aerosol pump (n=6 or 7), and a 30% lotion (n=8). First confirmed bite on any formula tested did not occur before 4.5 hours. Most test subjects (n-28 for all formulas, or 62%) demonstrated no sign of breakdown at 7.5+ hours.

Additional information in interpreting this data was received by BPB January 21, 1999 from Jean Killoren, Regulatory Coordinator.

DATA EVALUATION REVIEW OF THE DETERMINATION OF RELATIVE EFFICACY OF A WPC-DEVELOPED CITRIODIOL BASED NATURAL INSECT REPELLENT USING *AEDES AEGYPTI* MOSQUITOES §95-9

Product Manager: 90 **Reviewer:** Carol Frazer, Ph.D.
MRID No.: 446241-05 **Report Date:** 7/30/98
Testing Laboratory: Wisconsin Pharmacal Co.
Report No.: not provided
Author(s): unreadable
Species: humans
Weight: not applicable
Age: not given
Sex: 6-8 for each formula, sex not given
Source: not given
Test Materials: 305-LA, 305-LT, 305-LI
Pest Species: 200-400 mosquitoes, male and female, *Aedes aegypti*
Quality Assurance (40 CFR §160.12): Acceptable

Classification: Acceptable

Procedure (Deviation from §95): Test subjects washed their forearms with hand soap and then placed one forearm into respective test cage to determine a mosquito landing number. Required landings were at least 3 landings within 30 seconds to qualify as subject.

Volunteers then rolled up sleeves to the elbow, and skin above and below the target area (approximately 250 cm^2) was protected with cotton wrist bands. Hands protected with gloves. Test samples applied by weight at a rate of 0.4 g/250 cm^2, with formulations spread over the target area of subject's forearm with a gloved finger to provide uniform coverage. The aerosol samples applied at a rate as close to 0.4 gm/cm^2 as possible. Test subjects' forearms allowed to air dry at least 30 minutes prior to exposure. Test subjects were allowed to perform normal light-duty work between test periods.

Taking care not to rub their treated forearms against the cloth sleeve around the entry port, a technician assisted test subjects in exposing their treated forearms to mosquitoes in the test cage for 5 minutes or until first confirmed bite. Exposures repeated every 30 minutes for 5 minutes for a maximum of 8 hours or until a confirmed bite occurred. Both bites (blood is ingested, as evidence by abdominal swelling and color change) and probes (when mosquito lands and briefly penetrates the skin with mouth parts, but does not take blood) recorded.

Repellent deemed to have lost effectiveness after the first bite confirmed. Bites were confirmed when followed by an additional bite in the same exposure period or the next succeeding exposure period. The second bite was considered confirming and the breakdown time was established as the time the first bite occurred. When a confirmed bite occurred, testing was discontinued on that arm.

Results: All formulations gave a breakdown time of not less than 4.5 hours. With REPEL® NATURAL Insect Repellent Lotion (305-LA), 8 subjects had an average breakdown time of 7.38 hours, earliest breakdown of 6.5 hours, with an average of 0.19 bites per hour, and 3 subjects never getting bitten.

REPEL® NATURAL Insect Repellent Non-Aerosol Pump (305-LT), had 13 subjects in two groups with an average breakdown time in the first group of 7.50 hours, earliest at 6 hours, an average bites/hour of 0.16 and 5/7 having no bites whatsoever; the second group had an average breakdown time of 7.67 hours, earliest breakdown time at 6.50 hours, average bites/hour of 0.17 with 4/6 having no bites.

The REPEL® NATURAL Insect Repellent Aerosol (305-LI), 7 subjects, gives an average breakdown time of 6.64 hours, earliest at 4.5 hours, average bites/hour of 0.25 and 3/7 subjects not being bitten.

There may be a small problem with the 305-LT groups, however, as the subjects are indicated by initials and EC is listed in both groups with identical readings, i.e., date of test, # of bites, breakdown, etc. An MI and a JK are also listed twice, with the same date of testing, but the other data are different. The other initials are different in each group. If initials are to be used as identifiers, more needs to be included so there is no likelihood of mixing the data.

BPB's Comment: BPB considers this study acceptable to meet §95.9, but requires more studies to approve the label statement that this product repels mosquitoes.

PRÉCIS

Many of the reports, particularly the early ones, were simply declarative statements, with no data presented. Several of the later reports are detailed and some were published in peer-reviewed journals. All but the final test, that done by Wisconsin Pharmacal Co., did not use any of the products submitted for registration, and the similar test material (PMD-07) had at least 25% higher active ingredient.

The summaries above include 7 on mosquitoes: 3 cage-testing with *Aedes aegypti* (one of these tests also testing *Anopheles stephensii* and sandflies); one cage testing with Anopheles gambiae; one cage-testing with *Culex quinquefasciatus*; one field testing *Aedes aegypti*; and one field-testing with *Culex quinquefasciatus*, *Anopheles gambiae* and *Anopheles funestus*. In addition, two cage-testings were performed on sandflies *Phlebotomus papatas* and one cage-testing performed on stable flies *Stomoxys calcitrans*. Field testing was only performed with blackflies *Simulium woodi*. Additional cage-testing was performed on the midge *Culicoides variipennis* as well as field testing on the *impunctatus* species, and laboratory testing on the deer tick *Ixodes ricinis* (with filter paper and rabbit ear).

All of these tests indicated formulations of lemon eucalyptus test material efficacious against these insects for periods ranging from 2 to 11 hours. Repellent protection against many species of mosquito were evaluated using the lemon eucalyptus formulations, and this seems the most studied species. The decision to advertise this as protection against biting flies, however, may be somewhat premature. One test on stable flies, one test on blackflies, two on sandflies and two on the midge, one cage-testing and one field test. The blackfly field test was considered preliminary by the authors and they recommended further testing to measure efficacy and longevity of protection. No further testing was reported on this species.

All tests but the final one done by Wisconsin Pharmacal Co., are considered Unacceptable to label the products as repelling mosquitoes, ticks and biting flies. At least one field test and two cage-tests one using an Anopheles strain and one a Culex strain are required to acceptably register this mosquito repellent. The tick species tested using PMD-07 was the sheep tick, and there is no data on the deer tick, and whether that species is equally susceptible to this active ingredient. BPB will conditionally register these products as mosquito and tick repellents with the proviso that the additional mosquito studies, a tick study be performed. If Wisconsin Pharmacal Co., also wishes to add the stable fly, sandfly and midge as pests on their label, field tests on these additional species are required plus a lab test on the stable fly.

FIFRA

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X Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

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