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

OFFICE OF PREVENTION,
PESTICIDES AND TOXIC
SUBSTANCES

April 18, 2002

Memorandum

Subject: Secondary Review of Studies on the Insect Repellent Oil of Lemon Eucalyptus and Revisions to the Human Health Risk Assessment (PC 011550: WPC Brands; Case No. (62646) EPA File Symbol 305-LI.) DP Barcode D279876..

From: Roger Gardner, Toxicologist Roger Gardner 4/18/02
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Thru: Sheryl Reilly, Ph.D., Chief
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To: Jim Downing, Regulatory Action Leader
Biochemical Pesticides Branch
Biopesticides and Pollution Prevention Division (7511C)

Action Requested

Secondary review of Data Evaluation Records (DER) on the following studies:

MRID #	Study Type	Guideline #
45540101	Developmental Toxicity	870.3700
45540102	14-Day Repeat Dermal Toxicity	----
45540103	Special Study: Repellent Efficacy	----

The attached DERs were prepared by members of the Toxicology and Hazard Assessment Group, Life Sciences Division, Oak Ridge National Laboratory.

Determine revisions to the human health risk assessment on OLE according to the results of the new studies.

Recommendations and Conclusions

1. The lowest-observed-adverse-effect level (LOAEL) for systemic and dermal effects for Oil of Lemon Eucalyptus is 3000 mg/kg/ day, based on clinical signs indicative of neurological effects as well as dermal irritation at the application site. A previous 28-day dermal toxicity study showed that the no-observed-adverse-effect level (NOAEL) for systemic toxicity is 1000 mg/kg/day.

2. The maternal LOAEL for Oil of Lemon Eucalyptus is 1000 mg/kg body weight/day, based on transient clinical signs suggestive of a neurological effect (ataxia and impaired righting reflex). The maternal NOAEL was established at 300 mg/kg/day. A developmental LOAEL could not be established for this study. The developmental NOAEL is ≥1000 mg/kg body weight/day (highest dose tested).

3. Comparison of the LOAEL from the 14-day dermal toxicity study and the maternal LOAEL from the oral developmental toxicity study suggest that dermal absorption is likely to be 33% (i.e., 1000 mg/kg/day oral dose divided by the 3000 mg/kg/day dermal dose).

4. Transfer factors of the active ingredient under simulated use condition were 31% (aerosol spray on the arm), 42% (pump spray on the arm), 41% (aerosol on the leg), and 42% (pump spray on the leg).

5. The toxicity endpoint (clinical signs) were noted soon after the first dose in the oral developmental toxicity study and was reversible within a few days despite continued treatment at the LOAEL of 1000 mg/kg/day.

6. Proposed labels indicate that multiple applications may be necessary on each day of use and insect repellent usage information indicates individuals may apply repellent to their skin for an average of 9-13 days each year.

7. Typical application rates were estimated to be 0.14, 0.05 and 0.06 mg oil of lemon eucalyptus/cm^2 skin surface area treated for the aerosol, lotion and pump spray products, respectively.

8. Margins of exposure (MOE) exceed BPPD's level of concern (MOE<100) for a single application of the aerosol spray, after two applications of the lotion product for the subpopulation of children under 12 years of age, and after a single application of the pump spray (at two applications MOE= 98 for adult males).

9. Higher exposures may be common because more than 40% of the body surface area assumed in this assessment may be treated and results from the exposure study indicate higher application rates are possible (0.48 mg/cm^2 compared with 0.14 mg/cm^2 estimated for the aerosol and 0.16 vs. 0.06 mg/cm^2 for the pump spray).

I. Background

A previously submitted dermal developmental toxicity study (MRID 45056701) was classified as supplementary due to the dosing regimen. The OPPTS 870.3700 test guideline for developmental toxicity provides guidance for performing the study by the oral route of administration which is the most common route used for these studies, but guidance for those studies done by the primary route of human exposure, which may include the dermal route, is provided by reference to the dermal toxicity guideline (OPPTS 870.3200) and to the proceedings of a workshop on dermal developmental toxicity studies .[1] Beginning dosing as near as possible to the time of implantation in the rat study (gestation day 6) is typical for an oral study, but for dermal developmental toxicity studies, the preferred approach is to begin dermal dosing on gestation day 0, particularly if the test material has known or suspected low dermal absorption. The rationale for this is that the kinetics of dermal absorption may require a few days of treatment for a maximum daily absorbed dose to be reached. Therefore, the dermal developmental toxicity study with OLE is of limited value for assessing the hazard for endpoints which usually occur early in development (e.g., early and late resorptions), and a more complete assessment of potential developmental toxicity is needed for OLE.

Given the uncertainty associated with the previously submitted information on dermal absorption (i.e., no dermal absorption rate; MRID 44624209), a default assumption of 100% absorption was used in BPPD's risk characterizations. This default assumption overestimates dermal absorption, but appropriate information was not sufficient to estimate the extent of the error that is likely. Therefore, the 14-day dermal toxicity study (MRID 44540102) was conducted so that maternal toxicity results from the developmental toxicity study could be compared with systemic toxicity results from the subchronic dermal study to provide an estimate of dermal absorption. This approach is recommended as a first step in OPPTS Test Guideline 870.7600 for evaluating dermal absorption.

Finally, more specific exposure information was requested based on the use patterns for the aerosol and pump sprays to more realistically estimate exposures from those products (e.g., information that supports a modification of the exposure estimates by determining approximately how much of the OLE sprayed on clothing and skin surfaces is likely to become accessible for dermal absorption under normal conditions of use).

The three new studies (MRIDs 45540101 through -03) were submitted to address the issues related to full assessment of developmental toxicity potential, dermal absorption, and estimation of exposure..

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[1] See Kimmel, C.A. and E.Z. Francis. Proceeding of the workshop on the acceptability and interpretation of dermal developmental toxicity studies. *Fundamental and Applied* Toxicology 14:386-398. (1990). The oral route in a developmental toxicity study is preferred because systemic toxicity is more easily observed when establishing a NOAEL and LOAEL. In addition, the results can be compared with repeated -dose dermal studies to estimate a dermal absorption factor, which can be used to adjust exposure estimates. If an oral developmental toxicity study resulted in no maternal nor developmental toxicity (no endpoints) at a limit dose (1000 mg/kg/day), there would be no reason to quantitatively characterize a risk.

II. Data Summaries

A. Subchronic Dermal Toxicity (MRID 45540102)

In an acceptable non-guideline 14-day dermal toxicity study, Oil of Lemon Eucalyptus was applied to the shaved skin of groups of 15 female rats at dose levels of 5000 mg/kg/day 6 hours/day for 7 days or 3000 mg/kg/day 6 hours/day for 14 consecutive days. White mineral oil applied similarly to the shaved skin of another group of 15 female rats served as the control group.

One rat treated with 5000 mg/kg/day was found dead on day 6 and another in the same group was killed moribund on the same day. In the 5000-mg/kg/day group, clinical signs observed cage-side or during the detailed observation indicative of neurological effects included hypoactivity in 10 rats, hunched posture in 8, inactivity but alertness in 4, tremors in 6, and abnormal gait in 3. In the 3000-mg/kg/day group, hypoactivity was observed in four rats and hunched posture in one. Generally the onset of these signs occurred between days 4 and 6, lasted for 1-4 days, or were noted only during the detailed clinical examination (inactivity, tremors, and abnormal gait) and not during the daily cage-side observations. Clinical signs indicative of dermal irritation included desquamation and erythema, which was observed in 13 and 15 rats, respectively, in the 5000-mg/kg/day group and 15 and 14 rats, respectively, in the 3000-mg/kg/day

Rats in the 5000-mg/kg/day group gained only 35% as much weight as the controls during the first week of the study, but gained significantly more weight during the second week. Controls and 3000-mg/kg/day group female rats had similar weight gains but both groups lost weight during the second week of the study. Food consumption was not measured. The only postmortem parameter examined was liver weight; the absolute and relative (to body weight) liver weights were elevated by 14% and 10%, respectively, in the 5000-mg/kg/day group compared with that of controls. Absolute and relative liver weights of rats in the 3000-mg/kg/day group was slightly elevated compared with that of the controls.

The LOAEL for systemic and dermal effects for Oil of Lemon Eucalyptus is 3000 mg/kg/day, based on clinical signs indicative of neurological effects as well as dermal irritation at the application site. A previous 28-day dermal toxicity study showed that the NOAEL is 1000 mg/kg/day.

B. Developmental Toxicity Study (MRID 45540101)

In an acceptable developmental toxicity study (MRID 45540101), Oil of Lemon Eucalyptus (65.17%, *p*-methan-3,8-diol) was administered to groups of 25 pregnant rats by gavage on gestation days (GD) 6-20 inclusive. Dose levels were 0, 100, 300, and 1000 mg/kg/day administered undiluted with the gavage volume based on daily body weights. Controls received corn oil at a dosage of 1.04 mL/kg body weight. All surviving dams were sacrificed on GD 21 for evaluation of maternal and developmental parameters. All fetuses were given external examinations; one-half the fetuses in each litter was examined for visceral abnormalities and the remaining half was processed for skeletal examination.

No deaths occurred among dams in any treated or control group. Clinical signs, which were observed during the first few days of dosing and were suggestive of a neurological effect, consisted of ataxia and impaired righting reflex in all dose groups and decreased motor activity and lost righting reflex in the high-dose group. In addition, excessive salivation and a red substance around the mouth was also noted in all dose groups. In the high-dose group, absolute body weight was 3-5% ($p \leq 0.01$ or ≤ 0.05) less and weight gain was 52% ($p \leq 0.01$) less than that of controls during the first 3 days (GD 6-9) of dosing. Compensatory weight gain (+31%, $p \leq 0.01$) was observed for the GD 9-12 interval. In addition, food consumption by the high-dose group was 27% less than that of controls during the GD 6-9 dosing interval. Postmortem examination of the dams showed treatment-related increases in absolute and relative liver weight at all doses (26 and 30%, 16 and 13%, 6 and 7% for high-, mid-, and low-dose, respectively). Centrilobular hepatocyte hypertrophy was observed in the livers of 14 of 15 high-dose rats examined. The livers were not examined in mid- and low-dose rats.

The maternal lowest-observed-adverse-effect level (LOAEL) for Oil of Lemon Eucalyptus is 1000 mg/kg body weight/day, based on transient clinical signs suggestive of a neurological effect (ataxia and impaired righting reflex). The maternal NOAEL was established at 300 mg/kg/day.

No treatment-related adverse effects were observed for any cesarean section parameter (gravid uterine weight, number corpora lutea/dam, implantations/dam, percent pre- and post-implantation loss, total resorptions, resorptions/dam, and total number of live fetuses, mean fetal weight, and sex ratio). A statistically significant decrease was observed for the number of resorptions/dam in high-dose females. In addition, the percent pre-implantation loss, which occurred before treatment was initiated, showed a dose-related increase, whereas the post-implantation loss, which occurred after treatment was initiated, showed a dose-related decrease. A total of 379(24), 383(25), 393(25) and 367(24) fetuses(litters) in the 0-, 100-, 300-, and 1000-mg/kg/day groups were examined for external abnormalities, one-half the fetuses/litter were examined for visceral abnormalities by a microdissection technique, and one-half were processed for skeletal examination. No treatment-related external abnormalities, visceral abnormalities, or skeletal malformations/variations, including the number of ossification sites, were observed at any dose of the test material.

