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

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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Risk Assessment and Mammalian Toxicology Reviews for the Registration of a New Technical Product, Granola 97, (EPA File Symbol 004822-UOO) Containing the New Active Ingredient, p-Menthane-3,8-diol (Chemical No. 011550; DP Barcode D243976; Submission S538748)

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ACTION REQUESTED

S.C. Johnson & Son, Inc. submitted mammalian toxicity studies to BPPD, to support the registration of Granola 97, a technical pesticide product which contains the new active ingredient, p-Menthane-3,8-diol (99.3%; file symbol 004822-UOO). Attached to this memorandum are the human health risk assessment and the data evaluation reports for this pesticide.

Attachments

I. EXECUTIVE SUMMARY

The Biopesticides and Pollution Prevention Division (BPPD) has reviewed data submitted by S.C. Johnson & Son, Inc. to assess potential hazards and exposures that might result from the proposed use of a technical grade product, containing the new active ingredient, p-Menthane-3,8-diol, to be formulated into pesticide products that can be applied to human skin and clothing to repel mosquitos, black flies, and biting flies. Based on the review of submitted information, dose levels and toxicity endpoints were evaluated to characterize potential risks.

The technical grade active ingredient, p-Menthane-3,8-diol, is placed into Toxicity Category IV for acute oral toxicity, dermal toxicity and skin irritation, and Toxicity Category I for eye irritation. It was not a skin sensitizer. The no-observed-effect level (NOEL) from a 90-day dermal toxicity study in rats was established at a limit dose of 1000 mg/kg/day. The NOEL for immune suppression, as determined in a 28-day dermal study, via a primary antibody response to sheep red blood cells/plaque forming cell assay was > 3000 mg/kg/day in mice. The NOEL for maternal and developmental toxicity was established in rabbits at 3000 mg/kg/day by the dermal route. Mutagenicity studies evaluated p-Menthane-3,8-diol for its potential to cause point mutations in bacteria and mammalian cells, chromosomal aberrations in mammalian cells, and induction of micronuclei in polychromatic erythrocytes from mouse bone marrow, and found no genotoxicity at the doses tested, with and without metabolic activation.

Based on the evaluation of the submitted data, which is required for registration, there were no endpoints of concern identified which could be used in a risk assessment. Thus, there is reasonable certainty of no harm to the U.S. population or subpopulations, including infants and children, from the labeled use of the technical grade active ingredient. However, as end-use skin repellent products are developed and submitted for registration, further analysis of potential risks from exposure to this pesticide will be necessary. At that time, BPPD will consider the possible effects of the other ingredients and the dilution factor of the active ingredient in those formulations, and a determination of an appropriate safety factor for risk characterization will be made.

II. OVERVIEW

A. Chemical Overview

The technical grade active ingredient, p-Menthane-3,8-diol, is a new biochemical pesticide that is approximately 99% pure. This is to be formulated into insect repellents for use against mosquitos, deer ticks, body lice and biting flies.

B. Regulatory History (John Tice will fill this in).

III. SCIENCE ASSESSMENT

A. Physical and Chemical Properties

Information discussed in this section was reported in MRID 444387-12 and 13, and MRID 444893-01.

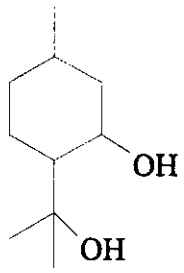
1. Chemical Identity

p-Menthane-3,8-diol (Pesticide Chemical Code 011550) is a colorless liquid. The chemical name includes the following synonyms:

p-Methane-3,8-diol
Cyclohexanemethanol: 2-hydroxy-alpha, alpha, 4-trimethyl

The CAS Registry number for p-Menthane-3,8-diol: 42822-86-6

The structural formula of p-Menthane-3,8-diol:



The molecular formula for p-Menthane-3,8-diol: $C_{10}H_{20}O_2$

2. Physical and Chemical Properties of p-Menthane-3,8-diol

TABLE 1.

Color	Opaque white
Physical State	Solid, specific observation temperature not noted
Odor	Faint mint
Melting Point	34.5°C
Boiling Point/Range	Not required; solid at room temperature
Density	0.989 g/mL at 24°C
Solubility	0.29 g/L at 25°C

Vapor Pressure	0.181 Pa, determined by gas saturation method
Dissociation Constant in Water	Not reported; product is not dispersible in water
Octanol/Water Partition Coefficient	Not required, intended use pattern not an environmental fate concern
pH	Not applicable; not dispersible with water
Stability	Stable to sunlight, heat (54°C), metal (iron, aluminum), and metal ions (iron II acetate, aluminum acetate)
Oxidizing/Reducing Action	Not discussed
Flammability	Flash point 139.8°C
Storage stability	Not applicable, product is a technical grade active ingredient
Viscosity	56.1 cP at 60°C
Miscibility	Not applicable, product not intended for dilution with petroleum solvents
Corrosion characteristics	Not applicable, product is a technical grade active ingredient

B. Human Risk Assessment

1. Hazard Assessment

There are sufficient data available to support a hazard assessment of p-Menthane-3,8-diol.

a. Acute Toxicity Studies

Five acute toxicity studies were submitted to support the registration of p-Menthane-3,8-diol. All studies were acceptable, and the results are listed in Table 2, below, and summarized as follows:

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TABLE 2.

Study	MRID No.	Results	Toxicity Category
Acute Oral-rat	444387-01	LD_{50}>5000 mg/kg	IV
Acute Dermal-rabbit	444387-02	LD_{50}>5000 mg/kg	IV
Acute Inhalation		Waived*	
Eye Irritation-rabbit	444387-03	Corrosive	I (unwashed eyes; II washed eyes)
Skin Irritation-rabbit	444387-04	Slight irritant	IV
Skin Sensitization-guinea pig (Modified Buehler)	444387-05	Not a sensitizer	N/A

* Acute inhalation toxicity data requirement is waived due to lack of exposure via this route.

1. **Acute Oral Toxicity (MRID 44387-01).** In an acute oral toxicity study, 5 groups of 5 female rats were dosed with 500, 875, 1250, 2000 and 5000 mg/kg body weight, and 1 group of 5 male rats were dosed at 5000 mg/kg. The primary clinical signs of toxicity were decreased activity in 1 female (500 mg/kg group) and in all 5 females in the 1250 mg/kg group, and reduced/no feces were observed in 2 rats in this dose group. Wobbly gait was observed in 5 female rats at 1250 mg/kg. Other clinical effects noted in the 3 other dose groups included breathing abnormalities, prostration, apparent hypothermia, hunched posture, urine stain, ocular discharge, decreased food consumption, and/or dark material around the facial area. All rats had normal body weight gains except 1 female in the 1250 mg/kg group. Two females in the 2000 mg/kg group and 2 females in the 5000 mg/kg group died by day 3 of the studies; none of the male rats died during the study. The timing of these observations was not specified, so it cannot be determined if these were immediate or delayed effects. In addition, at necropsy, the only notable effects were observed in the animals that died early in the study, and included: abnormal contents in the digestive tract, reddened mucosa of the stomach, dilated pelvis of the kidney, pale liver, blackish-purple spleen, distended ureters, and dark red thymus.

2. **Acute Dermal Toxicity (MRID 444387-02).** In this study, 5 male and 5 female NZW rabbits were tested at 5000 mg/kg. All rats survived and gained weight. Transient dark material around the mouth was observed in one animal. Significant dermal irritation was noted at the site of test material application, which included erythema, edema, dermal lesions, eschar, necrosis, desquamation and blanching, to varying degrees in all animals tested. No significant changes were observed at necropsy. Three incidences of cysts on the oviducts were observed; however, these findings were not considered to be related to the test material

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application, as they are "commonly found in rabbits of this strain," according to the study author.

3. **Primary Eye Irritation (MRID 444387-03).** Nine male NZW rabbits (5 males, 4 females) were treated with 0.1 mL of p-Menthane-3,8-diol. The treated eyes of 3 (1 male, 2 females) of the animals were rinsed with physiological saline approximately 30 seconds after instillation of the test material. All rabbits exhibited corneal opacity, iritis, and conjunctival irritation 1 hour after test material instillation, which persisted through 72 hours. In the group with unwashed eyes, corneal opacity persisted in 4 rabbits through day 7, and in 1 rabbit until the study was terminated at 28 days post instillation. In the rabbits with the washed eyes, corneal opacity and iritis was cleared by day 7. Conjunctival redness above normal level was observed in 2 animals until day 10. The persistence of significant corneal damage in the unwashed group for 28 days places the test substance in toxicity category I (corrosive) for eye irritation. **The product label should clearly emphasize the hazard and first aid treatment for accidental eye exposure.**

4. **Primary Skin Irritation (MRID 444387-04).** Six NZW rabbits (3 per sex) were treated dermally with 0.5 mL of the undiluted test substance, and the test site was covered with a semi-occlusive dressing for 4 hours. After the exposure period, the patches were removed and residual test material was wiped from the exposed skin with gauze moistened with distilled water. One hour after patch removal, very slight erythema and well-defined erythema were noted on 4/6 and 2/6 rabbits, respectively. The erythema cleared by 72 hours on 4/6 rabbits; the remaining 2 animals exhibited barely perceptible erythema at this time point. By day 7, no traces of irritation was observed in these animals.

5. **Dermal Sensitization (MRID 444387-05).** p-Menthane-3,8-diol was evaluated for dermal sensitization potential using a modified Buehler method. For the induction phase, 0.3 mL of the undiluted test material was applied to the shaved backs of 10 male and 10 female Hartley-derived albino guinea pigs under occlusion for 6 hours once each week for 3 weeks. The animals were left untreated for 2 weeks before challenge. The animals were challenged with 0.3 mL of the undiluted test material under occlusion at naive sites for 6 hours. A naive control group consisting of 5 male and 5 female guinea pigs was treated with 0.3 mL of the undiluted test material at challenge only. Reactions were scored at 24 and 48 hours post exposure.

The test animals demonstrated little (slight, patchy) or no erythema after each induction and challenge dose. The naive control animals also exhibited little or no erythema after the challenge dose. The results of this test were compared to historical positive controls, in which 10 animals/sex were treated with DNCB as a contact sensitizer (all animals exhibited slight-to-moderate, confluent erythema

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following challenge) within 6 months of the present study. The test substance did not cause contact sensitization under the conditions of this study.

b. **Immunotoxicity: 28-day Dermal Study (MRID 444387-09)**. In a dermal immunotoxicity study, female $B_6C_3F_1$ mice (10/dose) were exposed to undiluted p-Menthane-3,8-diol (a.i. 98.3%) at doses of 0.0, 1000, and 3000 mg/kg once per day for 28 days. Parameters tested were total body weight gains, weekly food consumption, absolute and relative spleen and thymus weights, and antibody plaque forming cell assay.

No mortality or clinically related signs of toxicity were observed. Mice exposed to p-Menthane-3,8-diol showed no statistically significant changes in body weight, or relative and absolute spleen and thymus weight compared with controls. Mice from both 1000 and 3000 mg/kg/day dosage groups did show statistically increased food consumption (17% and 16%, respectively) on day 21 but not on days 7, 14, or 28.

There are some problems interpreting the results of the plaque forming cell assay performed in this study. Exposure to 1000 mg/kg p-Menthane-3,8-diol resulted in a statistically significant 43% increase in antibody plaque forming cells/10^6 viable spleen cells. Total antibody plaque forming cells/spleen was increased 44% in the low dose group, but the enhancement was not statistically significant. Mice exposed to 3000 mg/kg showed no enhancement of either plaque forming cells/10^6 viable spleen cells or total plaque forming cells. Neither treatment group showed statistically significant changes in total number of viable cells per spleen and there were no differences in absolute and relative spleen and thymus weights in either test group.

The enhancement of the primary antibody response to sheep red blood cells in the low dose but not the high dose group, coupled with only two doses being tested, makes the LOEL appear to be lower than the NOEL. In the immune system, antigens can induce tolerance if injected in sufficiently large and frequent doses over a long enough period of time. Thus, one possible interpretation of the results observed in this study is that immune tolerance to sheep red blood cells was achieved at 3000 mg/kg/day.

However, for the purposes of hazard identification, the NOEL should be considered to be 3000 mg/kg/day. The reason for this is that since the plaque forming cell assay is currently only considered to be sufficiently validated as a test for immune *suppression*, and no suppression of immune response occurred at a limit dose of 1000 mg/kg/day, the stimulatory effect noted at 1000 mg/kg/day (a limit dose) is not considered to be an endpoint of concern. Thus, repeating the plaque forming cell assay at lower doses is not suggested for the purposes of risk assessment and registration of this technical pesticide product. It is advisable,

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however, to assess any formulations which include p-Menthane-3,8-diol for effects on the immune system.

This immunotoxicity dermal exposure study is classified as supplementary, as it does not meet the guideline requirements of §152-18. The study only partially fulfills the requirements outlined in the guideline, since only one immunologic parameter, humoral immune function measured by an antibody plaque forming cell assay, was tested. The study cannot be upgraded without the completion of the other studies included in that guideline. However, for the purposes of this risk assessment and the registration of p-Menthane-3,8-diol, further immunotoxicity testing is not required. The reasons for this are as follows: 1) the substance is a technical grade active ingredient, which will ultimately be incorporated into repellents for use on the skin and clothing; 2) no dermal sensitization was observed in a modified Buehler assay in guinea pigs (MRID 444387-05); 3) no effects on absolute and relative spleen and thymus weights, which are valid endpoints for immune suppression, occurred in the 28-day study nor in a 90-day dermal toxicity study (MRID 444387-10); and 4) the results of the 28-day immunotoxicity test indicated that no suppression of the primary antibody response to sheep red blood cells at a limit dose and higher. Thus, there is reasonable certainty that further immunotoxicity testing would not likely change the low level of concern for this endpoint.

c. **Subchronic (90-Day) Dermal Toxicity Study (MRID 444387-10).** In a 90-day subchronic dermal toxicity study, groups of 15 male and female Sprague-Dawley rats were treated with p-Menthane-3,8-diol (98.3%) at doses of 0, 1000 or 3000 mg/kg/day for 6 hours per day.