A developmental LOAEL could not be established for this study. The developmental NOAEL is ≥1000 mg/kg body weight/day.

C. Exposure Study (MRID 45540103)

Two proposed commercial products, Repel Essential Aerosol and Repel Essential Pump Spray were tested for the transfer of OLE to the skin under simulated conditions of use. The products contained 40% OLE. The products were sprayed on a substrate consisting of cotton with aluminum foil backing attached to the arm or leg. Transfer factors of the active ingredient for the aerosol or pump spray applied to the arm or leg under these condition was 31% (aerosol spray on the arm), 42% (pump spray on the arm), 41% (aerosol on the leg), and 42% (pump spray on the leg).

III. Discussion

A. Dose-response Assessment

1. Endpoint selection

The endpoints, no-observed-adverse-effect levels (NOAEL) and lowest-observed-adverse-effect levels (LOAEL), are summarized from the submitted toxicological data in Table 1.

TABLE 1: Toxicity Profile of Oil of Lemon Eucalyptus

Study Type	MRID No.	Results	Comments
Immunotoxicity - mouse	---	Waived	
Subchronic Toxicity, 21-Day Dermal - Rat	45045203	NOAEL = 1000 mg/kg/day (highest dose tested) LOAEL established in 14-day study (increased skin erythema, edema and flaking in treated animals)	Acceptable
Subchronic Toxicity, 14-Day Dermal - Rat	45540102	LOAEL = 3000 mg/kg/day (dermal irritation and signs indicative of neurological effects; hypoactivity and hunched posture) NOAEL = 1000 mg/kg/day from 21-day study	Acceptable
Developmental Toxicity (Dermal) - Rat	45056701	Maternal NOAEL>1 g/kg/day (HDT) LOAEL not established. Developmental: NOAEL>1 g/kg/day LOAEL not established (increased skin erythema, edema and flaking in treated animals)	Supplementary
Developmental Toxicity (Oral) - Rat	45540101	Maternal LOAEL = 1000 mg/kg /day, (transient clinical signs of neurological effects (ataxia and impaired righting reflex) Maternal NOAEL = 300 mg/kg /day Developmental LOAEL > 1000 mg/kg/day (HDT) Developmental NOAEL is ≥1000 mg/kg/day.	Acceptable
Bacterial Assay (Ames)	44624208	Non-mutagenic ± activation	Supplementary
Mouse Lymphoma	45045201	Non-mutagenic ± activation	Acceptable
Micronucleus Assay	45045202	Non-mutagenic	Acceptable

The maternal NOAEL of 300 mg/kg/day from the oral developmental toxicity study in rats is used for risk characterizations when dermal exposure estimates have been adjusted for dermal absorption, or dermal exposures can be compared directly to the dermal NOAEL of 1000 mg/kg/day without such adjustments. Most of the clinical signs were observed in susceptible rats soon after the first oral dose was administered (on gestation day 6) and were reversed within a few days (by gestation day 10 in most responding animals) despite continued treatment at 1000 mg/kg/day. The signs in dermally treated animals appeared after 5 treatments at 3000 mg/kg/day. These endpoints will be used to characterize acute, short and intermediate-term risks.

2. Dermal absorption

The estimate of a dermal absorption factor, which is based on the subchronic dermal toxicity and oral developmental toxicity studies, is a first approximation. The dermal irritation observed in the subchronic dermal studies is expected to alter absorption in some way, but a comparison of LOAELs from the two studies suggests that dermal absorption is less than 100%. The ratio of the maternal LOAEL from the developmental study to the dermal LOAEL in the subchronic study indicates that dermal absorption could be in the range of 33%. It should also be noted that clinical signs were observed immediately after oral doses were begun, while they did not appear until 3-6 days after the beginning of repeated daily dermal dosing. Therefore, the 33% dermal absorption factor is considered to be an upper bound estimate.

B. Exposure Assessment

1. Use patterns and estimating exposure

Oil of lemon eucalyptus is to be formulated into three products for use on skin and clothing. These products include an aerosol spray (40% a.i.), a lotion (30% a.i.) and a non-aerosol pump spray (40% a.i.).

The label for the 40% aerosol spray says "...repels mosquitos for up to one hour." The directions for use indicate that the container is to be held 6 to 8 inches from the skin or clothing and sprayed with a slow, sweeping motion. The product is not to be sprayed directly on the face, but the label recommends dispensing the repellent on the palm of the hand to spread on the face and neck. The label further advises, "Do not apply over cuts, wounds or irritated skin." If necessary, the label recommends reapplying every hour or as needed.

According to the label for the 30% lotion, it is to be applied to exposed skin to repel mosquitoes or biting insects, and if necessary, it should be reapplied every hour or as needed (repeated applications during each day of use).

The label for the 40% pump spray is similar to the other products except that there are no instructions regarding repeated applications.

Additional information on the use patterns for these OLE products was provided by reference to the DEET Joint Venture's (DJV) report on similar use of other insect repellent products containing DEET as the active ingredient (MRID 41968001). The following information was highlighted from that report by the registrant:

- Survey data indicates that approximately 37% of the U.S. population uses insect repellent products, and 60% of that use occurs during the months of June and July.
- The average number of days individuals used an insect repellent product during the months of June and July was reported in the survey as 7.5 for the general population and 5.6 for children, and annual average days of use were 12.5 and 9.3 for the general population and children, respectively.
- The estimated amounts of product applied to skin and clothes for a single application (skin only data was available for DEET products containing 100% a.i. but not for products containing less than 100% DEET) were reported to be 5.9 g (aerosol), 1.0 g (lotion), and 2.3 g (pump spray).

These points suggest short- and possibly intermediate-term exposure scenarios. An acute exposure scenario is not considered in this assessment because there are no acute endpoints indicated in the toxicity data.

Adjusting the amounts of product used by the percentage oil of lemon eucalyptus in those products in order to determine the amounts of active ingredient that will be applied yields 2.36 g (aerosol, adjusted for 40% a.i.), 0.3 g (lotion, adjusted for 30% a.i.), and 0.92 g (pump spray, adjusted for 40% a.i.). Using the transfer factors of 31-41% (median of 36%) for the aerosol and 42% for the pump spray products reported in the exposure study (MRID 45540103) as well as assuming a transfer factor of 100% for the lotion, the adjusted estimates would be 0.85 g a.i./application for the aerosol, 0.39 g/application for the pump spray, and 0.3 g a.i./application for the lotion. These calculations do not consider differences in label recommendations for frequency of use or the surface area exposed to the active ingredient.

According to the Agency's *Exposure Factors Handbook*, exposure estimates should be based on an estimated application rate expressed as mg a.i./cm^2 dermal surface area. Such estimates can be derived from directly observed amounts reported from the exposure study (MRID 45540103) and indirectly determined from the information provided in the DJV report.

In the exposure study, the aerosol or pump spray products were sprayed onto a foil-backed cotton cloth target material covering a person's limbs from the wrists to the upper arms or the legs from ankle to mid thigh. Since the submitted report did not describe the dimensions of the target materials it is assumed that the treated area of the target material is equivalent to that of an average male according to the *Exposure Factors Handbook* (skin surface area=1.94 m^2, the area treated is approximately 30% of the total or 5820 cm^2). The amounts of active ingredient sprayed, the appropriate transfer factor, and the assumed surface area are used to estimate a mg a.i./cm^2 application rate. For example, the exposure study indicated that 2100 mg a.i. was applied to the arms and the transfer factor was 30.1% for a total of 651 mg. Similar calculations for the aerosol applied to the legs indicated 2172 mg were applied. The total for arms and legs was 2783 mg applied to 5820 cm^2. The resulting application rate is 0.48 mg/cm^2 for the aerosol and a similarly derived value of 0.16 mg/cm^2 application rate was estimated for the pump spray product.

The DJV report described a usage study in which samples representing subpopulations of adult males (n=134, average age=32.3 years, average weight=181 lb [82.3 kg]), adult females (n=135, average age=37 years, average weight=148 lb [67.3 kg]), children 13-17 years old (n=136, average age=14.6 years, average weight=134 lb [61 kg]), and children under 12 years of age (n=137, average age=7.7 years, average weight=66 lb [30 kg]). Respective man total skin surface areas for these subpopulations are given in the Agency's *Exposure Factors Handbook* as 1.94, 1.69, 1.55 and 0.917 m^2.

The DJV use study indicated that individuals typically apply insect repellent mostly to their arms and legs. Reported results of the use survey are summarized in Table 2.

TABLE 2: Insect repellent use-patterns - areas people treat*

Area treated	Per cent applying repellent to area				
	Total	Adult male	Adult female	Children (12-17)	Children (12 and under)
Sample size	542	134	135	136	137
Left arm	94	98	92	96	91
Right arm	94	96	94	95	92
Right leg	72	60	70	76	92
Left leg	71	59	70	75	80
Neck	45	59	40	36	47
Left ankle	43	31	52	39	47
Right ankle	42	29	50	39	50
Face	21	25	17	18	23
Chest	13	13	15	9	16
Right foot	12	6	16	8	19
Left foot	12	6	16	9	18
Back	9	10	4	7	15
Hair	6	8	6	5	6

* MRID 41968001

Given these results, it is reasonable to assume that people commonly apply insect repellent to at least 40% of their skin. Table 3 summarizes the percentages by body part for adult men, women and children as reported in the *Exposure Factors Handbook.*

TABLE 3: Percentages of the total surface area represented by body parts typically treated with insect repellent.

Body part	Percentage of total surface for			
	Men	Women	Children (14-15)[a]	Children (7-8)[b]
Head[c]	2.6	2.4	—[g]	4.4[h]
Arms				9.8[i]
Upper arms[d]	3.7	3.6[f]	—[g]	—[j]
Forearms	5.9	6.7[f]	—[g]	—[j]
Hands	5.2	5.1	—[g]	4.7
Legs				20.3[i]
Thighs[e]	9.2	9.8	—[g]	—[j]
Lower legs	12.8	12.8	—[g]	—[j]
Total	39.4	38.4	40[g]	39.2

[a] Based on average age of sample for children aged 12-17 years from DJV report.

[b] Based on average age of sample for children <12 years of age from DJV report.

[c] Based on one-third of the value listed in the *Exposure Factors Handbook* assuming only the face is treated with insect repellent.

[d] Based on half the value listed in the Exposure Factors Handbook to account for treatment of skin above the elbow on arms.

[e] Based on half the value listed in the Exposure Factors Handbook to account for treatment of skin above the knee.