Decreased body weight (-8% day 36; -9% day 43, $p \leq 0.05$) and body weight gain (-30% days 29-36, $p \leq 0.05$) were observed in the high dose males. Low dose males displayed decreased (-71% days 64-71, $p \leq 0.05$) and increased (+260% days 71-78, $p \leq 0.01$) body weight gain. No other effects on body weight were observed.

Barely perceptible erythema and desquamation was reported in all low dose male and female animals. In addition, a number of high dose male and female animals displayed well-defined erythema (23% male, 33% female), slight edema (8% male, 0% female) and pinpoint to moderate eschar (77% male, 40% female). Dermal findings in the control group were limited to one female with desquamation.

Treatment-related microscopic lesions were observed in the kidneys from high dose males and in treated skin from high dose males and females. Hyaline droplets, likely due to alpha-$_{2u}$-globulin inclusions, were seen in kidneys of control males (20%, minimal to mild) and high dose males (100%, 73% moderate). Minimal acanthosis was observed in 53% of control males' treated skin, while minimal to

mild acanthosis was seen in 93% of high dose males. Chronic inflammation was observed in male control (20%, minimal to mild), female control (13%, minimal), and high dose male (100%, 67% mild) and female (100%, 60% mild) animals. In addition, parakeratosis was seen in 7% of high dose males and 27% of high dose females.

Statistically significant increased absolute liver weight (+18%, $p \leq 0.001$) and relative liver weight (+15%, $p \leq 0.001$) were observed in high dose females. Relative liver weight (+9%, $p \leq 0.05$), relative kidney weight (+12%, $p \leq 0.001$), and relative adrenal weight (+15%, $p \leq 0.05$) were increased in high dose males. There were no statistical differences noted for low dose male or female animals. No treatment-related effects were observed with regard to hematology, clinical chemistry, neurotoxicity, or ophthalmology.

Based on the data presented in this study, the NOEL is 1000 mg/kg/day; the LOEL is 3000 mg/kg/day. The LOEL is based on dermal observations in treated skin (increased erythema, edema and eschar) and histological observations in treated skin (increased acanthosis and inflammation). **This subchronic dermal toxicity study in rats is classified as acceptable.**

d. **Developmental Toxicity Study (MRID 444387-11)**. 25 pregnant Sprague-Dawley Crl:CD®BR rats per group were administered p-Menthane-3,8-diol (SCJ NB# 14735R108) (98.5% a.i., batch number 703002) by dermal application at doses of 0, 1, and 3 g/kg/day on gestation days (GD) 6-19, inclusive. All animals survived to scheduled sacrifice, and no treatment-related clinical signs of toxicity were observed during the study. The skin at the application site of treated animals also did not show signs of irritation. No statistically significant differences in absolute body weights occurred between the treated and control groups during the study, but slightly decreased body weight gains (91% of controls; $p \leq 0.05$ during gestation days 6-20) and similar decreases in food consumption (90%of controls; $p \leq 0.01$ during gestation days 6-9) were observed at the highest dose tested. At all other times during the study, food consumption and body weight gains by the treated groups were comparable to the controls. No abnormalities were noted at maternal necropsy. **Therefore, the maternal toxicity NOEL is >3 g/kg/day and the maternal toxicity LOEL was not identified.**

No dose- or treatment-related statistically significant effects on pregnancy rate, number of corpora lutea, pre- or postimplantation losses, resorptions/dam, fetuses/litter, fetal body weights, or fetal sex ratios were observed in the treated groups as compared to the controls. Two low-dose dams had complete litter resorption. No treatment-related external, visceral, or skeletal malformations/variations were observed in any litter. The number of litters in the 0, 1, and 3 g/kg/day groups containing fetuses with major malformations was 1/23,

2/21, and 1/22, respectively. All treated and control litters contained fetuses with minor variations in skeletal ossification. **Therefore, the developmental toxicity NOEL is >3 g/kg/day and the developmental toxicity LOEL was not identified. The study is acceptable.**

e. **Reproduction Toxicity**. Reproduction studies are not required (as a Tier 1 study) to support registration of biochemical pesticides. However, this information would be useful in the risk characterization of any end-use products, for determining an appropriate FQPA safety factor for infants and children. Without this study, and with only one developmental study in one species, it is possible that a ten-fold uncertainty factor will be applied to formulations containing p-Menthane-3,8-diol as the active ingredient.

f. **Mutagenicity Studies**. Four acceptable studies were conducted to evaluate the genotoxic potential of p-Menthane-3,8-diol (98.3% a.i.) including a bacterial gene mutation assay (OPPTS 870.5100), an *in vitro* mammalian cell gene mutation assay (OPPTS 870.5300), an *in vitro* chromosomal aberration test (OPPTS 870.5), and a mammalian erythrocyte micronucleus test (OPPTS 870.5395). These studies satisfy the Tier I requirements for genotoxicity data (40 CFR, §158.690(c)).

1. **Reverse gene mutation assay (Ames Test; MRID 444878-01).** Strains TA98, TA100, TA1535 and TA1537 of *Salmonella typhimurium* and strain WP2(uvrA) of *Escherichia coli* were exposed to p-Menthane-3,8-diol (Batch No. 703001, 98.3% a.i.) in DMSO at concentrations of 25 (WP2(uvrA) only), 75, 200, 600, 1800, and 5000 µg/plate (limit concentration) in the presence and absence of mammalian metabolic activation (S9-mix). Some reduction in the number of revertants per plate was seen both with and without S9-mix at 1000 µg/plate and higher concentrations in WP2(uvrA). The mean number of revertants per plate at 1000, 3333 and 5000 µg/plate in this strain was reduced, compared to the solvent control value of 21 both with and without S9-mix, to 8, 5, and 3, respectively, with S9-mix and to 7, 8 and 4, respectively, without S9-mix. **There was no evidence of induced mutant colonies over background.**

2. **Mammalian cell gene mutation assay at the thymidine kinase locus (MRID 444387-06).** L5178Y/TK$^{\pm}$ cells cultured *in vitro* were exposed to p-Menthane-3,8-diol (98.3% a.i., batch No. 703001) in DMSO at concentrations of 600, 800, 1000, 1250, 1500 and 2000 µg/mL in the absence of mammalian metabolic activation (S9-mix) and to concentrations of 500, 600, 800, 1000, 1250 and 1500 µg/mL in the presence of S9-mix. The 2000 µg/mL and 1500 µg/mL doses were too toxic to clone in the absence and presence of S9-mix, respectively, but no visible precipitate was seen in the treatment medium at any dose level. **There was no evidence of induced mutant colonies over background.**

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3. **Mammalian cell chromosomal aberration cytogenetics assay (MRID 444387-08).** Chinese hamster ovary CHO-K1 cell cultures were exposed to p-Menthane-3,8-diol (98.3% a.i., batch No. 703001) in DMSO in two independent assays. In the initial assay, concentrations of 50, 150, 500 and 1500 µg/mL, with and without metabolic activation (S9-mix), were evaluated following a 6 hour treatment and a 14 hour recovery period. In the repeat assay without S9-mix, concentrations of 250, 500, 1000 and 1500 µg/mL were evaluated after 20 hours continuous treatment and concentrations of 125, 250, 500 and 1000 µg/mL were evaluated after 44 hours of continuous treatment. In the repeat assay with S9-mix, concentrations of 250, 500, 1000 and 1500 µg/mL were evaluated after 6 hours treatment and either a 14 hour or 38 hour recovery period. **There was no evidence in the results of the two assays that chromosomal aberrations were increased by the test material.**

4. **Mouse Micronucleus Assay (MRID 444387-07).** In an ICR mouse bone marrow micronucleus assay, five mice/sex/dose were treated once i.p. with p-Menthane-3,8-diol in corn oil (98.3% a.i., batch No. 703001) at doses of 104, 208, 416 mg/kg or dermally over four days with 3 mL/kg total of neat agent. Bone marrow cells were harvested at 24 hours (all doses) and at 48 hours (416 mg/kg only) post-treatment. All mice in the 208 and 416 mg/kg groups were lethargic following treatment. Convulsions and prostration were also seen in all mice in the 416 mg/kg group. Seven of 15 males and 7/15 females in the 416 mg/kg group displayed piloerection. All mice in the dermal application group showed both hyperactivity and lethargy after treatment. **There was no significant increase in the frequency of micronucleated polychromatic erythrocytes in bone marrow at any dose, harvest time or route of exposure.**

f. **Metabolism Studies**. Not required (as a Tier I study) for the registration of biochemical pesticides.

2. Dose Response Assessment

a. **Endpoint Selection**: The endpoints, no-observed-effect levels (NOEL) and lowest-observed-effect levels (LOEL) are summarized from the submitted toxicological data as follows:

TABLE 3.

Toxicity Profile of p-Menthane-3,8-diol (98.3%)			
Study Type	**MRID No.**	**Results**	**Coregrade**
Immunotoxicity - mouse	444387-09	NOEL > 3000 mg/kg/day (HDT); LOEL not established	Supplementary
Subchronic Toxicity, Dermal - Rat	444387-10	NOEL = 1000 mg/kg/day; LOEL = 3000 mg/kg/day (increased skin erythema, edema and eschar)	Acceptable
Developmental Toxicity - Rat	444387-11	Maternal NOEL > 3 g/kg/day (HDT); LOEL not established. Developmental: NOEL > 3 g/kg/day (HDT), LOEL not established	Acceptable
Gene Mutation - *S. typhimurium/E. Coli* (WP2(uvrA))	444878-01	Non-mutagenic ± activation	Acceptable
Mouse Lymphoma	444387-06	Non-mutagenic ± activation	Acceptable
Micronucleus Assay	444387-07	Non-mutagenic ± activation	Acceptable
Chromosomal Aberration-CHO-K1 cells	444387-08	Non-mutagenic ± activation	Acceptable

b. Dermal Absorption: Not required as a Tier I study for biochemical pesticide registration. However, this information would be very useful for risk assessment purposes and determination of the MOEs for any end-use products that use p-Menthane-3,8-diol as the active ingredient, since these types of products would be repeatedly applied directly to the skin. Without this data, dermal absorption would be assumed to be 100%; of course, the effects of the other ingredients in a formulation, as well as the dilution factor of the active ingredient, would be considered in characterizing risk.

3. Exposure Assessment

a. Dietary exposure

There are no food uses proposed for p-Menthane-3,8-diol, so acute and chronic dietary risk assessments are not required.

b. Occupational and Residential Exposure

No occupational estimates are made in this assessment since p-Menthane-3,8-diol is a technical grade active ingredient, and is to be used to formulate insect repellents.

c. Aggregate exposure

The technical grade active ingredient, p-Menthane-3,8-diol, will be used to formulate pesticide products that can be applied to human skin and clothing to repel mosquitos, black flies, and biting flies. Based on this use pattern and the hazard assessment described above, an assessment of aggregate exposure has not been conducted for p-Menthane-3,8-diol.

4. Risk Characterization

a. Sensitivity of infants and children

There is only one developmental toxicity study (in one species) required as a Tier I study for the registration of biochemical pesticides. The study submitted for the registration of p-Menthane-3,8-diol was performed in the rabbit. This study indicated that there was no difference in sensitivity to p-Menthane-3,8-diol between rabbit fetuses and their mothers with respect to the dermal route of exposure. However, with only one developmental study, and no reproduction study, it is likely that in subsequent risk characterization of any formulations using p-Menthane-3,8-diol as the active ingredient, a ten-fold safety factor may be applied when characterizing risk (see discussion under 5. c. Determination of safety (U.S. Population, infants and children), below).

b. Non-occupational risk characterization

Margins of exposure (MOE) were not calculated. There was no level (endpoints) of concern identified from the submitted data, and since p-Menthane-3,8-diol is a technical grade product, non-occupational exposure is not expected.. At such time end-use skin repellent products containing this active ingredient are submitted to the Agency for registration, risk will be characterized based upon the other ingredients as well as the dilution of the active ingredient within the formulation (see discussion under 5. c. Determination of safety (U.S. Population, infants and children), below).

5. Other Food Quality Protection Act Considerations

a. Cumulative risk from exposure to substances with a common mechanism of toxicity

Section 408(b)(2)(D)(v) of the Food Quality Protection Act requires that, when considering whether to establish, modify, or revoke a tolerance, the Agency consider "available information" concerning the cumulative effects of a particular pesticide's residues and "other substances that have a common mechanism of toxicity." The Agency believes that "available information" in this context might include not only toxicity, chemistry, and exposure data, but also scientific policies and methodologies for understanding common mechanisms of toxicity and conducting cumulative risk assessments.

For most pesticides, although the Agency has some information in its files that may turn out to be helpful eventually determining whether a pesticide shares a common mechanism of toxicity with any other substances, EPA does not at this time have methodologies to resolve the complex scientific issues concerning common mechanisms of toxicity in a meaningful way. EPA has begun a pilot process to study this issue further through the examination of particular classes of pesticides. The Agency hopes that the results of this pilot process will increase the Agency's scientific understanding of this question such that EPA will be able to develop and apply scientific principles for better determining which chemicals have a common mechanism of toxicity and evaluating the cumulative effects of such chemicals. The Agency anticipates, however, that even as its understanding of the science of common mechanisms increases, decisions on specific classes of chemicals will be heavily dependent on chemical specific data, much of which may not be presently available.

Although at present the Agency does not know how to apply the information in its files concerning common mechanism issues to most risk assessments, there are pesticides as to which the common mechanism issues can be resolved. These substances include pesticides that are toxicologically dissimilar to existing chemicals (in which case the Agency can conclude that it is unlikely that a pesticide shares a common mechanism of activity with other substances) and pesticides that produce a common toxic metabolite (in which case common mechanism of activity will be assumed).

In this registration, the active ingredient, p-Menthane-3,8-diol, is a technical product which will be used to formulate insect repellents to be applied to human skin and clothing. Its activity as an insect repellent is considered to be nonspecific and as a repellent the biochemical is considered to have a non-toxic mechanism of

action. The non-toxic mechanism of activity, when used as an insect repellent, precludes attempting a cumulative risk assessment for this biochemical pesticide.

b. Endocrine disrupter effects

EPA is required to develop a screening program to determine whether certain substances (including all pesticides and inerts) "may have an effect in humans that is similar to an effect produced by a naturally occurring estrogen, or such other endocrine effect..." The Agency is currently working with interested stakeholders, including other government agencies, public interest groups, and industry and research scientists in developing a screening and testing program and a priority setting scheme to implement this program. Congress has allowed 3 years from the passage of FQPA (August 3, 1999) to implement this program. At that time, EPA may require further testing of p-Menthane-3,8-diol for endocrine effects.

c. Determination of safety (U.S. Population, infants and children)

Based on the evaluation of the submitted information, no endpoints of concern were identified from the studies required for registration of the technical grade active ingredient, p-Menthane-3,8-diol, which could be used in a risk assessment. Thus, there is reasonable certainty of no harm to the U.S. population or subpopulations, including infants and children, as the result of the uses of p-Menthane-3,8-diol to formulate repellents.

However, at such time as an end-use product is submitted to the Agency for registration, the potential effects of other ingredients and the dilution factor for the active ingredient within the formulation would have to be considered in the risk characterization. The reason for this is that even though there are no food uses for this pesticide, it is intended for direct application to the skin, including infants and children, and therefore, FQPA considerations apply. In this analysis, the appropriate safety factor will be determined, which by OPP policy includes a ten-fold uncertainty factor to be applied for infants and children unless appropriate data are available to its justify removal. For example, the chemical pesticides that OPP regulates require a database which includes two developmental studies (in different species) and a two-generation reproduction study; based on the results of those studies, a decision is made to remove or retain the ten-fold safety factor. However, the requirements for a biochemical pesticide registration include only one developmental study (in one species), and there are usually no data available which demonstrates whether young animals are differentially affected upon exposure to that pesticide. Therefore, the ten-fold FQPA safety factor would be probably be retained for biochemical pesticides, although the magnitude of exposure (e.g., if exposure is equivalent to 1/1000th of the appropriate NOEL) would be considered in this decision.

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DATA EVALUATION REPORT

P-MENTHANE-3,8-DIOL
(GRANOLA 97)

STUDY TYPES: ACUTE ORAL TOXICITY - RAT (81-1)
ACUTE DERMAL TOXICITY - RABBIT (81-2)
RIMARY EYE IRRITATION - RABBIT (81-4)
PRIMARY DERMAL IRRITATION - RABBIT (81-5)
DERMAL SENSITIZATION - GUINEA PIG (81-6)

SUMMARY: ACUTE TOXICITY ONE-LINERS (81-1, 81-2, 81-4 through 81-6)

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

Chemical Hazard Evaluation Group
Toxicology and Risk Analysis Section
Life Sciences Division
Oak Ridge National Laboratory
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Primary Reviewer:
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Date: 7-21-98

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Quality Assurance:
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Date: JUL 20 1998

Disclaimer

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

Oak Ridge National Laboratory, managed by Lockheed Martin Energy Research Corp. for the U.S. Department of Energy under contract number DE-AC05-96OR22464.

DATA REVIEW FOR ACUTE ORAL TOXICITY TESTING (§152-10; 870.1100)

EPA Reviewer: Sheryl K. Reilly, Ph.D. Sheryl K Reilly **Date** 1-25-99
Biopesticides and Pollution Prevention Division (7511C)

Study Title: An Acute Oral Toxicity Study of Granola 97 in Rats
MRID No.: 444387-01
File Jacket Symbol: 4822-UOO
DP Barcode: D243976
Study No.: 3068.64
Study Completion Date: October 16, 1997

Sponsor: S.C. Johnson & Son, Inc., Racine, WI 53403-2236
Testing Facility: Springborn Laboratories, Inc., Spencerville, OH 45887
Author: Bonnette, K.L.

Quality Assurance (40 CFR §160.12): Included

Test Material: Granola 97 (99% p-Menthane-3,8-diol); Lot 703001; clear viscous liquid
Species: Sprague-Dawley Crl:CD®BR VAF/Plus® Rats
Age: Males: Approximately 8 weeks; Females: approximately 9-13 weeks
Weight (prefasted): Males: 247-262 g; Females: 202-222 g
Source: Charles River Laboratories, Inc., Portage, MI

Conclusions:

1. **LD_{50} (mg/kg):** **> 5000 mg/kg**
   Males: > 5000 mg/kg
   Females: = 5308.4 mg/kg (95% C.L. 2325.7-12116.5 mg/kg)
   Combined: > 5000 mg/kg
2. **Tox. Category: IV**
3. **Classification: Acceptable**

Procedural Deviations from Subdivision M Guideline §152-10: None

Results:

Dosage (mg/kg)	Number of Deaths/Number Tested		
	Males	Females	Combined
500	--	0/5	-
875	--	0/5	-
1250	--	0/5	-
2000	--	2/5	-
5000	0/5	2/5	2/10

Observations: In an acute oral toxicity study, 5 groups of 5 female rats were dosed with 500, 875, 1250, 2000 and 5000 mg/kg body weight, and 1 group of 5 male rats were dosed at 5000 mg/kg. The primary clinical signs of toxicity were decreased activity in 1 female (500 mg/kg group) and all 5 females in the 1250 mg/kg group, and reduced/no feces were observed in 2 rats in this dose group. Wobbly gait was observed in 5 female rats at 1250 mg/kg. Other clinical effects noted in the 3 other dose groups included breathing abnormalities, prostration, apparent

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hypothermia, hunched posture, urine stain, ocular discharge, decreased food consumption, and/or dark material around the facial area. All rats had normal body weight gains except 1 female in the 1250 mg/kg group. Two females in the 2000 mg/kg group and 2 females in the 5000 mg/kg group died by day 3 of the studies; none of the male rats died during the study. The timing of these observations was not specified, so it cannot be determined if these were immediate or delayed effects.

Gross Necropsy: The most notable gross internal findings were observed in the animals that died and included abnormal contents in the digestive tract, reddened mucosa of the stomach, dilated pelvis of the kidney, pale liver, blackish-purple spleen, distended ureters, and dark red thymus. Gross necropsy was normal on the surviving rats.

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DATA REVIEW FOR ACUTE DERMAL TOXICITY TESTING (§152-11; 870.1200)

EPA Reviewer: Sheryl K. Reilly, Ph.D. Sheryl K Reilly **Date** 1-25-99
Biopesticides and Pollution Prevention Division (7511C)

Study Title: An Acute Dermal Toxicity Study of Granola 97 in Rabbits
MRID No.: 444387-02
File Jacket Symbol: 4822-UOO
DP Barcode: D243976
Study No.: 3068.65
Study Completion Date: October 16, 1997

Sponsor: S.C. Johnson & Son, Inc., Racine, WI 53403-2236
Testing Facility: Springborn Laboratories, Inc., Spencerville, OH 45887
Author: Bonnette, K. L.

Quality Assurance (40 CFR §160.12): Included

Test Material: Granola 97 (99% p-Menthane-3,8-diol); Lot 703001; clear viscous liquid
Species: New Zealand White Rabbits
Age: Approximately 12 weeks
Weight: Males: 2.6-2.7 kg; Females: 2.5-2.7 kg
Source: Myrtle's Rabbitry, Thompson Station, TN

Conclusion:

1. **Estimated LD_{50}** **> 5000 mg/kg**
   Males: > 5000 mg/kg
   Females: > 5000 mg/kg
   Combined: > 5000 mg/kg
2. **Tox. Category: IV**
3. **Classification: Acceptable**

Procedural Deviations from Subdivision M Guideline §152-11: None

Results:

Dosage (mg/kg)	Number of Deaths/Number Tested		
	Males	Females	Combined
5000	0/5	0/5	0/10

Observations: In this study, 5 male and 5 female NZW rabbits were tested at 5000 mg/kg. All rats survived and gained weight. Transient dark material around the mouth was observed in one animal. Significant dermal irritation was noted at the site of test material application, which included erythema, edema, dermal lesions, eschar, necrosis, desquamation and blanching, to varying degrees in all animals tested.

Gross Necropsy: No significant changes were observed at necropsy. Three incidences of cysts on the oviducts were observed; however, these findings are not considered to be related to the test material application, as they are "commonly found in rabbits of this strain," according to the study author.

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DATA REVIEW FOR PRIMARY EYE IRRITATION TESTING (§152-13, 870.2400)

EPA Reviewer: Sheryl K. Reilly, Ph.D. Sheryl K Reilly **Date** 1-25-99
Biopesticides and Pollution Prevention Division (7511C)

Study Title: A Primary Eye Irritation Study of Granola 97 in Rabbits
MRID No.: 444387-03
File Jacket Symbol: 4822-UOO
DP Barcode: D243976
Study No.: 3068.66
Study Completion Date: October 16, 1997

Sponsor: S.C. Johnson & Son, Inc., Racine, WI 53403-2236
Testing Facility: Springborn Laboratories, Inc., Spencerville, OH 45887
Author: Bonnette, K.L.

Quality Assurance (40 CFR §160.12): Included

Test Material: Granola 97 (99% p-Menthane-3,8-diol); Lot 703001; clear viscous liquid
Dosage: 0.1 mL
Species: New Zealand White Rabbits
Age: Males: Approximately 12 weeks; Females: Approximately 11-12 weeks
Weight: Males: 2.5-2.7 kg; Females: 2.5-2.7 kg

Source: Myrtle's Rabbitry, Thompson Station, TN

Conclusion:

1. **Toxicity Category: I (Corrosive)**
2. **Classification: Acceptable**

Procedural Deviations from Subdivision M Guideline §152-13: None

Observations	Number "positive"/number tested								
	Hours				Days				
	1	24	48	72	7	10	14	21	28
	Unwashed eyes*								
Corneal Opacity	6/6	6/6	6/6	6/6	4/6	1/6	1/6	1/6	1/6
Iritis	6/6	6/6	6/6	6/6	2/6	1/6	1/6	1/6	1/6
Conjunctivae:									
Redness	4/6	6/6	5/6	3/6	2/6	1/6	1/6	0/6	0/6
Chemosis	6/6	1/6	1/6	1/6	1/6	1/6	1/6	0/6	0/6
Discharge	6/6	1/6	0/6	0/6	1/6	0/6	1/6	0/6	0/6

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Observations	Number "positive"/number tested								
	Hours				Days				
	1	24	48	72	7	10	14	21	28
	Washed eyes*								
Corneal Opacity	3/3	3/3	3/3	3/3	0/3	0/3	0/3	-	-
Iritis	3/3	3/3	3/3	2/3	0/3	0/3	0/3	-	-
Conjunctivae:									
Redness	3/3	3/3	3/3	3/3	3/3	2/3	0/3	-	-
Chemosis	3/3	3/3	3/3	3/3	3/3	0/3	0/3	-	-
Discharge	3/3	2/3	0/3	2/3	0/3	0/3	0/3	-	-

*** Approximately 30 seconds after instillation of the test material, the treated eyes of 3 rabbits were washed with physiological saline.**

Summary: Nine male NZW rabbits (5 males, 4 females) were treated with 0.1 ml of Granola 97. The treated eyes of 3 (1 male, 2 females) of the animals were rinsed with physiological saline approximately 30 seconds after instillation of the test material. All rabbits exhibited corneal opacity, iritis, and conjunctival irritation 1 hour after test material instillation, which persisted through 72 hours. In the group with unwashed eyes, corneal opacity persisted in 4 rabbits through day 7, and in 1 rabbit until the study was terminated at 28 days post instillation. In the rabbits with the washed eyes, corneal opacity and iritis was cleared by day 7. Conjunctival redness above normal level was observed in 2 animals until day 10. The persistence of significant corneal damage in the unwashed group for 28 days places the test substance in toxicity category I (corrosive) for eye irritation. The product label should clearly emphasize the hazard and first aid treatment for accidental eye exposure.

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DATA REVIEW FOR PRIMARY DERMAL IRRITATION TESTING (§152-14, 870.2500)

EPA Reviewer: Sheryl K. Reilly, Ph.D. Sheryl K Reilly **Date** 1-25-99
Biopesticides and Pollution Prevention Division (7511C)

Study Title: A Primary Skin Irritation Study of Granola 97 in Rabbits
MRID No.: 444387-04
File Jacket Symbol: 4822-UOO
DP Barcode: D243976
Study No.: 3068.67
Study Completion Date: October 16, 1997

Sponsor: S.C. Johnson & Son, Inc., Racine, WI 53403-2236
Testing Facility: Springborn Laboratories, Inc., Spencerville, OH 45887
Author: Bonnette, K.L.