[f] There was no data reported for the upper arm and forearm separately. The reported total was 14% and these values were derived on the basis that the percentages for each were in the same proportions as those for men (i.e., forearm is approximately 48% of the total surface area for upper arm and forearm combined). 14 x 0.48 = 6.8.

[g] No values were included in the Exposure Factors Handbook, but the body weight for this sample (61 kg) is close enough to the adult female sample that the total skin surface area treated is assumed to be the same (approximately 40%).

[h] Data for children 6-7 years of age were used since none were available for 7-8 year olds.

[i] Areas were not reported as upper arm and forearm so an approximation was made by using 0.75 x percentage of the area represented by arms or legs (e.g., 27.1% of the total surface area x 0.75 = 20.3% of the total surface area for a child's legs which had repellent applied from the ankle to above the knee).

[j] No separate data for portions of extremities were available in the *Exposure Factors Handbook*.

Application rates can be calculated from the DJV report and results from Table 3, but because the amount of products used per application were not associated with each subpopulation, appropriate parameters for the overall sample of 542 individuals were determined. Weighted average skin surface area (1.52 m^2) and body weight (60 kg) were calculated to describe the general population. These parameters were used to determine an estimated application rate for aerosol, lotion and pump spray products. For example:

$$850 \text{ mg OLE} \div (15{,}200 \text{ cm}^2 \times 0.4) = 0.14 \text{ mg/cm}^2 \text{ for the aerosol product}$$

Similar calculations for the lotion and pump spray products indicated application rates of 0.05 and 0.06 mg/cm^2, respectively.

The estimated application rates from the exposure study were approximately 2.67 to 3.5-fold higher than those derived from the DJV report and information in the *Exposure Factors Handbook*. For purposes of this assessment, the estimated application rates derived from the DJV report are assumed to be typical and those from the laboratory exposure study are a worst case estimate of application rates for the aerosol and pump spray products.

The application rates (mg a.i./cm^2) and surface area exposed to OLE (cm^2), a mg/kg dose can be determined for comparison with the dermal NOAEL of 1000 mg/kg/day in risk characterization. For example, a child from the <12 year age group has a total surface area of 9170 cm^2, 40% of which (3668 cm^2) is treated at a rate of 0.14 mg/cm^2 for a total of 513.5 mg OLE. The dose rate for the subpopulation is 513.5 mg/30 kg body weight = 17.1 mg/kg/application. Similar calculations were done for each product and subpopulation, and the results are summarized in Table 4. It should be noted that the 17.1 mg/kg value could be adjusted by the 33% dermal absorption factor to get an adjusted dose of 5.6 mg/kg which would be compared with the oral NOAEL of 300 mg/kg. However, the resulting margins of exposure (MOE) would be the same.

TABLE 4: Estimates of exposures

Assumption	Adult males	Adult females	Children (13-17)	Children (≤ 12)
Body weight (kg)[a]	82.3	67.3	61	30
Treated surface area (m^2)[b]	7060	6760	6200	3668
Total amount applied (mg)				
Aerosol[c]	988.4	946.4	868	513.5
Lotion[d]	353	338	310	183.4
Pump Spray[e]	423.6	405.6	372	220.1
Estimated dose (mg/kg/application				
Aerosol	12	14.1	14.2	17.1
Lotion	4.3	5.0	5.1	6.1
Pump Spray	5.1	6.0	6.1	7.3

[a] Mean values from the DJV Survey
[b] Based on use information from the DJV report and surface area data in the *Exposure Factors Handbook.*
[c] Application rate = 0.14 mg/cm^2
[d] Application rate = 0.05 mg/cm^2
[e] Application rate = 0.06 mg/cm^2

2. Risk characterization

The toxicity endpoint (clinical signs) were noted soon after the first dose in the oral developmental toxicity study and was reversible within a few days despite continued treatment at the LOAEL of 1000 mg/kg/day. The same endpoint in dermally treated animals appeared after 5 treatments at 3000 mg/kg/day. Proposed labels indicate that multiple applications may be necessary on each day of use, and information from the DJV usage survey suggests that the repellents may be used on average from 9 to 13 days a year. This information also indicated that approximately 37% of the U.S. population uses insect repellent products. Typical application rates were estimated from the DJV Survey report to be 0.14, 0.05 and 0.06 mg/cm^2 for the aerosol, lotion and pump spray products, respectively. It is also assumed from DJV report information that a typical application may cover 40% of the total body surface area. It is reasonable to assume that higher exposures will occur because more than one application might be required each day and more than 40% of the body surface area may be treated.

Margins of exposure are determined by dividing the dermal NOAEL of 1000 mg/kg/day by the dose (mg/kg) calculated from exposure estimates. MOEs less than 100 exceed BPPD's level of concern. Resulting MOEs are summarized in Table 5.

TABLE 5: Margins of exposures for three products containing OLE

Product	Number of applications	Adult males	Adult females	Children (13-17)	Children (≤12)
Aerosol	1[a]	83	71	70	58
Lotion	1	233	200	196	164
	2[b]	116	100	98	82
	3[a]	78	67	65	55
Pump Spray	1	196	167	164	137
	2[c]	98	83	82	68
	3[a]	65	56	55	46

[a] MOE < 100 and exceeds level of concern for all subpopulations.
[b] MOE < 100 and exceeds level of concern for children <12 years of age.
[c] MOE < 100 and exceeds level of concern for all subpopulations except adult males.

Attachments

Data Evaluation Records (DER) for
a 14-Day Dermal Subchronic Toxicity Study (MRID 45540102),
an Oral Developmental Toxicity Study (MRID 4550101); and
a Special Study on Exposure (MRID 45540103)

DATA EVALUATION RECORD

CITRIODIOL (OIL of LEMON EUCALYPTUS) 011550

STUDY TYPE: 14-DAY REPEAT DERMAL TOXICITY
PC CODE: 011550

MRID 45540102

Prepared for

Biopesticides and Pollution Prevention Division
Office of Pesticide Programs
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Prepared by

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Task Order No. 108

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Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

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Oak Ridge National Laboratory, managed by UT-Battelle, LLC, for the U.S. Dept. of Energy under contract DE-AC05-00OR22725

Secondary EPA Reviewer: Roger Gardner
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Date 4/18/02

DATA EVALUATION RECORD
TXR#:

STUDY TYPE: 14-Day Dermal Toxicity - Non-Guideline

PC CODE: 011550 (*p*-methane-3,8-diol

DP BARCODE: 279874
SUBMISSION NO.: S607767

TEST MATERIAL (PURITY): Oil of Lemon Eucalyptus (citriodiol) (65.17%, *p*-methane-3-8, diol; total *cis* and *trans* isomers)

SYNONYMS: none

CITATION: Moore, G. 2001. 14-Day repeated dermal dose toxicity study in rats with Oil of Lemon Eucalyptus. Product safety Labs, 2394 Route 130, Dayton, NJ 08810. Laboratory study number 10315, October 30, 2001. MRID 45540102. Unpublished

SPONSOR: WPC Brands, Inc., 1 Repel Road, Jackson, WI 53037

EXECUTIVE SUMMARY: In a 14-day dermal toxicity study (MRID 45540102), Oil of Lemon Eucalyptus (*p*-methane-3,8-diol 65.17%, batch/lot #698058R)] was applied to the shaved skin of groups of 15 female Crl:CD®(SD)IGS BR VAF/Plus® rats at dose levels of 5000 mg/kg/day 6 hours/day for 7 days or 3000 mg/kg/day 6 hours/day for 14 consecutive days. White mineral oil applied similarly to the shaved skin of another group of 15 female rats served as the control group.

One rat treated with 5000 mg/kg/day was found dead on day 6 and another in the same group was killed moribund on the same day. In the 5000-mg/kg/day group, clinical signs observed cage-side or during the detailed observation indicative of neurological effects included hypoactivity in 10 rats, hunched posture in 8, inactivity but alertness in 4, tremors in 6, and abnormal gait in 3. In the 3000-mg/kg/day group, hypoactivity was observed in four rats and hunched posture in one. Generally the onset of these signs occurred between days 4 and 6, lasted for 1-4 days, or were noted only during the detailed clinical examination (inactivity, tremors, and abnormal gait) and not during the daily cage-side observations. Clinical signs indicative of dermal irritation included desquamation and erythema, which was observed in 13 and 15 rats, respectively, in the 5000-mg/kg/day group and 15 and 14 rats, respectively, in the 3000-mg/kg/day

Rats in the 5000-mg/kg/day group gained only 35% as much weight as the controls during the first week of the study, but gained significantly more weight during the second week. Controls and 3000-mg/kg/day group female rats had similar weight gains but both groups lost weight during the second week of the study. Food consumption was not measured. The only

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postmortem parameter examined was liver weight; the absolute and relative (to body weight) liver weights were elevated by 14% and 10%, respectively, in the 5000-mg/kg/day group compared with that of controls. Absolute and relative liver weights of rats in the 3000-mg/kg/day group was slightly elevated compared with that of the controls.

The LOAEL for systemic and dermal effects for Oil of Lemon Eucalyptus is 3000 mg/kg/day, based on clinical signs indicative of neurological effects as well as dermal irritation at the application site. A previous 28-day dermal toxicity study showed that the NOAEL is 1000 mg/kg/day.

This 14-day dermal toxicity study in the rat is **Acceptable/Non-Guideline** for defining the toxicological potential of the test material by the dermal route of exposure

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. **Test material:** Oil of Lemon Eucalyptus

Description:	Slightly viscous amber liquid
Lot/Batch #:	698058R
Purity:	65.17%, *p*-methane-3,8-diol, total *cis* and *trans* isomers
Compound Stability:	At least 1 year based on expiration date of August 23, 2002
CAS #:	Not provided
Structure:	Not available

2. **Vehicle and/or positive control:** Test material was applied undiluted; therefore, no vehicle was used; white mineral oil was applied to the skin of controls.

3. **Test animals:**

Species:	Female rats
Strain:	Crl:CD®(SD)IGS BR VAF/Plus®
Age/weight at study initiation:	12 weeks old: 227-266 g
Source:	Charles River Laboratories, Raleigh, NC
Housing:	Housed individually in suspended stainless-steel cages with mesh floors
Diet:	Purina Rodent Chow #5012, *ad libitum* (assumed)
Water:	Filtered tap water, *ad libitum*
Environmental conditions:	**Temperature:** 18-24°C **Humidity:** 41-68% **Air changes:** Not reported **Photoperiod:** 12 hours dark/12 light
Acclimation period:	13 days

B. STUDY DESIGN:

1. **In life dates:** Start: September 5, 2001; End: September 19, 2001

2. **Animal assignment**: Animals were assigned to the test groups noted in Table 1 based on a body weight stratification procedure. The high-dose group was treated for only 7 days.

TABLE 1: Study design

Test group	Dose	# Male	# Female
Control	5000 mg/kg/day mineral oil	0	15
Low	3000 mg/kg/day Oil of Lemon Eucalyptus	0	15
High[a]	5000 mg/kg/day Oil of Lemon Eucalyptus	0	15

Data taken from page 7, MRID 45540102.
[a]Treatment of the high-dose group was discontinued after day 7 because of the severity of the effects.

3. **Dose selection rationale**: The doses of 3000 and 5000 mg/kg/day levels were selected because a previous 28-day repeated dermal toxicity study showed no evidence of toxicity at the limit dose of 1000 mg/kg/day. The Sponsor wanted to define the toxicologic effects of Oil of Lemon Eucalyptus applied to the skin at doses above 1000 mg/kg/day.

4. **Preparation of test material:** Before treatment, the test material was heated to 50°C in a water bath, cooled to ~39°C and maintained at this temperature with continuous stirring during application. The vehicle (mineral oil) was also maintained at ~39°C during application.

5. **Preparation and treatment of animal skin:** During acclimation, the fur of each test animal was clipped from the dorsal area of the trunk from an area measuring about 100 cm^2 (from the shoulders to the hips) and the rats were fitted with an Elizabethan collar. One day later, sham wrappings were applied for 1 hour the first day, 3 hours the second day, and 6 hours the third through fifth days. The day before treatment started, the rats were weighed and shaved again and as often as needed during the study. Undiluted test substance or white mineral oil was applied directly to the skin and spread over the entire shaved area of rats in the high-dose and control groups and undiluted test substance was spread over to 3/5 of the shaved area of the low-dose group. The treated area was covered with a gauze pad, which was then secured with non-allergenic surgical tape. The rats were fitted with an Elizabethan collar to prevent ingestion of the test substance or removal of the wrapping. The dressings were removed after 6 hours and the application area was cleaned using a towel and 5% Dove® dishwashing liquid in tepid water. The area was gently rinsed in tepid water and patted dry. This process was repeated 7 days/week for 14 days for the control and 3000-mg/kg/day groups and for 7 days for the 5000-mg/kg/day group.

6. **Statistics:** Body weights and liver weights were analyzed by Bartlett's test; if Bartlett's test was not significant indicating homogeneity of variance, the data were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test if ANOVA was significant ($p \leq 0.05$). If Bartlett's test was significant indicating heterogeneity of variance, the data were analyzed by the nonparametric Kruskal-Wallis test followed by Dunn's multiple comparison test if Kruskal-Wallis was significant ($p \leq 0.05$).

Pairwise statistical significance was indicated by $p \leq 0.05$.

C. METHODS:

1. Observations:

1a. **Cageside observations:** Animals were observed twice daily for mortality and for clinical signs once daily about 1 hour after removing the wrapping and cleaning the application site on days 1-6 and 8-13. Particular attention was focused on gait, posture, and increased salivation during observation. The rats were observed pretreatment on day 7 to evaluate status.

1b. **Clinical Examinations:** Detailed clinical examinations were conducted on days 7 and 14 about 1 hour after removing the wrapping and cleaning the application site. Particular attention focused on the skin, fur, eyes, mucous membranes, secretions, excretions, respiration, circulation, nervous system, and behavior patterns.

1c. **Neurological evaluations:** A neurological evaluation was not performed; this is a non-guideline study.

2. **Body weight:** Animals were weighed twice during acclimation, 1 day prior to initiation of the study and on days 8 and 15 (prior to terminal sacrifice) or after death.

3. **Food consumption:** Food consumption was not determined during this study.

4. **Ophthalmoscopic examination:** Eyes were not examined during this study.

5. **Hematology and clinical chemistry:** Blood was not collected for hematology or clinical chemistry evaluations.

6. **Urinalysis:** Urine was not collected during this study.

7. **Sacrifice and pathology:** All animals were sacrificed on day 15 by carbon dioxide asphyxiation; the livers were excised, weighed and discarded. The carcasses also were discarded.

II. RESULTS:

A. OBSERVATIONS:

1. **Clinical signs of toxicity**: Clinical signs noted during cage-side observations or during the detailed clinical observation are summarized in Table 2. In the 5000-mg/kg/day group, clinical signs indicative of a neurological effect were observed particularly during the first 7 days. During the daily cage-side observations, hypoactivity was observed for 1-3 days in 10 rats between days 4 and 6 of the study. Hunched posture was observed for 1 to 4 days in six rats between days 3 and 6. Anogenital staining was observed in the two rats that died. During the detailed examination on day 7, four rats were inactive but alert, four had slight tremors and two had severe tremors, eight had hunched postures, and three had abnormal gaits. Treatment of the 5000-mg/kg/day group was discontinued after day 7 because of the

deaths and the severity of the clinical signs; therefore, a recovery was observed between days 7 and 15. On day 14 (7 days after treatment was terminated), only two rats in the 5000-mg/kg/day group were inactive (but alert), one rat showed slight intermittent tremors, two rats had abnormal or flattened posture, and one rat showed evidence of excessive defecation. On day 15, tremors were noted in two rats, inactivity in one, and abnormal posture in one.

In the 3000-mg/kg/day group, hypoactivity was observed for 1 day (day 5, 6, or 11) each in four rats , and hunched posture was observed in one rat on day 6 only. During the detailed examination on days 7 and 12, none of the signs indicative of a neurological effect were observed in the 3000-mg/kg/day group. The red/brown eye or nasal discharge was observed in one rat. On day 15, one rat was inactive.

TABLE 2. Cage-side and detailed clinical observations in female rats treated topically with Oil of Lemon Eucalyptus

Observation observations	White Mineral Oil 5000 mg/kg/day	Oil of Lemon Eucalyptus 3000 mg/kg/day	Oil of Lemon Eucalyptus 5000 mg/kg/day[a]
Number of animals observed	**15**	**15**	**15**
Daily cage-side			
Desquamation	0	15 (3-8 days)	13 (3-8 days)
Erythema		14 (3-10 days)	15 (3-10 days)
Hypoactive		4 (1 day)	10 (1-3 days)
Hunched posture		1 (1 day)	6 (1-4 days)
Anogenital staining		0	2 (1-2 days)
Dead or killed moribund		0	2 (day 6)
Day 7			
Detailed Clinical Examination - open field			
Inactive but alert	0	0	4
Tremors	0	0	6
Hunched posture	0	0	8
Abnormal gait	0	0	3
Detailed Clinical Examination – handling			
Red/brown ocular or nasal discharge	1	1	4
Drooping eyelids	0	0	1
Desquamation at dose site	0	13	13
Erythema at dose site	0	2	13
Day 14			
Detailed Clinical Examination – open field			
Inactive but alert	0	0	2
Tremors	0	0	1
Abnormal or flattened posture	0	0	2
Excessive defecation	0	0	1
Day 15			
Detailed Clinical Examination – open field			
Inactive but alert	0	1	1
Tremors	0	0	2
Abnormal or flattened posture	0	0	1
Detailed Clinical Examination – handling			
Drooping eyelids	1	3	0

Data taken from Tables 1 (pp. 13-16) and 3 (pp. 18-21), MRID 45540102.
[a]This group was treated for only 7 days.

2. **Mortality**: On day 6 of the study, one rat in the 5000-mg/kg/day group was found dead and another in the same group was sacrificed moribund. Clinical signs were similar to those observed in surviving rats; the cause of death was not determined.

3. **Neurological evaluations:** A neurological evaluation was not conducted in this special study.

4. **Dermal Irritation:** Desquamation and erythema at the dose site were seen in all rats in the 3000- and 5000-mg/kg/day group except desquamation was not observed in the two rats that died (5000-mg/kg/day group). Desquamation was first observed on day 6 and erythema on day 3 of both treated groups. Erythema in the 5000-mg/kg/day was observed primarily on the ventral surface of the animals during the day 7 detailed clinical examination.

B. BODY WEIGHT AND WEIGHT GAIN: Mean body weights and weight gain data are summarized in Table 3. Mean body weights for the three groups were similar on days 1, 8, and 15. During the first 7 days, rats in the 5000-mg/kg/day group gained only 35% as much weight as mineral oil controls; however, the 5000-mg/kg/day group gained significantly more weight than controls after treatment was discontinued. Controls lost weight during the second week. Body weights and weight gain by 3000-mg/kg/day group females were similar to those of controls; this group also lost weight during the second week of the study.

TABLE 3. Average body weights and body weight gain in female rats treated topically with Oil of Lemon Eucalyptus

Days on study	White Mineral oil 5000 mg/kg/day	Oil of Lemon Eucalyptus 3000 mg/kg/day	Oil of Lemon Eucalyptus 5000 mg/kg/day[a]
Body weight gain (g)			
1	248.2 ± 8.0	246.7 ± 10.0	246.5± 8.8
8	256.4 ± 9.3	252.9 ± 12.8	249.6 ± 12.3 (97)
15	250.1 ± 15.0	250.5 ± 17.1	258.2 ± 8.4 (103)
Weight gain (g)			
37263	8.2 ± 5.9	6.1 ± 11.0	2.9 ± 8.0 (35 0)
37482	-6.3 ± 13.7	-2.3 ± 10.2	8.6 ± 7.5**
37270	1.9 ± 13.0	3.8 ± 15.3	11.5 ± 6.8 (605)

Data taken from Table 4, pages 22-24, MRID 45540102.
[a]Treatment was discontinued after day 7.
[b]Numbers in parentheses are percent of control calculated by the reviewer

C. FOOD CONSUMPTION AND EFFICIENCY:

1. **Food consumption**: Food consumption was not measured in this special study.

2. **Food efficiency**: Food efficiency was not calculated in this special study.

D. OPHTHALMOSCOPIC EXAMINATION: The eyes were not examined.

E. BLOOD ANALYSES:

Blood was not drawn for either hematology of clinical chemistry evaluations.

F. URINALYSIS: Urine was not collected for analysis.

G. SACRIFICE AND PATHOLOGY:

1. Organ weight: Absolute and relative liver weights are summarized in Table 4. The terminal body weight of rats treated with 5000 mg/kg/day of the test substance was slightly greater than that of the mineral oil control. The absolute and relative (to body weight) liver weights were significantly increased by 14 and 10%, respectively, in rats treated with 5000 mg/kg/day of the test substance compared with the mineral controls. Absolute and relative liver weights at 3000 mg/kg/day were slightly greater that those of the control rats; statistical significance was not achieved.

TABLE 4. Liver weights in female rats treated topically with Oil of Lemon Eucalyptus

Parameter	White Mineral oil 5000 mg/kg/day	Oil of Lemon Eucalyptus 3000 mg/kg/day	Oil of Lemon Eucalyptus 5000 mg/kg/day
Terminal body weight (g)	250.1 ± 15.0	250.5 ± 17.1	258.2 ± 8.4 (103)[a]
Absolute liver weight (g)	10.83 ± 0.99	11.40 ± 1.39	12.30 ± 1.05** (114)
Liver to body weight ratio × 1000	43.3 ± 2.7	45.4 ± 4.0	47.6 ± 3.2** (110)

Data taken from Table 5, pages 25-27, MRID 45540102,
[a]Numbers in parentheses are percent of control calculated by the reviewer.
**$p \leq 0.01$, statistically significant, treated group compared with controls.