Quality Assurance (40 CFR §160.12): Included

Test Material: Granola 97 (99% p-Menthane-3,8-diol); Lot 703001; clear viscous liquid
Dosage: 0.5 mL
Species: New Zealand White Rabbits
Age: Approximately 11 weeks
Weight: Males: 2.2-2.3 kg; Females: 2.4 kg
Source: Myrtle's Rabbitry, Thompson Station, TN

Conclusion:

1. **Toxicity Category: IV (Mild or Slight irritant)**
2. **Classification**: **Acceptable**

Procedural Deviations from Subdivision M Guideline §152-14: None

Results: Six NZW rabbits (3 per sex) were treated dermally with 0.5 mL of the undiluted test substance, and the test site was covered with a semi-occlusive dressing for 4 hours. After the exposure period, the patches were removed and residual test material was wiped from the exposed skin with gauze moistened with distilled water. One hour after patch removal, very slight erythema and well-defined erythema were noted on 4/6 and 2/6 rabbits, respectively. The erythema cleared by 72 hours on 4/6 rabbits; the remaining 2 animals exhibited barely perceptible erythema at this time point. By day 7, no traces of irritation was observed in these animals. The PDIS = 1.1 (Slight irritant).

DATA REVIEW FOR DERMAL SENSITIZATION STUDY (§152-15; 870.2600)

EPA Reviewer: Sheryl K. Reilly, Ph.D. Date 1-25-99
Biopesticides and Pollution Prevention Division (7511C)

Study Title: A Dermal Sensitization Study of Granola 97 in Guinea Pigs, Modified Buehler Design
MRID No.: 444387-05
File Jacket Symbol: 4822-UOO
DP Barcode: D243976
Study No.: 3068.68
Study Completion Date: October 16, 1997

Sponsor: S.C. Johnson & Son, Inc., Racine, WI 53403-2236
Testing Facility: Springborn Laboratories, Inc., Spencerville, OH 45887
Author: Bonnette, K.L.

Quality Assurance (40 CFR §160.12): Included

Test Material: Granola 97 (99% p-Menthane-3,8-diol); Lot 703001; clear viscous liquid
Positive Control Material: 1-Chloro-2,4-dinitrobenzene (DNCB)
Species: Hartley Albino Guinea pigs
Age: Approximately 7 weeks
Weight: Males: 383-499 g; Females: 356-452 g
Source: Harlan Sprague Dawley, Inc., Haslett, MI
Method: Modified Buehler Method

Conclusion:

1. **There is no indication that this product is a dermal sensitizer in guinea pigs.**
2. **Classification**: **Acceptable**

Procedural Deviations from §152-15: None

Procedure: Granola 97 was evaluated for dermal sensitization potential using a modified Buehler method. For the induction phase, 0.3 mL of the undiluted test material was applied to the shaved backs of 10 male and 10 female Hartley-derived albino guinea pigs under occlusion for 6 hours once each week for 3 weeks. The animals were left untreated for 2 weeks before challenge. The animals were challenged with 0.3 mL of the undiluted test material under occlusion at naive sites for 6 hours. A naive control group consisting of 5 male and 5 female guinea pigs was treated with 0.3 mL of the undiluted test material at challenge only. Reactions were scored at 24 and 48 hours post exposure.

Results: The test animals demonstrated little (slight, patchy) or no erythema after each induction and challenge dose. The naive control animals also exhibited little or no erythema after the challenge dose. The results of this test were compared to historical positive controls, in which 10 animals/sex were treated with DNCB as a contact sensitizer (all animals exhibited slight-to-moderate, confluent erythema following challenge) within 6 months of the present study. The test substance did not cause contact sensitization under the conditions of this study.

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ACUTE TOX ONE-LINERS

1. **REGISTRATION NO.:** 4822-UOO
2. **PC CODE:** 011550
3. **TEST MATERIAL:** Granola 97 (99% p-Menthane-3,8-diol); Lot 703001; clear viscous liquid

Study/Species/Lab Study #/Date	MRID	Results	Tox. Cat.	Core Grade
Acute oral toxicity rat/Springborn Laboratories, Inc. 3068.64/10-16-97	444387-01	LD_{50} > 5000 mg/kg	IV	A
Acute dermal toxicity rabbit/Springborn Laboratories, Inc. 3068.65/10-16-97	444387-02	LD_{50} > 5000 mg/kg	IV	A
Primary eye irritation rabbit/Springborn Laboratories, Inc. 3068.66/10-16-97	444378-03	Corrosive; All rabbits had corneal opacity, iritis, and conjunctival irritation one hour after test material instillation. **Unwashed eyes:** Corneal opacity and iritis persisted in one rabbit through the end of the study. Conjunctival redness, chemosis, and discharge were resolved by day 21. **Washed eyes:** Corneal opacity and iritis cleared by day 7. Conjunctival redness above normal level was observed in 2 animals until day 10.	I	A
Primary dermal irritation rabbit/Springborn Laboratories, Inc. 3068.67 /10-16-97	444387-04	Slight irritant; very slight erythema and well defined erythema on 4/6 and 2/6 rabbits at 1 hour that cleared by 72 hours on 4/6 rabbits and by day 7 on 2/6 rabbits.	IV	A
Dermal sensitization guinea pig/Springborn Laboratories, Inc. 3068.68/10-16-97	444387-05	Not a sensitizer (Modified Buehler)	--	A

Core Grade Key: **A =Acceptable, S = Supplementary, U = Unacceptable, V = Self Validated**

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DATA EVALUATION REPORT

GRANOLA 97 (p-MENTHANE-3,8-DIOL)

STUDY TYPE: IMMUNOTOXICITY - DERMAL EXPOSURE - MICE (§152-18)

Prepared for

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

Prepared by

Chemical Hazard Evaluation Group
Toxicology and Risk Assessment Section
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Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 22

Primary Reviewer: Melissa D. Halpern, Ph.D.	Signature: Date:	Robert H. Ross 4-13-98
Secondary Reviewers: H. T. Borges, Ph.D., MT(ASCP), D.A.B.T	Signature: Date:	H T Borges 4-13-98
Robert H. Ross, M.S., Group Leader	Signature: Date:	Robert H. Ross 4-13-98
Quality Assurance: LeeAnn Wilson, M.S.	Signature: Date:	L. A. Wilson 4-13-98

Disclaimer

This Data Evaluation Report may have been altered by the Biopesticides and Pollution Prevention Division subsequent to signing by Oak Ridge National Laboratory personnel.

Oak Ridge National Laboratory, managed by Lockheed Martin Energy Research Corp. for the U.S. Department of Energy under contract number DE-AC05-96OR22464

DATA REVIEW FOR IMMUNOTOXICITY TESTING (Subdivision M §152-18; OPPTS 880.3550)

EPA Reviewer: Sheryl K. Reilly, Ph.D. Sheryl K Reilly, **Date** 1-25-99
Biological and Pollution Prevention Division (7511C)

Study Title: Immunotoxicity Screening Study in Mice Exposed Dermally to Granola 97
MRID No.: 444387-09
File Jacket Symbol: 4822-UOO
DP Barcode: D243976
Submission: S538748
P.C. Code: 004822
Study No.: 1
Project No.: L08686SN1
Study Completion Date: October 17, 1997

Sponsor: SC Johnson, 1525 Howe Street, Racine, WI, 53403
Testing Facility: IIT Research Institute, Life Sciences Research, 10 West 35th Street, Chicago, IL 60616
Authors: R.V. House, W.D. Johnson, and J.F. Krueger

Quality Assurance (40 CFR 160.12): Included

EXECUTIVE SUMMARY: In a dermal immunotoxicity study, female $B_6C_3F_1$ mice (10/dose) were exposed to undiluted p-Menthane-3,8-diol (a.i. 98.3%) at doses of 0.0, 1000, and 3000 mg/kg once per day for 28 days. Parameters tested were total body weight gains, weekly food consumption, absolute and relative spleen and thymus weights, and antibody plaque forming cell assay.

No mortality or clinically related signs of toxicity were observed. Mice exposed to p-Menthane-3,8-diol showed no statistically significant changes in body weight, or relative and absolute spleen and thymus weight compared with controls. Mice from both 1000 and 3000 mg/kg/day dosage groups did show statistically increased food consumption (17% and 16%, respectively) on day 21 but not on days 7, 14, or 28.

There are some problems interpreting the results of the plaque forming cell assay performed in this study. Exposure to 1000 mg/kg p-Menthane-3,8-diol resulted in a statistically significant 43% increase in antibody plaque forming cells/10^6 viable spleen cells. Total antibody plaque forming cells/spleen was increased 44% in the low dose group, but the enhancement was not statistically significant. Mice exposed to 3000 mg/kg showed no enhancement of either plaque forming cells/10^6 viable spleen cells or total plaque forming cells. Neither treatment group showed statistically significant changes in total number of viable cells per spleen and there were no differences in absolute and relative spleen and thymus weights in either test group.

The enhancement of the primary antibody response to sheep red blood cells in the low dose but not the high dose group, coupled with only two doses being tested, makes the LOEL appear to be lower than the NOEL. In the immune system, antigens can induce tolerance if injected in sufficiently large and frequent doses over a long enough period of time. Thus, one possible interpretation of the results observed in this study is that immune tolerance to sheep red blood cells was achieved at 3000 mg/kg/day.

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However, for the purposes of hazard identification, the NOEL should be considered to be 3000 mg/kg/day. The reason for this is that since the plaque forming cell assay is currently only considered to be sufficiently validated as a test for immune *suppression*, and no suppression of immune response occurred at a limit dose of 1000 mg/kg/day, the stimulatory effect noted at 1000 mg/kg/day (a limit dose) is not considered to be an endpoint of concern. Thus, repeating the plaque forming cell assay at lower doses is not suggested for the purposes of risk assessment and registration of this technical pesticide product. It is advisable, however, to assess any formulations which include p-Menthane-3,8-diol for effects on the immune system.

This immunotoxicity dermal exposure study is classified as supplementary, as it does not meet the guideline requirements of §152-18. The study only partially fulfills the requirements outlined in the guideline, since only one immunologic parameter, humoral immune function measured by an antibody plaque forming cell assay, was tested. The study cannot be upgraded without the completion of the other studies included in that guideline. However, for the purposes of this risk assessment and the registration of p-Menthane-3,8-diol, further immunotoxicity testing is not required. The reasons for this are as follows: 1) the substance is a technical grade active ingredient, which will ultimately be incorporated into repellents for use on the skin and clothing; 2) no dermal sensitization was observed in a modified Buehler assay in guinea pigs (MRID 444387-05); 3) no effects on absolute and relative spleen and thymus weights, which are valid endpoints for immune suppression, occurred in the 28-day study nor in a 90-day dermal toxicity study (MRID 444387-10); and 4) the results of the 28-day immunotoxicity test indicated that no suppression of the primary antibody response to sheep red blood cells at a limit dose and higher. Thus, there is reasonable certainty that further immunotoxicity testing would not likely change the low level of concern for this endpoint.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Granola 97 (p-menthane-3,8-diol)

   Description: white crystalline solid
   Lot/Batch #: 703001
   Purity: 98.3%
   Stability of compound: stable under ambient conditions

2. Positive control: cyclophosphamide diluted in sterile saline.

3. Test animals

   Species: mouse, $B_6C_3F_1$ (female)
   Age and weight at study initiation: 6 weeks; 16.5 - 18.8 g
   Source: Charles River Laboratories Inc., Portage, MI
   Housing: Singly in stainless steel wire mesh cages
   Diet: Purina Rodent Chow #5002 *ad libitum*
   Water: tap water *ad libitum*
   Environmental conditions:
   Temperature: 20 - 24°C
   Humidity: 32 - 74%
   Air changes: unknown

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Photoperiod: 12-hour light/dark cycle
Acclimation period: 1 week

B. STUDY DESIGN

1. In life dates: Start: 7/24/97; end: 8/21/97

2. Animal assignment: Animals were assigned to treatment groups using a computerized randomization process constrained by body weight. Mice were individually identified by ear punch and assigned to one of four groups (Table 1).

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TABLE 1. Study design[a]			
Test group	**Dose to animals (mg/kg/day)**	**Treatment duration (days)**	**Number of animals**
Sham control[b]	0	28	10
Low	1000	28	10
High	3000	28	10
Positive control[c]	0	1	10

[a]Data taken from p. 10 (MRID 44438709.)
[b]Dosed with 3000 mg/kg distilled water.
[c]Received a single dose of 80 mg/kg cyclophosphamide 24 hours prior to euthanasia and assay.

3. Dose selection rationale: Not stated.

4. Test substance preparation and analysis: Test substance sufficient for the days dosing was dispensed and warmed slightly to liquefy. These aliquots were kept on a hot plate in the animal room to maintain the liquid state. Tests for homogeneity and stability were not included.

5. Dose application: The dorsal scapular fur of all mice (including positive controls) was clipped prior to initial dosing. Shaving was repeated weekly during the test period. Correcting for density, the liquefied test substance was administered at 1.036 and 3.109 mL/kg of body weight. Sham control mice received a daily dermal application of 3.109 mL/kg distilled water. Both test substance and water were administered with an automated pipettor and were allowed to spread according to their own physical properties. Positive control mice received no dermal treatments but received a single IP dose of 80 mg/kg cyclophosphamide in sterile saline on day 28. None of the treatment sites were covered after application of the test substance.