2. Gross pathology: The animals were not examined for gross lesions.

3. Microscopic pathology: No tissues, including liver, were excised and processed for microscopic examination.

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATOR(S)' CONCLUSIONS: The investigators concluded that questionable to mild clinical signs occurred in the 3000-mg/kg/day dose group, and clear and severe clinical signs including death were observed in the 5000-mg/kg/day group. The severity of effects prompted the investigators to discontinue the 5000-mg/kg/day treatment after day 7. The investigator considered the stringent wrapping procedure as the cause of the poor weight gain in controls and possibly exacerbated the poor weight gain by the 5000-mg/kg/day group during the first week of treatment

B. REVIEWER COMMENTS: This study was conducted to determine the dose of Oil of Lemon Eucalyptus required to produce toxicity when applied topically. Topical treatment of female rats with 5000-mg/kg/day of the test substance resulted in the death of one rat and another being sacrificed moribund 6 days after treatment started. Treatment was discontinued after the 7th day in this group. Clinical signs, some of which were indicative of neurological effects (hypoactivity, inactivity, tremors, hunched, and abnormal gait) and dermal irritation (desquamation and erythema) were noted in the 5000-mg/kg/day group. There was a reversal of clinical signs in almost all rats in the 5000-mg/kg/day after treatment was stopped. Clinical signs indicative of neurological effects were noted but were less severe in the 3000-mg/kg/day group than in the 5000-mg/kg/day group. However, signs indicative

of dermal irritation were similar at both doses. In a developmental toxicity study (MRID 45540101), maternal animals dosed with 1000 mg/kg/day Oil of Lemon Eucalyptus by gavage also exhibited neurological signs during the first few days of dosing. Although the clinical signs were transient in both studies, the reviewer considers them to be adverse.

A notable decrease in weight gain occurred in the 5000-mg/kg/day group during the first week of treatment; a rapid recovery occurred after treatment was terminated. The study author attributed the weight loss in the control and 3000-mg/kg/day groups to the wrappings. The reviewer believes that the weight loss was due to normal week-to-week fluctuations, because the rats should have adapted to the wrappings after undergoing this procedure for almost 2 weeks including the acclimation period. This study also showed that topical treatment with 5000 mg/kg/day Oil of Lemon Eucalyptus resulted in increased absolute and relative liver weights. The liver was not examined microscopically to determine if hepatocyte hypertrophy occurred as in pregnant rats administered Oil of Lemon Eucalyptus by gavage (developmental toxicity study, MRID 45540101).

In conclusion, **the LOAEL systemic and dermal effects for Oil of Lemon Eucalyptus is 3000 mg/kg/day, based on clinical signs indicative of neurological effects as well as dermal irritation at the application site. A previous 28-day dermal toxicity study showed that the NOAEL is 1000 mg/kg/day.**

The reviewer also concludes that, based on the neurological effects observed in the current study and in the developmental toxicity study (MRID 45540101), a developmental neurotoxicity should be conducted with this substance.

C. <u>STUDY DEFICIENCIES:</u> The only notable deficiency was that the test substance was applied neat and did not contain white mineral oil applied to the skin of control rats.

DATA FOR ENTRY INTO ISIS

Subchronic Dermal (28 day) Study - rodents (870.3200)

PC code	MRID	Study	Species	Duration	Route	Admin	Dose range mg/kg/day	Doses mg/kg/day	NOAEL mg/kg/day	LOAEL mg/kg/day	Target organ	Comments
11550	5e+07	Special	rat	14 days	dermal	dermal	3000-5000	5000 (mineral oil), 3000, 5000 (Oil of Lemon Eucalyptus)	NA	3000	Nervous system	Systemic
11550	5e+07	Special	rat	14 days	dermal	dermal	3000-5000	5000 (mineral oil), 3000, 5000 (Oil of Lemon Eucalyptus)	NA	3000	Skin	Dermal

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DATA EVALUATION RECORD

CITRIODIOL (OIL of LEMON EUCALYPTUS)
PC CODE 040503/011550

STUDY TYPE: DEVELOPMENTAL TOXICITY – RAT
[OPPTS 870.3700a (§83-a)]
MRID 45540101

Prepared for

Biopesticides and Pollution Prevention Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Toxicology and Hazard Assessment Group
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 108

Primary Reviewer:
K.A. Davidson, Ph.D., D.A.B.T.

Signature: _____
Date: FEB 0 7 2002

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Carol S. Forsyth, Ph.D., D.A.B.T.

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Robert H. Ross, M.S., Group Leader

Signature: _____
Date: FEB 0 7 2002

Quality Assurance:
Susan Chang, M.S.

Signature: _____
Date: FEB 0 7 2002

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Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory, managed by UT-Battelle, LLC, for the U.S. Dept. of Energy under contract DE-AC05-00OR22725

Secondary EPA Reviewer: Roger Gardner
Biopesticides and Pollution Prevention Division (7509C)

Signature: Roger Gardner
Date 4/18/02

DATA EVALUATION RECORD
TXR#:

STUDY TYPE: Prenatal Developmental Toxicity Study - Rat [OPPTS 870.3700a (§83-3a)]; OECD 414.

PC CODE: 011550 (methane-3,8-diol)
040503 (Oil of Lemon Eucalyptus)

DP BARCODE: 279872
SUBMISSION NO.:

TEST MATERIAL (PURITY): Oil of Lemon Eucalyptus (citriodiol) (65.17%, *p*-methane-3-8, diol; total *cis* and *trans* isomers)

SYNONYMS: none reported

CITATION: Trenton, N. 2001. Oral (gavage) developmental toxicity study of Oil of Lemon Eucalyptus (OLE) in rats. Argus Research, 905 Sheehy Drive, Building A, Horsham. PA 19044. Laboratory Project ID 720-006. October 30, 2001. MRID 45540101. Unpublished.

SPONSOR: WPC Brands, Inc., 1 Repel Road, P.O. Box 198, Jackson, WI 53037

EXECUTIVE SUMMARY: In a developmental toxicity study (MRID 45540101), Oil of Lemon Eucalyptus (65.17%, *p*-methan-3,8-diol, Lot No. 698058R) was administered to groups of 25 pregnant Crl:CD®(SD)IGS BR VAF/Plus® rats by gavage on gestation days (GD) 6-20 inclusive. Dose levels were 0, 100, 300, and 1000 mg/kg/day administered undiluted with the gavage volume based on daily body weights. Controls received corn oil at a dosage of 1.04 mL/kg body weight. All surviving dams were sacrificed on GD 21 for evaluation of maternal and developmental parameters. All fetuses were given external examinations; one-half the fetuses in each litter was examined for visceral abnormalities and the remaining half was processed for skeletal examination.

No deaths occurred among dams in any treated or control group. Clinical signs, which were observed during the first few days of dosing and were suggestive of a neurological effect, consisted of ataxia and impaired righting reflex in all dose groups and decreased motor activity and lost righting reflex in the high-dose group. In addition, excessive salivation and a red substance around the mouth was also noted in all dose groups. In the high-dose group, absolute body weight was 3-5% ($p \leq 0.01$ or ≤ 0.05) less and weight gain was 52% ($p \leq 0.01$) less than that of controls during the first 3 days (GD 6-9) of dosing. Compensatory weight gain (+31%, $p \leq 0.01$) was observed for the GD 9-12 interval. In addition, food consumption by the high-dose group was 27% less than that of controls during the GD 6-9 dosing interval. Postmortem examination of the dams showed treatment-related increases in absolute and relative liver weight at all doses (26 and 30%, 16 and 13%, 6 and 7% for high-, mid-, and low-dose, respectively).

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Centrilobular hepatocyte hypertrophy was observed in the livers of 14 of 15 high-dose rats examined. The livers were not examined in mid- and low-dose rats.

The maternal lowest-observed-adverse-effect level (LOAEL) for Oil of Lemon Eucalyptus is 1000 mg/kg bw/day, based on transient clinical signs suggestive of a neurological effect (ataxia and impaired righting reflex). The maternal NOAEL was established at 300 mg/kg/day.

No treatment-related adverse effects were observed for any cesarean section parameter (gravid uterine weight, number corpora lutea/dam, implantations/dam, percent pre- and post-implantation loss, total resorptions, resorptions/dam, and total number of live fetuses, mean fetal weight, and sex ratio). A statistically significant decrease was observed for the number of resorptions/dam in high-dose females. In addition, the percent pre-implantation loss, which occurred before treatment was initiated, showed a dose-related increase, whereas the post-implantation loss, which occurred after treatment was initiated, showed a dose-related decrease. A total of 379(24), 383(25), 393(25) and 367(24) fetuses(litters) in the 0-, 100-, 300-, and 1000-mg/kg/day groups were examined for external abnormalities, one-half the fetuses/litter were examined for visceral abnormalities by a microdissection technique, and one-half were processed for skeletal examination. No treatment-related external abnormalities, visceral abnormalities, or skeletal malformations/variations, including the number of ossification sites, were observed at any dose of the test material.

A developmental LOAEL could not be established for this study. The developmental NOAEL is ≥1000 mg/kg bw/day.

This developmental toxicity study in the rat is classified **Acceptable/Guideline** and satisfies the guideline requirement for a developmental toxicity study (OPPTS 870.3700; OECD 414) in the rat.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. **Test material:** Oil of Lemon Eucalyptus

Description:	Viscous, pale yellow liquid
Lot/Batch #:	698058R
Purity:	65.17 % (55-75%) *p*-methane-3-8, diol; total *cis* and *trans* isomers
Compound Stability:	At least 1 year based on expiration date of March 30, 2002
CAS #of TGAI:	Not provided
Structure:	Not available

2. **Vehicle and/or positive control:** Mazolla® Corn Oil, Lot/Batch # SEP1901A

3. **Test animals:**

Species:	Rat	
Strain:	Crl:CD®(SD)IGS BR VAF/Plus®	
Age/weight at study initiation:	Females: 70 days old, 226-250 g; Males 94 days old; 532-801 g at time of cohabitation	
Source:	Charles River Laboratories, Inc., Raliegh, NC	
Housing:	Except during cohabitation, females were housed individually in stainless-steel cages with wire bottoms,	
Diet:	Cetified Rodent Diet® #5002 (PMI Nutrition International, St. Louis, MO), *ad libitum*	
Water:	Tap water processed by reverse osmosis, *ad libitum*	
Environmental conditions:	**Temperature:**	20-22°C
	Humidity:	45.2-68.5%
	Air changes:	10/hr
	Photoperiod:	12 hours dark/12 hours light
Acclimation period:	5 days	

B. PROCEDURES AND STUDY DESIGN:

1. **In life dates**: Start: July 1, 2001; End: July 26, 2001

2. **Mating:** Mature virgin females were paired (1:1) with males of the same stock, strain, and source for up to 5 days. Mating was confirmed by the presence of a copulatory plug *in situ* and/or the presence of sperm in the vaginal smear. The study author did not state specifically, but the day evidence of mating was confirmed is assumed to be gestation day (GD) 0.