6. Statistics: Terminal body weight, spleen and thymus weight, cell viability, and antibody plaque forming assay data were log- or logit-transformed. Group data were assessed using ANOVA and Dunnett's test.

C. METHODS

1. Observations: All mice were observed for mortality and morbidity twice daily on weekdays and once daily on weekends. Mice were observed weekly for adverse clinical signs.

2. Body weight: Random mice were weighed the day following receipt. All mice were weighed on Day 0 and weekly thereafter for the duration of the study. In addition, all mice were weighed prior to euthanasia and these weights were used to determine organ-to-body weight ratios.

3. Food consumption: Food consumption was recorded weekly.

4. Sacrifice and pathology: Mice were sacrificed by CO_2 asphyxiation. All animals survived until scheduled termination of the study.

a.. Gross necropsy: The spleen and thymus from each animal were removed and weighed.

b. Tissue preparation/histopathology: No organs or tissues were preserved.

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c. Cell viability: Spleen cell viability was determined via trypan blue exclusion. Neither thymus nor bone marrow cell viability was assessed.

5. Primary Antibody Response (Plaque forming cell assay): On day 25, all mice were injected i.v. with 4 x 10^7 washed sheep red blood cells (SRBC) suspended in Dulbecco's phosphate-buffered saline. Four days post immunization, mice were euthanized and the spleen and thymus were aseptically removed and weighed. Single cell suspensions were prepared by rubbing the spleens through nylon mesh, triturating through a 23-gauge needle and allowing cellular debris to settle. Spleen cells were suspended in RPMI-1640 media supplemented with Hepes buffer, 10 μg/mL gentamicin, and 2 mM L-glutamine. Spleen cell preparations were diluted 1/30 and 1/120 in RPMI. Aliquots from each dilution were added to tubes containing Bacto-agar/DEAE-dextran, washed SRBC and guinea pig complement. The tubes were mixed and the mixtures poured into petri dishes. A glass cover slip was placed on each mixture to form a homogeneous monolayer. The plates were incubated in 5% CO_2 at 37 °C and 95% humidity for 3 hours. The resulting antibody forming cells were enumerated using a plaque counter. Plaques were counted, multiplied by the dilution factor, and the cell suspension volume to determine antibody plaque forming cells/spleen.

II. RESULTS

A. OBSERVATIONS

1. Clinical signs of toxicity: No clinical signs of toxicity were observed in this study. Alopecia was observed on one sham control mouse during weeks 2-4.

2. Mortality: No deaths occurred during the study.

B. BODY WEIGHT AND WEIGHT GAIN: No effect on body weight (Table 2) or body weight gain (Table 3) was observed for any treatment group.

TABLE 2. Mean body weights (g) of mice exposed dermally to Granola 97[a]

Day of study	Treatment group (mg/kg Granola 97)			
	Sham control	Low dose 1000	High dose 3000	Positive control
0	17.4	17.4	17.3	17.3
7	19.3	19.0	19.4	18.8
14	20.1	19.8	20.1	20.0
21	20.5	20.5	20.5	20.5
28	21.1	21.3	21.6	21.0

[a]Data taken from Table 2, p. 18; MRID 44438709

TABLE 3. Mean weekly body weight gain (g) of mice exposed dermally to Granola 97[a]				
Day of study	Treatment group (mg/kg Granola 97)			
	Sham control	Low dose 1000	High dose 3000	Positive control
7	1.9	1.7	2.1	1.5
14	0.8	0.8	0.7	1.2
21	0.4	0.7	0.4	0.4
28	0.6	0.7	1.1	0.5
28	21.1	21.3	21.6	21.0

[a]Data taken from Table 3, p. 19 (MRID 44438709)

C. FOOD CONSUMPTION: Mean weekly food consumption was statistically increased in both the low and high dose treatment groups on day 21 (Table 4). There were no differences at other time points tested.

TABLE 4. Mean food consumption of mice exposed dermally to Granola97[a]				
Day of study	Treatment group (mg/kg Granola 97)			
	Sham control	Low dose 1000	High dose 3000	Positive control
7	70	74	71	65
14	84	99	96	90
21	86	101*	100*	96
28	95	104	105	97

[a]Data taken from Table 4,p. 20; MRID 44438709
*$p \leq 0.05$

D. BLOOD WORK

1. Hematology: Not performed.

2. Clinical chemistry: Not performed.

E. GROSS NECROPSY

1. Organ weights: There were no observed effects on relative or absolute spleen and thymus weight in either treatment group (Table 5). The statistically significant decrease observed in the positive control group was consistent with cyclophosphamide treatment.

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TABLE 3. Absolute and relative organ weights in mice exposed dermally to Granola 97[a]				
Day of study	Treatment group (mg/kg Granola 97)			
	Final body weight (g)	Absolute thymus weight (g)	Absolute spleen weight	Relative spleen weight
Sham control	21.7	0.050	0.092	0.42
Low dose	21.9	0.044	0.093	0.42
High dose	22.2	0.042	0.089	0.42
Postive control	22.1	0.040	0.077*	0.35*

[a] Data taken from Table 5, p. 21 (MRID 44438709)
* $p \leq 0.05$

2. Histology: Not performed.

F. IMMUNOTOXICITY TESTS

1. Plaque forming cell assays

The number of antibody plaque forming cells (APFC)/10^6 viable spleen cells were statistically increased 43% in the low dose group relative to sham controls (Table 6). There were no statistically significant changes observed in APFC/10^6 viable cells in the high dose group. APFC/spleen was increased in the low dose group 44%, but the increase was not statistically significant. Viable cells/spleen was unchanged in both low and high dose groups. The decreases observed in viable cells/spleen, APFC/spleen and APFC/10^6 viable cells in the positive control group were consistent with cyclophosphamide treatment.

TABLE 3. Immunologic parameters after dermal exposures to Granola 97				
Test	Treatment (mg/kg)			
	Sham control	Low dose 1000	High dose 3000	Positive control
viable cells/spleen ($\times 10^7$)	6.5 (1.1)[b]	6.5 (1.5)	6.0 (1.1)	5.0* (6.1)
APFC/spleen	115380 (37935)	165780 (61658)	101250 (31511)	2970* (1590)
APFC/10^6 viable cells	1735 (369)	2489* (571)	1683 (425)	60* (33)

[a] Data taken from Table 5, p. 22; MRID 44438709.
[b] Data shown as the mean (SD).
* $p \leq 0.05$

III. DISCUSSION AND STUDY DEFICIENCIES

A. DISCUSSION: The data presented in this study show that the body weight and absolute or relative spleen and thymus weight of $B_6C_3F_1$ mice dermally exposed to Granola 97 for 28 days at 1000 and 3000 mg/kg was not affected by treatment. Food consumption was statistically increased in both treatment groups on day 21, but remained consistent with sham controls at all other time points tested. No treatment related clinical abnormalities were observed.

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Mice treated with the higher dose of Granola 97 showed no changes in humoral immune response measured by antibody plaque forming cells directed against sheep red blood cells. However, mice treated with the lower dose had a 44% increase of antibody plaque forming cells/spleen and a statistically significant 43% increase of antibody plaque forming cells/10^6 viable cells.

The authors of this study concluded that the enhanced immune response observed in the low dose group is not likely biologically significant since no enhancement was observed in the high dose group. However, immunomodulatory compounds are known for their capricious nature, and can have surprisingly different effects over a range of doses. Consequently, they do not always display a traditional dose response. It would not be without precedent for immune enhancement to be observed at one dose with immune suppression at another. Because this study observed the effects of only two doses of Granola 97, it is unclear if a more conventional dose response would be seen if mice were exposed to doses lower than 1000 mg/kg and doses intermediate between 1000 and 3000 mg/kg.

There are a number of major problems associated with this study and the results are difficult to intepret. The enhancement of the primary antibody response to sheep red blood cells in the low dose but not the high dose group, coupled with only two doses being tested, makes a determination of the LOEL to be lower than the NOEL. However, the effect seen at 1000 mg/kg/day was significant, and thus for the purposes of this study, the LOEL should be considered to be 1000 mg/kg/day (enhanced primary antibody response to sheep red blood cells), and a NOEL was not established.

B. <u>STUDY DEFICIENCIES</u>

1. No rationale for dose selection was stated.

2. Only 2 doses of test substance were studied (not a typical dose-response study).

3. Treatment areas were not covered after application of the test substance, thus dosing by the oral route may have occurred, although single-housing of the animals would have minimized this occurrence.

4. The NOEL and LOEL could not be determined because immune effects were seen at the low dose but not the high dose.

5. Additional tests, required by OPPTS 880.3550, were not included in this report. Studies required but not included were:

   a. Hematology
   b. Clinical biochemistry
   c. Tissue preparation
   d. Thymus and bone marrow cell viability
   e. Immunoglobulin quantification
   f. Specific cell-mediated immunity (either MLC, DTH or CTL assay), and
   g. Non-specific cell-mediated immunity (NK activities, total and differential peritoneal cell counts, and evaluation of phagocytosis)

In order to upgrade this study to acceptable, these studies would have to be performed and the deficiencies addressed. However, at the limit dose of 1000 mg/kg/day, no *suppression* of immunity occurred, and there was *no effect on relative and absolute spleen and thymus weights.* Since the plaque forming cell assay is currently only considered to be validated for the detection of *immune suppression*, then it is not necessary to repeat the plaque forming cell assay at lower doses.

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DATA EVALUATION REPORT

GRANOLA 97 (p-menthane-3,8-diol)

STUDY TYPE: SUBCHRONIC DERMAL TOXICITY - RAT (82-3)

Prepared for

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DATA EVALUATION RECORD

STUDY TYPE: Subchronic Dermal Toxicity-Rat; OPPTS 870.3250 [§82-3)]

DP BARCODE: D243976
P.C. CODE: 011550

SUBMISSION CODE: S538748
TOX. CHEM. NO.: unknown

TEST MATERIAL (PURITY): Granola 97 (98.3%)

SYNONYMS: p-menthane-3,8-diol

CITATION: Rush, R.E. (1997) A 90-day dermal toxicity study of Granola 97 in rats. Springborn Laboratories, Inc., Health and Environmental Sciences, 640 North Elizabeth Street, Spencerville, OH, 45887. Study # SCJ 14735R108, October 14, 1997. MRID 44438710. Unpublished.

SPONSOR: S.C. Johnson and Son, Inc., 1525 Howe Street, Racine, WI, 53403-2236.

EXECUTIVE SUMMARY: In a 90 day subchronic dermal toxicity study (MRID 44438710), groups of 15 male and female Sprague-Dawley rats were treated with Granola 97 (98.3%) at doses of 0, 1000 or 3000 mg/kg/day for 6 hours per day.

Decreased body weight (-8% day 36; -9% day 43, $p \leq 0.05$) and body weight gain (-30% days 29-36, $p \leq 0.05$) were observed in the high dose males. Low dose males displayed decreased (-71% days 64-71, $p \leq 0.05$) and increased (+260% days 71-78, $p \leq 0.01$) body weight gain.. No other effects on body weight were observed.

Barely perceptible erythema and desquamation was reported in all low dose male and female animals. In addition, a number of high dose male and female animals displayed well-defined erythema (23% male, 33% female), slight edema (8% male, 0% female) and pinpoint to moderate eschar (77% male, 40% female). Dermal findings in the control group were limited to one female with desquamation.

Treatment-related microscopic lesions were observed in the kidneys from high dose males and in treated skin from high dose males and females. Hyaline droplets, likely due to alpha-$_{2u}$-globulin inclusions, were seen in kidneys of control males (20%, minimal to mild) and high dose males (100%, 73% moderate). Minimal acanthosis was observed in 53% of control males' treated skin, while minimal to mild acanthosis was seen in 93% of high dose males. Chronic inflammation was observed in male control (20%, minimal to mild), female control (13%, minimal), and high dose male (100%, 67% mild) and female (100%, 60% mild) animals. In addition, parakeratosis was seen in 7% of high dose males and 27% of high dose females.

Statistically significant increased absolute liver weight (+18%, $p \leq 0.001$) and relative liver weight (+15%, $p \leq 0.001$) were observed in high dose females. Relative liver weight (+9%, $p \leq 0.05$), relative kidney weight (+12%, $p \leq 0.001$), and relative adrenal weight (+15%, $p \leq 0.05$) were increased in high dose males. There were no statistical differences noted for low dose male or female animals.

No treatment-related effects were observed with regard to hematology, clinical chemistry, neurotoxicity, or ophthalmology.

Based on the data presented in this study, the NOEL is 1000 mg/kg/day; the LOEL is 3000 mg/kg/day. The LOEL is based on dermal observations in treated skin (increased erythema, edema and eschar) and histological observations in treated skin (increased acanthosis and inflammation).

This subchronic dermal toxicity study in rats is classified as **acceptable (guideline)** (§82-3) and satisfies the Subdivision F guideline requirements.

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Granola 97

   Description: clear, oily liquid/clear, solid material
   Lot/Batch #: 703001
   Purity: a.i. 98.3%
   Stability of compound: not stated
   CAS #: 42822-86-6
   Structure: not provided

2. Vehicle and/or positive control

   no vehicle used

3. Test animals

   Species: Rat
   Strain: Sprague-Dawley Crl: CDBR VAF/Plus
   Age/weight at study initiation: males: 7 weeks, 209-277g ; females: 7 weeks, 150-190g.
   Source: Charles River Laboratories, Inc., Portage, Michigan.
   Housing: individually, in suspended stainless steel, wire mesh cages
   Diet: PMI Certified Rodent Chow #5002, *ad libitum*

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Water: tap water, *ad libitum*
Environmental conditions
Temperature: 65-79^0 F
Humidity: 30-70%
Air changes: 10-15 per hour
Photoperiod: 12 hour light/dark cycle
Acclimation period: 12 days

B. STUDY DESIGN

1. In life dates

Start: 4/16/1997; End: 7/21/1997

2. Animal assignment

Animals were assigned to one of three groups based on body weights using a computer randomization program (Table 1). Fifteen rats/sex/dose were utilized.

Table 1. Study Design

Dose mg/kg/day	No. of animals	
	male	female
0 (control)	15	15
1000 (low dose)	15	15
3000 (high dose)	15	15

3. Dose selection rationale

The doses utilized in the study were chosen "to meet and exceed the EPA's Limit Test dose level of 1000 mg/kg/day and to provide an adequate safety factor relative to anticipated human exposure."

4. Test substance preparation and analysis

Bulk containers of Granola 97 and distilled water used for controls were kept in a 37-45^0 C oven to maintain a liquid state. On each day of dosing, a container of Granola 97 was removed and dispensed into daily dosing aliquots. These aliquots were placed on a heated stir plate and stirred continuously during dosing.

5. Dose application

An area of fur equivalent to approximately 15% of the body surface was clipped from the dorsal area of the rats two days before initiation of dosing. The animals were clipped at least weekly thereafter. Granola 97 or distilled water for controls were applied and evenly spread over the clipped area of each animal using a syringe and ball-tipped gavage needle. After application, the treatment area was covered with four-ply gauze secured with elastic wrap. The cut ends of the elastic wrap were secured with athletic tape. Due to wrapping related mortality in 5 animals during the first 17 days of the trial, this wrapping procedure was modified. A less restricting binder consisting of a harness and one piece of tape accompanied by athletic tape applied to the hindpaws was utilized.

After each 6 hour application, the wrapping material was removed and the treatment site was wiped with gauze soaked in deionized tap water.

Animals were treated daily except during the functional observation battery intervals (days 43-45 and 85-87). Five animals/sex/group were evaluated on each day.

At the end of the testing period, animals were sacrificed "when possible" via CO_2 asphyxiation. No indication was made of the mode of euthanasia when this was not possible.

6. Statistics

Data was analyzed using ANOVA. When statistically significant differences were determined, control to treatment group comparisons were performed using the Tukey-Kramer method.

C. METHODS

1. Observations

Mortality and moribundity were checked twice daily. Animals were also observed for overt toxic effects prior to dosing and approximately 30-120 minutes after wrap removal. Once a week, detailed clinical observations were performed.

2. Body weight

Animals were weighed at study initiation and once per week throughout the study.

3. Food consumption

Individual food consumption was calculated on the same days as body weights, once per week throughout the study.

4. Ophthalmoscopic examination

Ophthalmological examinations were performed by a board-certified veterinary ophthalmologist prior to study initiation (day -2) and near the study's conclusion (day 94). Eyes were dilated using 0.5% Mydriacyl opthalmic solution prior to biomicroscopic and indirect opthalmoscopic examination.

5. Blood was collected after overnight fasting from the orbital plexus for hematology and clinical analysis from all surviving animals. The CHECKED (X) parameters were examined.

a. Hematology

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)*	X	Mean corpusc. HGB conc.(MCHC)
X	Erythrocyte count (RBC)*	X	Mean corpusc. volume (MCV)
X	Platelet count*	#	Reticulocyte count
	Blood clotting measurements*		
X	(Thromboplastin time)		
	(Clotting time)		
X	(Prothrombin time)		

* Required for subchronic studies based on Subdivision F Guidelines
# Reticulocyte slides were prepared, but not examined.

b. Clinical chemistry

	ELECTROLYTES		OTHER
X	Calcium*	X	Albumin*
X	Chloride*	X	Blood creatinine*
	Magnesium	X	Blood urea nitrogen*
X	Phosphorus*	X	Total Cholesterol
X	Potassium*	X	Globulins
X	Sodium*	X	Glucose*
	ENZYMES	X	Total bilirubin
X	Alkaline phosphatase (ALK)	X	Total serum protein (TP)*
	Cholinesterase (ChE)	X	Triglycerides
	Creatine phosphokinase		Serum protein electrophores
	Lactic acid dehydrogenase (LDH)		
X	Serum alanine amino-transferase (also SGPT)*		
X	Serum aspartate amino-transferase (also SGOT)*		
	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

* Required for subchronic studies based on Subdivision F Guidelines

6. Urinalysis

Urinalysis was not required and not performed.

7. Neurotoxicity Screening

A functional observation battery was conducted on animals during days 43-45 and 85-87 and included the following observations:

a. Home cage observations: body posture, tremors, convulsions, piloerection, respiratory rate/pattern, eyelid closure, unusual urination, abnormal feces and bizarre behavior.

b. Removal from home cage: ease of removal, reactivity to being handled, vocalization, general appearance, lacrimation, salivation, pupil size, exophthalmus, urine staining and fecal staining.

c. Open field observations: body posture, tremors, convulsions, gait, alertness of unperturbed animals, excessive/repetitive actions, urination, defecation, rearing and bizarre behavior.

d. Manipulative tests: tail pinch, startle response, righting ability, pupil response, approach response, touch response, grip strength and landing foot splay.

8. Sacrifice and pathology

All animals that died and those sacrificed on schedule were subjected to gross pathological examination. All tissues and organs collected from control and high dose animals, all gross lesions from all mid dose animals and selected tissues from mid dose animals found dead during the study were processed for histopathology. The CHECKED (X) tissues were collected for histological examination. The (XX) organs, in addition, were weighed.

X	DIGESTIVE SYSTEM	X	CARDIOVASC./HEMAT.	X	NEUROLOGIC
	Tongue	x	Aorta*	xx	Brain*
x	Salivary glands*	xx	Heart*	x	Periph. nerve*
x	Esophagus*	x	Bone marrow*	x	Spinal cord (3 levels)[T]
x	Stomach*	x	Lymph nodes*	x	Pituitary*
x	Duodenum*	x	Spleen*	x	Eyes (optic n.)
x	Jejunum*	xx	Thymus*		
x	Ileum*				GLANDULAR
x	Cecum*		UROGENITAL	xx	Adrenal gland*
x	Colon*	xx	Kidneys*+	x	Lacrimal gland[T]
x	Rectum*	x	Urinary bladder*	x	Mammary gland[T]
xx	Liver*+	xx	Testes*+	xx	Parathyroids[++]
	Gall bladder*	x	Epididymides	xx	Thyroids[++]
x	Pancreas*	x	Prostate		
		x	Seminal vesicle		OTHER
	RESPIRATORY	xx	Ovaries	x	Bone
x	Trachea*	x	Uterus*	x	Skeletal muscle
xx	Lung*			x	Skin (treated & untreated)
	Nose			x	All gross lesions and masses*
	Pharynx				
	Larynx				

* Required for subchronic studies based on Subdivision F Guidelines
+ Organ weight required in subchronic and chronic studies.
T = required only when toxicity or target organ

II. RESULTS

A. OBSERVATIONS

No treatment-related mortality was observed. Five deaths (1 female control, 2 low dose females and 2 high dose males) during the first 17 days of the treatment period were attributed to the original wrapping procedure.

Treatment-related dermal reactions were observed (Table 2) in this study. An increase in barely perceptible erythema, desquamation and focal/pinpoint eschar was observed in low and high dose males. A few incidences of well-defined erythema, very slight edema and mild to moderate eschar were observed in the high dose males. In females, an increase in barely perceptible to well-defined erythema and desquamation was seen in both low and high dose groups. A few incidences of focal/pinpoint to mild eschar were also noted in the high dose females.

TABLE 2. Incidence of dermal observations after expsure to Granola 97

Dermal observation	Dosage Groups					
	Control (0 mg/kg/day)		Low (1000 mg/kg/day)		High (3000 mg/kg/day)	
	male	female	male	female	male	female
erythema, grade 1	0	0	13	12	13	15
erthema, grade 2	0	0	0	1	1	4
maximized, grade 4	0	0	0	0	2	1
edema, grade 1	0	0	0	0	1	0
eschar, focal/pinpoint	0	0	2	0	8	5
eschar, mild	0	0	0	0	1	1
eschar, moderate	0	0	0	0	1	0
desquamation	0	1	14	13	13	15

Data taken from p. 33 and 34, MRID 44438710

B. BODY WEIGHT AND WEIGHT GAIN

Small, but statistically significant ($p \leq 0.05$) decreases in body weight of high dose males were observed on days 36 (-8%) and 43 (-9%). No decreases were seen at the other time points tested and there were no changes in body weight in low dose males or in any female dosed group.

Low dose males showed a decrease in body weight gain for days 64-71 (-71%, $p \leq 0.05$) but an increase for days 71-78 (+260%, $p \leq 0.01$). High dose males had a decrease in body weight gain for days 29-36 only (-30%, $p \leq 0.05$). Female mice showed no changes in body weight gain during the study.

C. FOOD CONSUMPTION AND COMPOUND INTAKE

1. Food consumption

Mean food consumption was increased in high dose females for days 57-64 (+4%, $p \leq 0.01$), 71-78 (+5%, $p \leq 0.05$) and 78-85 (+10%, $p \leq 0.05$). No changes were observed for low dose females or males of any dose group.

D. OPHTHALMOSCOPIC EXAMINATION

No ocular lesions associated with the test substance were observed.

E. Blood work

1. Hematology

No statistically significant differences were observed except for increased segmented neutrophils in low dose males and high dose females (both +100%, $p \leq 0.05$), and increased activated partial thromboplastin time in high dose females (+6%, $p \leq 0.05$). Although these values were statistically increased above controls, they were still within normal ranges seen historically at the testing site and are not considered treatment-related.

2. Clinical chemistry

In males, phosphorus was increased in the low dose group (+13%, $p \leq 0.05$), while total protein, globulin and urea nitrogen were increased in the high dose group (+5%, $p \leq 0.05$; +8%, $p \leq 0.01$; +6%, $p \leq 0.01$; respectively). Glucose was decreased in both low and high dose males (-17%, $p \leq 0.001$; and -20%, $p \leq 0.001$). In females, chloride was decreased in the high dose group (-2%, $p \leq 0.05$). In both low and high dose females, globulin was increased (+6%, $p \leq 0.05$; +11%, $p \leq 0.001$), A/G ratio (-8%, $p \leq 0.01$; -12%, 0.001) and glucose (-14%, $p \leq 0.001$; -15%, $p \leq 0.001$) were decreased. Although these values were statistically significantly different than controls, they remained within normal ranges seen historically at the testing site and are not considered treatment-related.

F. NEUROTOXICITY

No evidence of treatment-related neurological effects were observed.

G. SACRIFICE AND PATHOLOGY

1. Organ weight

Selected organ weights are summarized in Table 3. Absolute liver weight was statistically significantly increased in high dose females (+18%, $p \leq 0.001$) and relative liver weight was increased in both high dose males (+9%, $p \leq 0.05$) and females (+15%, $p \leq 0.001$). Relative kidney weight (+12%, $p \leq 0.001$) and adrenal weight (+15%, $p \leq 0.05$) was increased only in high dose males.

No changes in absolute or relative organ weights were observed in low dose male or low dose female groups.

TABLE 3. Selected absolute and relative organ weights (g) for rats exposed dermally to Granola 97

Organ	Males			Females		
	Control	Low dose	High dose	Control	Low dose	High dose
Liver	**Absolute organ weights**					
	13.84	13.28	14.30	7.58	7.96	8.92**
Liver/total final body weight	**Relative organ weights**					
	3.22	3.11	3.53*	3.28	3.36	3.77**
Kidney/total final body weight	0.87	0.90	0.97**	0.92	0.94	0.94
Adrenal/final brain weight	2.81	3.12	3.23*	3.65	3.68	3.79

* $p \leq 0.05$
** $p \leq 0.001$
Data taken from Tables 10,11 and 12, pgs 92-102 MRID 44438710

2. Gross pathology

No treatment-related gross lesions were observed in either animals surviving until termination of the study or in the five animals found dead during the study.

3. Microscopic pathology -

a. Non-neoplastic

Treatment-related skin lesions were noted in the treated skin of high dose male and female animals (Table 4). These lesions included an increase in the incidence and severity of acanthosis, chronic inflammation and parakeratosis.

Treatment-related lesions were also observed in the kidneys of high dose male animals. Eosinophilic hyaline droplets were present within the cytoplasm of the tubular lumen in the renal cortex of 3/15 control males and 15/15 high dose males. Because of the morphology of the droplets and the occurrence in only male rats, the authors suggested that the droplets were alpha-$_{2u}$-globulin inclusions. High dose males also showed an increase in the incidence and severity of chronic progressive nephropathy. The authors suggested that these lesions were a secondary effect of the injury that results from the hyaline droplet formation.