3. **Animal assignment:** Upon arrival male and female rats were assigned randomly to individual housing. Mated females were randomly assigned to each dose group based on body weights recorded on GD 0. The dose groups are listed in Table 1

TABLE 1. Animal assignment				
Dose (mg/kg bw/day)[a]	0	100	300	1000
Dose volume (mL/kg bw/day)[b]	1.04 (mineral oil)	0.10	0.31	1.04
# Females	25	25	25	25

Data taken from page 18, MRID 45540101.
[a]Test material was administered neat in the volumes and doses indicated.
[b]The dose volume was calculated based on 1000 mg = mL and dividing by the specific gravity (0.961).

4. **Dose selection rationale**: The dose levels were selected based on the results from a range-finding study using pregnant females given undiluted Oil of Lemon Eucalyptus at gavage doses of 100, 300, 650, or 1000/mg/kg/day on GD 6-20. Controls were given corn oil at a volume equal to that of the high-dose group. Liver weights were increased at all doses. Ataxia, excessive salivation, and reduced weight gain during the first few days were observed at ≥300 mg/kg/day. Feed consumption were reduced at ≥650 mg/kg/day, and decreased motor activity, impaired righting reflex, bradypnea and/or mydriasis were observed at 1000 mg/kg/day. No effects were observed on fetuses at any dose. Based on the range-finding studies, the doses for the definitive developmental toxicity study were 100, 300, and 1000 mg/kg/day.

5. **Dosage preparation and analysis**: The test material was administered undiluted as supplied by the Sponsor. The material was heated to 60°C to dissolve all crystals and maintained at 39°C with constant stirring during dosing. Analysis of homogeneity and concentration was not applicable; stability was not analyzed after heating the test material up to 60°C.

Results -

Homogeneity analysis: Not applicable

Stability analysis: Data on the stability of the test substance after heating were not found in the report

Concentration analysis: The dosage volume was calculated by converting mg to mL (1000 mg = 1mL) and dividing by the specific gravity (0.961).

Analytical of homogeneity and concentration was not applicable; the actual dosage to the animals was calculated.

6. **Dosage administration**: All doses were administered once daily by gavage, on GD 6-20. The volume administered was adjusted based on the body weight on the day of dosing. It appears that undiluted test substance was administered to the rats and the volume administered was different for each dose group. In addition, it also appears that corn oil was administered to controls only.

C. OBSERVATIONS:

1. **Maternal Observations and Evaluations**: The animals were checked twice daily for mortality throughout the study starting with the acclimation period. The rats were observed for clinical signs once daily during acclimation, once on GD 0, and twice daily from GD 6-

20. Observations were made before dosing and about 60 minutes after dosing. Body weights were recorded weekly during acclimation, on GD 0, daily on GD 6-20, and at sacrifice on GD 21. Food consumption data were recorded on GD 0, 6, 9, 12, 15, 18, 20, and 21.

All dams were sacrificed by carbon dioxide asphyxiation on GD 21 and subjected to gross examination of the thoracic, abdominal, and pelvic viscera. The liver and gross lesions were excised and retained in 10% buffered formalin, the uteri of non-pregnant rats were pressed between glass plates to confirm the absence of implantation sites, and the uteri were retained in 10% buffered formalin. The livers of 15 randomly-selected control and high-dose group females were examined microscopically. The number of corpora lutea were counted and the number and distribution of implantation sites, live and dead fetuses, and early and late resorptions were recorded.

2. **Fetal evaluations**: The fetuses were individually weighed, sexed, and examined for gross external abnormalities. One-half the fetuses in each litter were examined for visceral abnormalities using a variation of the microdissection technique of Staples, and one-half the fetuses in each litter were stained with alizarin red S for examination of skeletal abnormalities.

D. DATA ANALYSIS:

1. **Statistical analyses:** Clinical observations and other proportional data were analyzed by the Variance Test for Homogeneity of the Binomial Distribution. Maternal and fetal body weight and weight changes, feed consumption data, sex ratios, percent resorptions, and fetal abnormalities including ossification sites were analyzed using parametric tests (Bartlett's Test and Analysis of Variance (ANOVA)) followed by Dunnett's Test ($p>0.001$ for Bartlett's test) for pairwise comparisons. The Kruskal-Wallis Test was used if Bartlett's test was significant ($p \leq 0.001$) and if there were $\leq 75\%$ ties; Dunn's Method of Multiple Comparison for pairwise comparisons followed when Kruskal-Wallis Test was significant. If the number of ties was >75%, Fisher's Exact test was used. Cesarean section count data were analyzed using Kruskal-Wallis Test as described above.

2. **Indices:** The study author did not report pre- or post-implantation loss.

3. **Historical control data:** Historical control data from 13 studies conducted between June 1998 to September 1999, were provided to allow comparison with concurrent controls.

II. RESULTS:

A. MATERNAL TOXICITY:

1. **Mortality and clinical observations:** No females died during the study. Notable clinical observations are summarized in Table 2. Significantly increased incidences of clinical signs were observed in high-dose females; red perioral substance was the only clinical sign that was observed in significantly more rats in all dose groups than in the control group. High-dose females showed evidence of excessive salivation, ataxia (muscle incoordination), decreased motor activity and impaired or lost righting reflex within the first hour after dosing and during the first few days of dosing. A few rats in the 100- and 300-mg/kg/day groups also showed evidence of excessive salivation, ataxia, and impaired righting reflex, which are considered related to the test substance, because these signs were not observed in controls. Further the clinical observations suggest neurological effects associated with administration of the test material; however, the study author did not consider the observations to be toxicologically significant.

TABLE 2. Clinical observations in females administered Oil of Lemon Eucalyptus (GD 6-20)

Observations	Dose Administered (mg/kg/day)			
	0	100	300	1000
Red perioral substance	0/0[a]	29/16**	30/15**	34/17**
Excessive salivation	0/0	3/3	5/4	38/15**
Ataxia (muscle incoordination)	0/0	1/1	2/2	17/14**
Decreased motor activity	0/0	0/0	0/0	14/12**
Impaired righting reflex	0/0	2/1	2/2	14/12**
Lost righting reflex	0/0	0/0	0/0	3/3**

Data taken from Table 1, page 34, MRID 45540101.
[a]Total number of observations/number of rats with observation
**$p \leq 0.01$, statistically significant, treated group compared with controls.

2. **Body weight:** Body weight and weight gain data are summarized in Table 3. Absolute body weights of high-dose female rats were slightly but significantly reduced by 3-5% ($p \leq 0.01$ or ≤ 0.05) compared with controls on GD 7, 8, and 9, and the corrected body weight of mid-dose females was 4% ($p \leq 0.05$) greater than that of controls on GD 21. No other statistically significant differences in absolute body weights were noted for any group at any time during the study. In high-dose females, weight gain also was significantly decreased (-52%, $p \leq 0.01$) from GD 6-9, significantly greater by 31% ($p \leq 0.01$) from GD 9-12, and significantly less than that of controls from GD 18-21.

TABLE 2. Mean (±SD) maternal body weight and weight change (g)				
Gestation Day/Interval	Dose in mg/kg bw/day (# of Dams)			
	0 (25)	100 (25)	300 (25)	1000 (25)
Body Weight (g)				
GD 0	237.2 ± 6.6	237.2 ± 6.7	237.7 ± 6.5	237.5 ± 6.8
GD 6	266.3 ± 10.3	266.4 ± 10.2	266.8 ± 11.9	264.4 ± 12.5
GD 9	279.2 ± 11.4	278.2 ± 12.1	279.6 ± 12.5	270.7 ± 10.1* (97)
GD 12	295.8 ± 11.8	296.1 ± 13.8	298.4 ± 13.5	292.3 ± 11.2
GD 21	417.0 ± 25.9	415.8 ± 23.5	429.1 ± 26.1	405.3 ± 25.3
GD 21 (corrected)[a]	303.3 ± 23.7	305.1 ± 17.4	316.3 ± 16.4* (104)	298.1 ± 16.7
Weight Change (g)				
GD 0-6	29.2 ± 9.5	29.2 ± 7.2	29.1 ± 9.6	27.0 ± 9.9
GD 6-9	12.9 ± 4.7	11.8 ± 6.1	12.8 ± 4.8	6.2 ± 6.1** (48)
GD 9-12	16.5 ± 4.7	17.9 ± 5.4	18.8 ± 4.5	21.6 ± 5.0** (131)
GD 12-21[b]	121.2	119.7	130.7	113.0
GD 6-21 (corrected)	37.3 ± 18.7	38.7 ± 12.3	49.5 ± 11.2** (133)	33.7 ± 15.2
Gravid uterine weight	113.4 ± 12.6	110.7 ± 12.3	112.8 ± 14.8	107.2 ± 13.1

Data obtained from Tables 2-3 (pp. 35-37, MRID 45540101.
[a]Corrected body weight is the absolute body weight on GD 21 minus gravid uterine weight.
[b]Weight change calculated by the reviewer.
*p≤0.05, **p≤0.01, statistically significant, treated groups compared with controls.

3. **Food consumption:** When calculated as g/animal/day, food consumption by high-dose females during GD 6-9 was 27% ($p<0.01$) less than that of controls. When calculated as g/kg body weight/day, food consumption by high-dose females was 24% ($p\leq0.01$) less than that of controls during GD 6-9 and 8% greater during GD 9-12. Mid- and low-dose females consumed slightly but significantly more food than controls at sporadic intervals when calculated by either method. The effects in the low- and mid-dose groups are not considered treatment related because of the lack of a dose-related trend.

4. **Gross pathology:** No treatment-related gross lesions were reported for this study. Mean absolute and relative (% body weight) liver weights were significantly ($p\leq0.01$) elevated by 26 and 30%, respectively, in high-dose females; mid-dose weights were significantly elevated by 16 and 13%, respectively. Low-dose relative liver weight was elevated by 7%; the 6% increase for absolute liver weight did not achieve statistical significance. Microscopic examination showed minimal to mild centrilobular hepatocyte hypertrophy in the liver of 14 of 15 ($p\leq0.01$) high-dose females examined compared with none of the 15 controls. The livers of low- and mid-dose group females were not examined microscopically.

5. **Cesarean section data:** Cesarean section data are summarized in Table 4. No statistically significant differences were observed for numbers of corpora lutea/dam, implantations/dam, live fetuses/dam, mean fetal weight, or sex ratio. A decreasing dose-related trend was noted for the total number of resorptions and the total number of early resorptions. The number of resorptions/dam and the number of early resorptions/dam also showed a dose-related

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decrease that was statistically significant at the high-dose level. The percent pre-implantation loss showed a dose-related increase, whereas the percent post-implantation loss showed a dose-related decrease that did not achieve statistical significance at any dose. These data show no adverse effects as a result of treatment with the test substance.

TABLE 4 Cesarean section observations

Observation	Dose (mg/kg bw/day)			
	0	100	300	1000
# Animals Assigned	25	25	25	25
# Animals Pregnant	24	25	25	24
Pregnancy Rate (%)	96.0	100	100	96.0
# Nonpregnant	1	0	0	1
Maternal Wastage				
# Died	0	0	0	0
# Aborted	0	0	0	0
# Premature Delivery	0	0	0	0
Corpora Lutea/Dam	18.4 ± 1.9[a]	18.5 ± 2.2	19.0 ± 2.9	18.4 ± 3.2
Implantations/Dam	16.6 ± 2.0	16.0 ± 1.6	16.2 ± 1.9	15.4 ± 1.9
Total # Litters	24	25	25	24
Total # Live Fetuses Live Fetuses/Dam	379 15.8 ± 2.0	383 15.3 ± 1.7	393 15.7 ± 2.2	367 15.3 ± 2.0
Total # Dead Fetuses	0	0	0	0
Total # Resorptions	20	18	13	4
Early	19	17	13	4
Late	1	1	0	0
Resorptions/Dam	0.8 ± 0.7	0.7 ± 1.0	0.5 ± 1.0	0.2 ± 0.4**
Early	0.8 ± 0.6	0.7 ± 1.0	0.5 ± 1.0	0.2 ± 0.4**
Late	0.00 ± 0.2	0.00 ± 0.2	00.0 ± 0.00	0.00 ± 0.00
Mean Fetal Weight (g/litter)	5.18 ± 0.26	5.21 ± 0.30	5.23 ± 0.26	5.13 ± 2.27
Males (g)	5.29 ± 0.27	5.31 ± 0.34	5.36 ± 0.26	5.25 ± 0.32
Females (g)	5.06 ± 0.29	5.10 ± 0.26	5.08 ± 0.31	5.02 ± 0.26
Sex Ratio (% Male)	46.8 ± 13.0	48.8 ± 11.0	52.8 ± 15.0	48.9 ± 13.6
Preimplantation Loss (%)[b]	9.8	13.5	14.7	16.3
Postimplantation Loss (%)[b]	4.8	4.4	3.1	0.6

Data obtained from Table 9 (pp. 42-44), MRID 45540101.
[a]Mean ± standard deviation
[b]Calculated by the reviewer:
% preimplantation loss = [(mean no. corpora lutea/dam - mean no. implantations/dam)/ mean no. corpora lutea/dam] × 100;
% post-implantation loss = [(mean no. implantations/dam - mean no. live fetuses/dam)/ mean no. implantations/dam] × 100.
**$p \leq 0.01$, statistically significant, treated groups compared with the control.

B. <u>DEVELOPMENTAL TOXICITY:</u> The total number of fetuses(litters) affected with any alteration was 4(4), 5(4), 4(2), and 4(3) in the 0-, 100-, 300-, and 1000-mg/kg/day groups, respectively.

TABLE 5c. Skeletal examinations				
Observations	Dose (mg/kg bw/day)			
	0	100	300	1000
#Fetuses(litters) examined	197(24)[a]	198(25)	200(25)	190(24)
Cervical vertebrae: rib at 7th cervical vertebra	3(3)	1(1)	3(2)	3(2)
Thoracic vertebrae: centrum, bifid	0(0)	3(2)	0(0)	1(1)
Ribs: wavy	0(0)	1(1)	0(0)	0(0)

Data obtained from Table 13, pag 47, MRID 45540101.
[a]Fetal (litter) incidence

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS: The study author concluded that the treatment-related clinical observations (ataxia, decreased motor activity, and impaired or lost righting reflex) were not toxicological but pharmacological effects, because the observations were transient and did not appear to affect the well-being of the animals. Maternal body weight gain and food consumption in the high-dose group was reduced during the first 3 days of dosing and weight gain was again reduced during the last 3 days of dosing. The changes in absolute and relative liver weight and mild hepatocyte hypertrophy were considered adaptive response. The study author also noted that cesarean-section data and fetal(litter) parameters were unaffected by treatment with the test substance. Therefore, the maternal lowest-observed-adverse-effect level (LOAEL) was 1000 mg/kg/day and the maternal no-observed-adverse-effect level (NOAEL) was 300 mg/kg/day. The developmental toxicity NOAEL was >1000 mg/kg/day.

B. REVIEWER COMMENTS:

1. Maternal toxicity:

All dams administered Oil of Lemon Eucalyptus from GD 6-20 at doses up to 1000 mg/kg/day survived until sacrifice on GD 21. During the first few days of dosing, excessive salivation, ataxia, and impaired righting reflex, were observed in dams at all dose levels; decreased motor activity and lost righting reflex were observed at the high-dose only. The study author did not consider these observations to be toxicologically significant because they were transient and did not affect the well-being of the animals. Excessive salivation could be caused by irritation or taste of the test substance. The red substance around the mouths of treated rats in all groups is treatment related. The substance was not identified, but is not considered to be indicative of an adverse effect.

Other treatment-related maternal effects were transient reductions in absolute body weights, weight gain, and food consumption in the high-dose group. There effects occurred primarily during the first 3 days of dosing. There appeared to be a compensatory effect during the second 3 days (GD 9-12), when weight gain exceed that of the control group. The decrease in weight gain from GD 18-21 is considered incidental and not due to treatment with the test substance. Further, the corrected body weights at GD 21 were similar for the high-dose and

1. **External examination**: A total of 379(24), 383(25), 393(25), and 367(24) fetuses(litters) in the 0-, 100-, 300-, and 1000-mg/kg/day groups, respectively, were examined for external abnormalities. No treatment-related external findings were noted. An abnormality of the eyes was observed in one fetus in one litter in the control group. External abnormalities are summarized in Table 5a.

2. **Visceral examination:** A total of 182(24), 185(25), 193(25), and 177(24) fetuses(litters) in the 0-, 100-, 300-, and 1000-mg/kg/day groups, respectively, were examined for visceral defects. No treatment-related defects were noted. An interventricular septal defect was observed and the pulmonary artery was constricted in one fetus of one litter in the 300-mg/kg/day group. Visceral defects are summarized in Table 5b.

3. **Skeletal examination:** A total of 197(24), 198(25), 200(25), and 190(24) fetuses(litters) in the 0-, 100-, 300-, and 1000-mg/kg/day groups, respectively, were examined for skeletal abnormalities. The only findings reported were a rib at the seventh cervical vertebra, bifid centrum in the thoracic vertebra, and wavy ribs at very low incidence in one or more groups. In addition, no treatment-related effects were reported for the number of ossification sites in the fetuses(litters) at any dose level. Skeletal abnormalities are summarized in Table 5c

TABLE 5a. External examinations

Observations	Dose (mg/kg bw/day)			
	0	100	300	1000
#Fetuses(litters) examined	379(24)[a]	383(25)	393(25)	367(24)
#Fetuses(litters) affected with any alteration	4(4)	5(4)	4(2)	4(3)
Eyes: bulge, depressed, bilateral	1(1)	0(0)	0(0)	0(0)

Data obtained from Tables 10-11 (pp. 44-45), MRID 45540101.
[a]Fetal (litter) incidence

TABLE 5b. Visceral examinations

Observations	Dose (mg/kg bw/day)			
	0	100	300	1000
#Fetuses(litters) examined	182(24)[a]	185(25)	193(25)	177(24)
Heart: interventricular septal defect	0(0)	0(0)	1(1)	0(0)
Blood vessels: pulmonary artery constricted	0(0)	0(0)	1(1)	0(0)

Data obtained from Table 12, page 46, MRID 45540101.
[a] Fetal (litter) incidence

1. **External examination**: A total of 379(24), 383(25), 393(25), and 367(24) fetuses(litters) in the 0-, 100-, 300-, and 1000-mg/kg/day groups, respectively, were examined for external abnormalities. No treatment-related external findings were noted. An abnormality of the eyes was observed in one fetus in one litter in the control group. External abnormalities are summarized in Table 5a.

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Eyes: bulge, depressed, bilateral	1(1)	0(0)	0(0)	0(0)

Data obtained from Tables 10-11 (pp. 44-45), MRID 45540101.
[a]Fetal (litter) incidence

TABLE 5b. Visceral examinations

Observations	Dose (mg/kg bw/day)			
	0	100	300	1000
#Fetuses(litters) examined	182(24)[a]	185(25)	193(25)	177(24)
Heart: interventricular septal defect	0(0)	0(0)	1(1)	0(0)
Blood vessels: pulmonary artery constricted	0(0)	0(0)	1(1)	0(0)

Data obtained from Table 12, page 46, MRID 45540101.
[a] Fetal (litter) incidence

TABLE 5c. Skeletal examinations				
Observations	Dose (mg/kg bw/day)			
	0	100	300	1000
#Fetuses(litters) examined	197(24)[a]	198(25)	200(25)	190(24)
Cervical vertebrae: rib at 7th cervical vertebra	3(3)	1(1)	3(2)	3(2)
Thoracic vertebrae: centrum, bifid	0(0)	3(2)	0(0)	1(1)
Ribs: wavy	0(0)	1(1)	0(0)	0(0)

Data obtained from Table 13, pag 47, MRID 45540101.
[a]Fetal (litter) incidence

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS: The study author concluded that the treatment-related clinical observations (ataxia, decreased motor activity, and impaired or lost righting reflex) were not toxicological but pharmacological effects, because the observations were transient and did not appear to affect the well-being of the animals. Maternal body weight gain and food consumption in the high-dose group was reduced during the first 3 days of dosing and weight gain was again reduced during the last 3 days of dosing. The changes in absolute and relative liver weight and mild hepatocyte hypertrophy were considered adaptive response. The study author also noted that cesarean-section data and fetal(litter) parameters were unaffected by treatment with the test substance. Therefore, the maternal lowest-observed-adverse-effect level (LOAEL) was 1000 mg/kg/day and the maternal no-observed-adverse-effect level (NOAEL) was 300 mg/kg/day. The developmental toxicity NOAEL was >1000 mg/kg/day.

B. REVIEWER COMMENTS:

1. Maternal toxicity:

All dams administered Oil of Lemon Eucalyptus from GD 6-20 at doses up to 1000 mg/kg/day survived until sacrifice on GD 21. During the first few days of dosing, excessive salivation, ataxia, and impaired righting reflex, were observed in dams at all dose levels; decreased motor activity and lost righting reflex were observed at the high-dose only. The study author did not consider these observations to be toxicologically significant because they were transient and did not affect the well-being of the animals. Excessive salivation could be caused by irritation or taste of the test substance. The red substance around the mouths of treated rats in all groups is treatment related. The substance was not identified, but is not considered to be indicative of an adverse effect.

Other treatment-related maternal effects were transient reductions in absolute body weights, weight gain, and food consumption in the high-dose group. There effects occurred primarily during the first 3 days of dosing. There appeared to be a compensatory effect during the second 3 days (GD 9-12), when weight gain exceed that of the control group. The decrease in weight gain from GD 18-21 is considered incidental and not due to treatment with the test substance. Further, the corrected body weights at GD 21 were similar for the high-dose and

control groups. A decrease in food consumption was associated with the decrease in weight gain during the first 3 days of dosing. Although these changes are statistically significant, they are so small in relation to the overall body weights of animals in the study that they are unlikely to be of toxicological significance.