TABLE 4. Selected microscopic pathology for rats exposed dermally to Granola 97						
Pathological finding	Mles			Fmales		
	Control n=15	Low dose n=2	High dose n=15	Control n=15	Low dose n=2	High dose n=15
Treated skin						
Acanthosis minimal mild	8 8 0	0 0 0	14 5 9	0 0 0	1 1 0	12 6 6
Inflammation chronic-minimal chronic-mild chronic-active	3 1 2 0	0 0 0 0	15 4 10 1	2 2 0 0	1 1 0 0	15 6 9 0
Parakeratosis minimal mild	0 0 0	0 0 0	1 0 1	0 0 0	0 0 0	4 4 0
Kidneys						
Hyaline droplets minimal mild moderate	3 2 1 0	0 0 0 0	15 1 3 11	0 0 0 0	0 0 0 0	0 0 0 0
CP* nephropathy minimal mild	2 2 0	0 0 0	10 4 6	2 2 0	0 0 0	0 0 0

*chronic progressive
Data taken from Appendix O, pages 340-350, MRID 44438710

b. Neoplastic

No neoplastic lesion were reported.

III. DISCUSSION

A. DISCUSSION

The data presented in this 90 day subchronic dermal study show that moderate toxic effects are observed in rats exposed to 3000 mg/kg/day of Granola 97. These effects include dermal observations (increased erythema, edema and eschar) and histological changes in treated skin (increased acanthosis and inflammation) and kidneys (hyaline droplet formation and chronic progressive nephropathy in males). No significant toxic effects were observed in animals exposed to 1000 mg/kg/day.

No treatment-related mortalities were seen in this study. Exposure to Granola 97 produced a variety of effects in a number of other parameters, but these observations are unlikely to be biologically significant. Decreased body weight and/or body weight gain in low and high dose males was observed only at a few time points, and the final differences in these parameters were not statistically significant. Female animals showed no changes in body weight or body weight gain, but food consumption was increased at certain time points in the high dose females. While any treatment-related weight loss in the female high dose group may have been masked by this increased food consumption, overall food consumption was not significantly different over the course of the study.

Differences in hematologic and clinical chemistry values were also observed. However, no clear dose-related trends were established and the differences, while statistically valid, were not biologically significant. Changes in relative and absolute organ weights were noted as well, but again no dose-related trends were obvious.

The author of this study states that the NOEL is 3000 mg/kg/day. The author suggests that the increased incidence and severity of hyaline droplet formation and chronic progressive nephropathy in male rats are associated with alpha-$_{2u}$ -globulin inclusions and these inclusions are specific to the male rat and have no known significance in humans. The reviewers agree with the assumptions concerning the nephropathy. However, 3000 mg/kg clearly produced an increase in the incidence and severity of acanthosis and inflammation in treated skin. In addition, the increased severity of erythema, eschar and edema in the high dose groups suggest toxic effects. Consequently, the reviewer disagrees with the author's conclusions and establishes the NOEL as 1000 mg/kg/day and the LOEL as 3000 mg/kg/day based on dermal effects.

B. STUDY DEFICIENCIES

Minor: Only two doses of Granola 97 were tested, but a NOEL and LOEL could be established from the data presented. The lack of an intermediate dose does not compromise the study.

DATA EVALUATION REPORT

GRANOLA 97

STUDY TYPE: DEVELOPMENTAL TOXICITY – RAT (83-3a)

Prepared for

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

Prepared by

Chemical Hazard Evaluation Group
Toxicology and Risk Analysis Section
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 22

Primary Reviewer:
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Quality Assurance:
Susan Chang, M.S.

Signature: _____
Date: _____

Disclaimer

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

Managed by Lockheed Martin Energy Research, Corp., for the U.S. Department of Energy under Contract No. DE-AC05-96OR22464.

GRANOLA 97

Developmental Toxicity Study (83-3a)

EPA Reviewer: Sheryl Reilly, Ph.D.
Biological and Pollution Prevention Division

Roger Gardner for Date: 11/20/98

DATA EVALUATION RECORD

STUDY TYPE: Developmental Toxicity - Rat
OPPTS 870.3700 [§83-3a]

DP BARCODE: D243976
P.C. CODE: 061954

SUBMISSION CODE: S538748
TOX. CHEM. NO.:

TEST MATERIAL (PURITY): Granola 97 (SCJ NB# 14735R108) (98.5% a.i.)

SYNONYMS: p-Menthane-3,8-diol

CITATION: Wakefield, A.E. (1997) Rat prenatal developmental toxicity study with Granola 97 (SCJ NB# 14735R108). Covance Laboratories, Inc., 9200 Leesburg Pike, Vienna, VA 22182-1699. Laboratory Study Identification, Covance 6106-116. October 30, 1997. MRID 44438711. Unpublished.

SPONSOR: S.C. Johnson & Son, Inc., 1525 Howe Street, Racine, WI 53403-2236

EXECUTIVE SUMMARY: In a developmental toxicity study (MRID 44438711), 25 pregnant Sprague-Dawley Crl:CD®BR rats per group were administered Granola 97 (SCJ NB# 14735R108) (98.5% a.i.) by dermal application at doses of 0, 1, and 3 g/kg/day on gestation days (GD) 6-19, inclusive. The doses were adjusted based on a specific gravity of 0.98 and the control group received tap water. On GD 20, all dams were sacrificed and all fetuses were examined for external malformation/variations. Approximately one-half of each litter was processed for visceral examination and the remainder stained for skeletal and cartilage evaluation.

All animals survived to scheduled sacrifice. No treatment-related clinical signs of toxicity were observed in any animal during the study. The skin at the application site of animals in both treated groups did not show signs of irritation. No statistically significant differences in absolute body weights occurred between the treated and control groups during the study. Body weight gains were significantly (91% of controls; $p \leq 0.05$) lower in the high-dose group during GD 6-20 as compared to the controls. The reduced body weight gain by the high-dose group was a result of significantly lower (90%of controls; $p \leq 0.01$) food consumption than the controls during the GD 6-9 interval. At all other times during the study, food consumption and body weight gains by the treated groups were comparable to the controls. No abnormalities were noted at maternal necropsy.

Therefore, the maternal toxicity NOEL is >3 g/kg/day and the maternal toxicity LOEL was not identified.

No dose- or treatment-related statistically significant effects on pregnancy rate, number of corpora lutea, pre- or postimplantation losses, resorptions/dam, fetuses/litter, fetal body weights, or fetal sex ratios were observed in the treated groups as compared to the controls. Two low-dose dams had complete litter resorption.

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No treatment-related external, visceral, or skeletal malformations/variations were observed in any litter. The number of litters in the 0, 1, and 3 g/kg/day groups containing fetuses with major malformations was 1/23, 2/21, and 1/22, respectively. All treated and control litters contained fetuses with minor variations in skeletal ossification.

Therefore, the developmental toxicity NOEL is >3 g/kg/day and the developmental toxicity LOEL was not identified.

This study is classified as Acceptable (guideline) and satisfies the guideline requirements for a developmental toxicity study (83-3a) in rats.

COMPLIANCE: Signed and dated Quality Assurance, Good Laboratory Practice, and Data confidentially statements were included.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Granola 97 (SCJ NB# 14735R108)

   Description: clear, viscous liquid
   Lot/Batch No.: 703002
   Purity: 98.5% a.i.
   Stability of compound: not stated, responsibility of sponsor
   CAS No.: 42822-86-6
   Structure: not provided

2. Vehicle and/or positive control

   Tap water served as the control material. The test material was applied neat without the use of a vehicle.

3. Test animals

   Species: Rat
   Strain: Sprague-Dawley Crl:CD®BR
   Age and weight at study initiation: 11 weeks; 205-280 g on GD 0
   Source: Charles River Laboratories, Inc., Portage, MI (females); Kingston, NY (males)
   Housing: Animals were housed individually in a stainless-steel, hanging, wire-mesh cage.
   Diet: PMI® Certified Rodent Diet® #5002 and tap water were available *ad libitum*.
   Environmental conditions:
   - Temperature: 19.5-25.8°C
   - Humidity: 40.1-62.4%
   - Air changes: ≥10/hour
   - Photoperiod: 12-hour light/12-hour dark

   Acclimation period: 1 week

B. PROCEDURES AND STUDY DESIGN

This study was designed to assess the developmental toxicity potential of Granola 97 when administered dermally to rats on gestation days 6 through 19, inclusive.

1. In life dates

Start: June 11, 1997; end: July 5, 1997

2. Mating

One female was mated with one male of the same strain until mating was confirmed by the presence of sperm in a vaginal lavage or a retained copulatory plug. The day evidence of mating was observed was designated GD 0.

3. Animal assignment and dose selection are presented in Table 1. Confirmed-mated females were assigned to groups using a table of random numbers.

TABLE 1. Animal assignment

Group	Dose (g/kg/day)	Number of animals
Control	0	25
Low	1	25
High	3	25

Data taken from text table p. 14, MRID 44438711.

4. Dose selection rationale

Doses were based on data from pharmacology, other toxicology, and kinetics studies. The study author stated that the high-dose level was close to the maximum feasible dermal dose. No other details on dose selection were included in the study.

5. Dosing

The test material was applied neat, as supplied. Prior to the first dose, and as needed thereafter (at least weekly), animals were shaved on the dorsal surface of the back. Test material was administered to the site once daily on GD 6-19. The application site was covered with 4-ply gauze and a dermal harness was placed on the animal. At least 6 hours but not more than 6 hours and 55 minutes after dose application, the site was wiped with gauze to remove any residue. Doses were based on the most recently recorded body weight.

6. Dose solution preparation and analysis

Doses were adjusted based on the specific gravity of 0.98.

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C. OBSERVATIONS

1. Maternal observations and evaluations

The animals were checked twice daily for mortality and moribundity. Observation for clinical signs of toxicity was performed once daily at the time of dermal harness removal. A thorough physical examination was conducted at each weighing interval and included an examination of the treatment site for irritation. Maternal body weights and food consumption were measured on GD 0, 3, 6, 9, 12, 15, 18, and 20. Dams were sacrificed on GD 20 by carbon dioxide inhalation and exsanguination and examined grossly. The uterus from each gravid female was excised, weighed, and examined for the number and placement of implantation sites, live and dead fetuses, early and late resorptions, and any abnormalities of the placenta or amniotic sac. The ovaries were examined for the number of corpora lutea.

2. Fetal evaluations

Each fetus was sexed, weighed, examined for external abnormalities, and sacrificed via intraperitoneal injection of sodium pentobarbital. Approximately one-half of all fetuses from each litter were randomly selected and processed for visceral examination. The remaining fetuses were eviscerated and processed for skeletal and cartilage evaluation.

D. DATA ANALYSIS

1. Statistical analysis

Mean maternal body weight, body weight change, food consumption, uterine weights, and cesarean section data of the treated groups were compared to the control group using a One-Way analysis of Variance. Levene's test was used to determine homogeniety of variances. If the variances of the untransformed data were heterogeneous, analyses were performed on rank-transformed data. Dunnett's t-test served as the *post-hoc* group comparison test. Mean live fetal weights were analyzed by One-Way Analysis of Covariance using the number of fetuses in each litter as the covariate.

2. Historical control data were not provided to allow comparison with concurrent controls.

II. RESULTS

A. MATERNAL TOXICITY

1. Mortality and clinical signs

All animals survived to scheduled sacrifice. No treatment-related clinical signs of toxicity were observed in any animal during the study. The skin at the application site of animals in both treated groups did not show signs of irritation.

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2. Body weight

Selected maternal body weights and body weight gains are given in Table 2. No statistically significant differences in absolute body weights occurred between the treated and control groups during the study. Body weight gains were significantly (91% of controls; $p \leq 0.05$) lower in the high-dose group during the treatment period as compared to the controls.

TABLE 2. Maternal body weight and body weight gain (g)

Day of gestation	0 g/kg/day	1 g/kg/day	3 g/kg/day
0	250.5 ± 14.4	253.3 ± 14.5	251.1 ± 15.2
6	289.5 ± 14.0	291.8 ± 17.4	286.8 ± 15.6
18	384.0 ± 26.8	374.9 ± 42.6	374.3 ± 20.1
20	417.2 ± 33.3	403.7 ± 52.6	402.9 ± 22.7
weight gain 6-20	127.74 ± 24.07	111.96 ± 44.73	116.05 ± 15.56*
weight gain 0-20	166.70 ± 25.71	150.48 ± 47.32	151.73 ± 18.96
Corrected body weight[a]	329.77 ± 19.71	327.77 ± 31.56	316.69 ± 20.60

Data taken from Tables 2A, 2B, and 5, pp. 28, 30, and 37, respectively, MRID 44438711.
*Significantly different from control, $p \leq 0.05$.
[a]Corrected body weight = terminal body weight - gravid uterine weight.

3. Food consumption

Food consumption data are summarized in Table 3. The high-dose group had significantly lower (90% of controls; $p \leq 0.01$) food consumption than the controls during the GD 6-9 interval. At all other times during the study, food consumption by the treated groups was comparable to the controls.

TABLE 3. Maternal food consumption (g/animal/day)

Gestation interval	0 g/kg/day	1 g/kg/day	3 g/kg/day
0-6	24.4 ± 1.7	24.9 ± 2.4	24.5 ± 1.7
6-9	26.8 ± 1.9	25.2 ± 3.2	24.2 ± 1.6**
6-20	29.8 ± 2.2	29.1 ± 3.8	29.8 ± 2.2
0-20	28.2 ± 1.9	27.8 ± 3.1	28.2 ± 1.9

Data taken from Table 3, pp. 32-33, MRID 44438711.
**Significantly different from control, $p \leq 0.01$.

4. Gross pathology

No treatment-related abnormalities were observed at maternal necropsy.