Postmortem evaluation of the dams revealed no treatment-related gross lesions, but the absolute and relative liver weights were elevated at all doses with statistical significance being achieved at the mid and high dose levels for the relative liver weights only (% body weight). Microscopic examination revealed centrilobular hepatocyte hypertrophy in the liver of high-dose group females, and these observations are not considered toxicologically significant because only relative liver weights were significantly affected. Furthermore, the only histological changes noted were an increased incidence of slight or mild hepatocellular hypertrophy.

The maternal toxicity LOAEL for Oil of Lemon Eucalyptus is 1000 mg/kg/day based on transient clinical signs suggestive of a neurological effect. The NOAEL was established at 300 mg/kg/day.

2. **Developmental toxicity:**

   a. **Deaths/resorptions:** No fetal deaths occurred at any dose level, and the total number of resorptions, total number of early resorptions, resorptions/dam, and early resorptions/dam were decreased with increasing dose. This finding is not an adverse effect. The percent pre-implantation loss was increased with increasing dose; however, the loss occurred before initiating treatment with the test substance. The percent post-implantation loss, which could be affected by treatment with the test substance, showed a decreasing dose-related trend, which is not an adverse effect.

   b. **Altered growth:** Treatment of the dams with the test substance had no effect of fetal weight or the number of ossifications sites.

   c. **Developmental variations:** No treatment-related developmental variations were observed in this study.

   d. **Malformations:** No treatment-related malformations were observed in this study.

A developmental LOAEL could not be established for this study. The developmental NOAEL is ≥1000 mg/kg bw/day.

C. **STUDY DEFICIENCIES:**

The rats were dosed with the undiluted test substance; therefore, the volume administered to each rat varied according to dose and body weight. In addition, the vehicle (white mineral oil) was not present in the test substance administered.

DATA FOR ENTRY INTO ISIS

Developmental Study - rats (870.3700a)

PC code	MRID	Study	Species	Duration	Route	Admin	Dose range mg/kg/day	Doses mg/kg/day	NOAEL mg/kg/day	LOAEL mg/kg/day	Target organ	Comments
	45540101	developmental	rats	GD 6-20	oral	gavage	100-1000	0, 100, 300, 1000	<100	100	neurological clinical signs	Maternal
	45540101	developmental	rats	GD 6-20	oral	gavage	100-1000	0, 100, 300, 1000	≥1000	not established	none	Developmental

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DATA EVALUATION RECORD

CITRIODIOL (OIL OF LEMON EUCALYPTUS)

STUDY TYPE: SPECIAL STUDY - REPEL EFFICACY
MRID 45540103

Prepared for

Biopesticides and Pollution Prevention Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Toxicology and Hazard Assessment Group
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Work Assignment No. 108

Primary Reviewer:
K.A. Davidson, Ph.D., D.A.B.T.

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Date: JAN 18 2002

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Carol Forsyth , Ph.D., D.A.B.T.

Signature: _____
Date: JAN 18 2002

Robert H. Ross, M.S., Group Leader

Signature: Robert H. Ross
Date: JAN 18 2002

Quality Assurance:
Lee Ann Wilson, M.A.

Signature: L. A. Wilson
Date: JAN 18 2002

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory, managed by UT-Battelle, LLC, for the U.S. Dept. of Energy under contract DE-AC05-00OR22725

Biopesticides and Pollution Prevention Division (7509C) **Date**_____
EPA Secondary Reviewer: Roger Gardner **Signature:** Roger Gardner
Biopesticides and Pollution Prevention Division (7509C) **Date** 4/18/02

DATA EVALUATION RECORD

STUDY TYPE: Special Study - Repel Efficacy

PC CODE:

DP BARCODE: D279876/000305-LI
SUBMISSION NO.:

TEST MATERIAL (PURITY): Oil of Lemon Eucalyptus (100%); 40% by weight in formulation

SYNONYMS:

CITATION: Bestari, K. 2001. Determination of active ingredient transfer factor for repel essential aerosol and pump spray products to arms and legs under simulated use conditions. Centre for Toxicology, University of Guelph, Guelph, Ontario N1G 2W1, Canada. Laboratory study No. 2001-CT-WPC. November 7, 2001. MRID 45540103. Unpublished.

SPONSOR: WPC Brands, 1 Repel Road, P.O. Box 198, Jackson, WI 53037

EXECUTIVE SUMMARY:

Two proposed commercial products, Repel Essential Aerosol (Lot # 01043001) and Repel Essential Pump Spray (424011), were tested for the transfer of the active ingredients, Oil of Lemon Eucalyptus (OLE) (100%, a.i., Lot # 199078R) to the skin under simulated conditions. The products contained 40% OLE. The products were sprayed on a substrate consisting of cotton with aluminum foil backing attached to the arm or leg. The aerosol or pump spray was applied to the substrate for 4 sec (aerosol to arm), 6 sec (pump spray to arm), 9 sec (aerosol to leg), or 15 sec (pump spray to leg). The transfer of active ingredients under these condition was 31%, 42%, 41%, and 42%, respectively.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. **Test Material:** Oil of Lemon Eucalyptus (OLE) in Repel Essential Aerosol and Pump Spray

Description:	
Lot/Batch #:	Oil of lemon eucalyptus: 199078R; Repel Essential Aerosol: 01043001; Repel Essential Pump Spray: 424011
Purity:	100% (40% a.i. in formulation)
Compound Stability:	~1 year based on receipt and expiration dates
CAS # for TGAI:	
Vehicle/Solvent used:	none used (formulation includes propellant and inert ingredients)
Control formulations:	Repel Essential Aerosol (inert ingredients + propellant): 01043002; Propellant only:01043003; Repel Essential Pump Spray (inert ingredients only): 424012

2. **Relevance of Test Material to Proposed Formulation(s):**

Oil of Lemon Eucalyptus is the active ingredient in Repel Essential Aerosol and Repel Essential Pump Spray. The purpose of this study is to determine the transfer of the active ingredient to the arms and legs under conditions that simulate normal usage.

B. STUDY DESIGN

1. Protocol

The Repel Essential Aerosol or Pump Spray was sprayed on the arm from wrist to upper arms or the leg from ankle to mid thigh that had been covered with a substrate consisting of cotton material (100% unbleached T-shirt material) backed with aluminum foil and attached with tape. The spray was applied for 4 seconds (aerosol to arm), 6 seconds (pump spray to arm), 9 seconds (aerosol to leg), or 15 seconds (pump spray to leg) at a distance of 6-8 inches in an outdoors area protected from the wind. Immediately after spraying, the cotton material, aluminum foil, and tape were removed from the arm or leg and placed in a fume hood for 5 hours to allow evaporation of the propellant and volatile inert ingredients. The total amount of substance sprayed on the arm or leg was determined by weighing the canister before and after spraying. The theoretical amount of active ingredient sprayed was calculated based on its fractional amount in the product. The actual amount of active ingredient sprayed on the arm or leg was determined by weighing the substrate before application, immediately after application, and after the 5- hour evaporation period and then correcting for the amount of active ingredient evaporated during the 5-hour and the fraction of propellant and inert residues remaining after evaporation. The weight of the substrate was also corrected for fluctuations in weight gain/loss during the experiment (control blank). The fraction of OLE loss during evaporation was determined by applying a thin layer of the active ingredient to a glass substrate and measuring (weight difference) the amount of residue remaining after 5 hours at room temperature. Each experiment was conducted in triplicate.

2. Calculations

Correction Factor (A) = amount (g) of a.i. after 5-hour evaporation/amount of a.i. at time 0

Amount of propellant & inert ingredient residues (B) = average amount (g) - average control blank

Total product residue (C) = amount of total product residue after evaporation - average control blank

Transfer factor (%) = ((C-B)A/weight of a.i. sprayed) × 100

II. RESULTS

The results for determining the repel efficacy of the aerosol and pump spray to the arms and legs are summarized in Table 1. The results of this study show that 30.7% to 41.9% of the active ingredient sprayed from the canister is transferred during use under simulated conditions.

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS:

The investigators noted that information regarding the transfer of active ingredient following spray application of insect repellants was lacking. Therefore, they investigated this issue for two proposed commercial products containing OLE in a aerosol and a pump spray delivery system. They found that approximately 31% of the active ingredient in the aerosol reaches the arms, whereas about 41% of the active ingredient in the aerosol reaches the legs and 42% in the pump spray reaches the arms and legs under the conditions of this study.

B. REVIEWER COMMENTS:

Repel Essential Aerosol and Repel Essential Pump Spray contain OLE as the active ingredient. When sprayed as an aerosol on the arm 31% of the active ingredient in the aerosol reached the arm and 41% of the OLE in the aerosol reached the legs and 42% of the OLE in the pump spray reached the arms and legs. The residue remaining on the substrate used in the study was adjusted for propellant and/or inert ingredients, evaporation of active ingredient, change in weight of the substrate. The test to determine how much active ingredient would evaporate during the 5-hour period was not conducted inside a fume hood, but in an open room and it may have been different from that of a fume hood. There was considerable agreement in the pretreatment weight of the substrate, suggesting very small source of variability in the results. There was some variability due to the fraction of residue remaining after the 5-hour evaporation period. In spite of the sources of variability, the overall results were consistent and showed that 31-42% of the active ingredient in the formulation can be transferred to the skin under the conditions of this study.

OIL OF LEMON EUCALYPTUS

TABLE 1. Transfer factor for Repel Essential Aerosol or Pump Spray

Study	Spray time (seconds)	Total product sprayed from canister (g)[a]	Amt. a.i. sprayed from canister (g)[b]	Total product residue (g)[c] (C)	Correction factor[d] (B)	Correction factor[e] (A)	Transfer factor[f] (%)
Repel Essential Aerosol sprayed to the arm	4	5.2 ± 0.2	2.1 ± 0.1	0.6764 ± 0.2244	0.0949	0.9237	30.7 ± 12.8
Repel Essential Pump Spray to the arm	6	1.6 ± 0.2	0.6 ± 0.1	0.2212 ± 0.0686	-0.0216	0.9237	41.6 ± 9.3
Repel Essential aerosol sprayed on the leg	9	13.1 ± 0.6	5.2 ± 0.2	2.2839 ± 0.2452	0.2925	0.9237	41.3 ± 6.2
Repel Essential Pump Spray to the leg	15	4.1 ± 0.5	1.6 ± 0.2	0.6570 ± 0.1080	0.0257	0.9237	41.9 ± 4.2

Data taken from pages 21, 24, 27, and 30, MRID 4550103.
[a]Weight difference of canister before and after application.
[b]Amount of active ingredient applied (total product applied × % active ingredient (40%)).
[c]Amount of product on the substrate after 5 hours evaporation less the control blank
[d]Adjustment for inert residues remaining after the 5-hour evaporation period less the control blank.
[e]Correction factor for evaporation of active ingredient during the 5-hour evaporation period.
[f]Transfer factor (%) = ([(C-B)/A]/Amount a.i. sprayed from canister) × 100.