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5. Cesarean section data

Data obtained at cesarean section are given in Table 4. No dose- or treatment-related statistically significant effects on pregnancy rate, number of corpora lutea, pre- or postimplantation losses, resorptions/dam, fetuses/litter, fetal body weights, or fetal sex ratio were observed in the treated groups as compared to the controls. One control dam had only two implantation sites with a single viable fetus. The slightly higher percentage of resorptions/dam and fewer live fetuses/litter in the low-dose group are a result of two animals with total litter resorption. If these two dams are excluded from the group means, the values are similar to the control group levels. Because the high-dose group was not similarly affected, the differences in the low-dose group are not considered to be treatment-related.

TABLE 4. Cesarean section observations

Observation	0 g/kg/day	1 g/kg/day	3 g/kg/day
No. Animals Assigned	25	25	25
No. Animals Mated/Inseminated	23	23	22
Pregnancy Rate (%)	92	92	88
Maternal Mortality	0	0	0
Delivered early/aborted	0	0	0
Total Corpora Lutea	393	365	378
Corpora Lutea/Dam	17.1	15.9	17.2
Total Implantation	347	313	341
Implantation/Dam	15.1	13.6	15.5
Preimplantation loss (%)	12.6	16.6	9.3
Postimplantation loss (%)	6.1	15.8	8.2
Total Live Fetuses	332	284	313
Live Fetuses/litter	14.4	12.3	14.2
Mean Fetal Weight (g)	3.76	3.78	3.64
Sex Ratio (% Male)	53	49	50
Total Resorptions	15	29	28
Resorptions/Dam (%)[a]	0.7 (6.1)	1.3 (15.8)	1.3 (8.2)
Early resorptions/dam[a]	0.7	0.7	0.8
Late resorptions/dam[a]	0.0	0.6	0.5
Total Dead Fetuses	0	0	0
Dams with all resorptions	0	2	0

Data taken from Tables 6 and 7, pp. 39-41 and 45, respectively, MRID 44438711.
[a]Includes dams with all resorptions.

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B. DEVELOPMENTAL TOXICITY

No treatment-related external, visceral, or skeletal malformations/variations were observed in any litter. The number of litters in the 0, 1, and 3 g/kg/day groups containing fetuses with major malformations was 1/23, 2/21, and 1/22. All litters contained fetuses with minor variations in skeletal ossification.

1. External examination

No treatment-related external malformations/variations were observed in any fetus from any treated litter. One control fetus had macrophthalmia, one low-dose fetus had anasarca, and another low-dose fetus from a different litter was acaudate with anal atresia.

2. Visceral examination

No treatment-related visceral malformations/variations were observed in any fetus from any treated litter. The single fetus from a control group dam was examined viscerally. A common finding in control and treated litters was renal pelvic cavitation. One low-dose fetus had multiple malformations.

3. Skeletal examination

All treated and control litters contained fetuses with skeletal variations. Because one control dam had a single viable fetus which was processed for visceral examination, only 22 control group litters were available for skeletal examination. The most common variations were bipartite vertebral centra, sternebrae unossified or incompletely ossified, and incomplete ossification of the skull. The number of litters in the high-dose group containing fetuses with sternebrae asymmetrically ossified was significantly ($p = 0.047$) greater than the control group with 1/22, 1/21, and 6/22 litters in the 0, 1, and 3 g/kg/day groups, respectively, containing affected fetuses. One fetus in a low-dose litter had only the first sacral vertebrae present with the second through the fourth sacral vertebrae and all caudal vertebrae absent.

III. DISCUSSION

A. INVESTIGATORS' CONCLUSIONS

The study author concluded that dermal administration of Granola 97 to pregnant rats during organogenesis did not result in overt maternal or fetal toxicity. The NOAEL for maternal and fetal toxicity is 3 g/kg/day.

B. REVIEWER'S DISCUSSION

1. MATERNAL TOXICITY

Maternal toxicity was not observed during the study. Slightly decreased overall body weight gains occurred in the high-dose group and were most pronounced during the GD 6-9 interval; body weight gains during other intervals of the

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treatment period were comparable to the control group levels. The reduced weight gain was most likely due to the decrease in food consumption during days 6-9 of the treatment period. While body weight gains for GD 6-20 and food consumption for GD 6-9 reached statistical significance for the high-dose group, the changes are of questionable biological significance. The reductions were transient, were not dose-related, and were within 10% of the control group value. Therefore, the reduced body weight gain in the high-dose group resulting from decreased food consumption is not considered to be a treatment-related toxicity.

The study author did not justify the dermal route of treatment except to state that this is the expected route of human exposure. No information was included that showed dermal absorption of Granola 97 occurred to an appreciable amount. In a 90-day dermal study in rats (MRID 44438710), the main finding was inflammation and acanthosis at the application site on males and females administered 3 g/kg/day for 6 hr/day. There was also a trend towards increasing severity of kidney lesions in male rats indicating some dermal absorption may occur, but absorption is probably minimal. Although the doses used in both the current and subchronic studies exceed the limit dose, experimental evidence of dermal absorption is necessary before the current study can be considered valid. Therefore, further justification of the dermal route and pharmacokinetic data showing dermal absorption are required before this study is acceptable.

Although three doses are generally recommended to adequately assess dose-response relationships, only two doses were used in the current study. Lack of a third dose is not considered a deficiency because no toxicity occurred at the high dose and because the high dose was above the limit dose.

2. DEVELOPMENTAL TOXICITY

a. Deaths/resorptions

Maternal treatment with Granola 97 during gestation did not result in fetal death or an increase in resorptions.

b. Altered growth

Fetal growth was not affected by maternal treatment with Granola 97.

c. Developmental variations

No treatment-related variations were observed in any fetuses. Although the number of high-dose litters containing fetuses with asymmetrically ossified sternebrae was significantly greater than the controls, this is not considered a compound-related effect. The variation was not dose-related and there were only seven fetuses in a total of six affected litters. Historical control data would have been helpful to determine the background incidence range of this anomaly.

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d. Malformations

Major malformations did not result from maternal treatment with Granola 97 during gestation. Two fetuses from different low-dose litters had multiple malformations, but these are considered incidental to treatment.

C. STUDY DEFICIENCIES

There are no deficiencies in this study.

DATA EVALUATION REPORT

p-MENTHANE-3,8-DIOL

STUDY TYPE: *Salmonella/escherichia*/mammalian Activation Gene Mutation Assay; OPPTS 870.5265[1] [§84-2]

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

Chemical Hazard Evaluation Group
Toxicology and Risk Analysis Section
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 22

Primary Reviewer: Bradford L. Whitfield, Ph.D.	Signature: Date:	B L Whitfield 9/25/98
Secondary Reviewers: Cheryl B. Bast, Ph.D., D.B.A.T.	Signature: Date:	Cheryl B Bast OCT 0 2 1998
Robert H. Ross, M.S., Group Leader	Signature: Date:	Robert H. Ross OCT 0 2 1998
Quality Assurance: Susan Chang, M.S.	Signature: Date:	Robert H Ross for S Chang OCT 0 2 1998

Disclaimer

[1] 870.5100 - Reverse mutation *E. coli* WP2 and WP2uvrA
870.5140 - Gene mutation *Aspergillus nidulans*
870.5250 - Gene mutation *Neurospora crassa*

EPA Work Assignment Manager: Reilly
Biopesticides & Pollution Prevention Division

Sheryl K Reilly, Date 1-25-99

DATA EVALUATION RECORD

STUDY TYPE: *Salmonella/Escherichia*/mammalian activation gene mutation assay; OPPTS 870.5265[1] [§84-2]

DP BARCODE: D243976
CASE: 061954

SUBMISSION CODE: S538748
TOX. CHEM. NO.: not provided

TEST MATERIAL (PURITY): Granola 97 (p-methane-3,8-diol)(98.3% a.i.)

SYNONYMS: p-Menthane-3,8-diol; SCJ NB # 14735R108

CITATION: Wagner, V.O. and E.W. Walton (1997) Bacterial reverse mutation assay with Granola 97 (SCJ NB # 14735R108). MA BioServices, Inc., 9630 Medical Center Drive, Rockville, MD 20850. Laboratory study number G97BF49.502, November 18, 1997. MRID 44487801. Unpublished

SPONSOR: S.C. Johnson & Son, Inc., 1525 Howe Street, Racine, WI 53403-2236

EXECUTIVE SUMMARY: In a reverse gene mutation assay in bacteria (MRID 44487801), strains TA98, TA100, TA1535 and TA1537 of *S. typhimurium* and strain WP2(uvrA) of *E. coli* were exposed to Granola 97 (Batch No. 703001, 98.3% a.i.) in DMSO at concentrations of 25 (WP2(uvrA) only), 75, 200, 600, 1800, and 5000 µg/plate in the presence and absence of mammalian metabolic activation (S9-mix). The S9-fraction was obtained from Aroclor 1254 induced male Sprague-Dawley rat liver.

Granola 97 was tested up to a limit concentration of 5000 µg/plate. In the preliminary cytotoxicity assay, no thinning of the background lawn was seen at any concentration up to and including 5000 µg/plate in any of the five tester strains, with or without S9-mix. Some reduction in the number of revertants per plate was seen both with and without S9-mix at 1000 µg/plate and higher concentrations in WP2(uvrA). The mean number of revertants per plate at 1000, 3333 and 5000 µg/plate in this strain was reduced, compared to the solvent control value of 21 both with and without S9-mix, to 8, 5, and 3, respectively, with S9-mix and to 7, 8 and 4, respectively, without S9-mix. Granola 97 was not mutagenic to any of the five tester strains at any concentration tested, with or without S9-mix. Solvent and positive control values were appropriate for the respective strains. **There was no evidence of induced mutant colonies over background.**

This study is classified as acceptable (guideline). It satisfies the requirement for FIFRA Test Guideline 84-2 for *in vitro* mutagenicity (bacterial reverse gene mutation) data.

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[1]870.5100 - Reverse mutation *E. coli* WP2 and WP2uvrA
870.5140 - Gene mutation *Aspergillus nidulans*
870.5250 - Gene mutation *Neurospora crassa*

Co

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Granola 97

Description: thick, clear, colorless liquid
Lot/Batch #: 703001
Purity: 98.3% a.i.
Stability of compound: responsibility of sponsor
CAS #: 42822-86-6
Structure: not provided
Solvent used: DMSO
Other comments: store at 37±2°C, protected from light

2 Control materials

Negative:
Solvent/final concentration: DMSO/50 µL/plate
Positive:
Nonactivation:
Sodium azide 1.0 µg/plate TA100, TA1535
2-Nitrofluorene 1.0 µg/plate TA98
9-Aminoacridine 75 µg/plate TA1537
Methyl methanesulfonate 1000 µg/plate WP2(uvrA)

Activation:
2-Aminoanthracene 1.0 µg/plate (TA strains)
2-Aminoanthracene 10.0 µg/plate WP29uvrA

3. Activation: S9 derived from male Sprague-Dawley rats

x Aroclor 1254 x induced x rat x liver
__ phenobarbital __ non-induced __ mouse __ lung
__ none __ other __ other

S9 mix composition: in 100 mM phosphate buffer, pH 7.4

5 mM glucose-6-phosphate
4 mM NADP
8 mM $MgCl_2$
33 mM KCl
10% S9-fraction

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4. Test organisms: *S. typhimurium* strains

__ TA97 _x_ TA98 _x_ TA100 __ TA102 __ TA104
_x_ TA1535 _x_ TA1537 __ TA1538

E. coli strain WP2(uvrA)

Properly maintained? Y
Checked for appropriate genetic markers (rfa mutation, R factor)? Y (The testing laboratory's criteria for a valid study require the bacteria to have the appropriate genetic markers. The authors stated that all criteria for a valid study were met. They did not state directly that the bacteria were checked for the appropriate genetic markers.)

5. Test compound concentrations used

Preliminary cytotoxicity test: (single plating)
Nonactivated and activated conditions: 6.7, 10, 33, 67, 100, 333, 667, 1000, 3333, 5000 µg/plate (all strains)
Main mutagenicity test: (triplicate platings)
Nonactivated and activated conditions: 25 µg/plate (WP2(uvrA) only); 75, 200, 600, 1800, 5000 µg/plate (all strains)

B. TEST PERFORMANCE

1. Type of Salmonella assay

_x_ standard plate test
__ pre-incubation (__ minutes)
__ "Prival" modification *(i.e. azo-reduction method)*
__ spot test
__ other [describe]

2. Protocol

For each plate, 500 µL of S9-mix (or 500 µL of 100 mM phosphate buffer), 100 µL of an overnight culture of the desired bacteria tester strain and 50 µL of solvent or test material were added to 2 mL of molten selective top agar at 45±2°C (media composition is given in the Appendix). The mixture was vortexed and poured onto the surface of 25 mL of minimal bottom agar. After the agar had solidified, the plates were inverted and incubated for 48 to 72 hours at 37±2°C. All platings were in triplicate. Revertant colonies on the plates were either counted immediately after incubation or the plates were stored at 4±2°C until they could be counted. Revertant colonies were usually counted by an automated colony counter; however, manual counting was used in the preliminary cytotoxicity assay, when toxicity was apparent or if a test article precipitate interfered with automatic counting. All plates for a given tester strain and activation condition were counted by the same method.

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To be considered positive, the test material must cause a dose-related increase in the mean number of revertants per plate over at least two increasing concentrations of test material in at least one tester strain. The increase in mean number of revertants must be at lease three times the solvent control value for strains TA1535 and TA1537 and at least two times the solvent control value for strains TA98, TA100 and WP2(uvrA).

II. REPORTED RESULTS

A. PRELIMINARY CYTOTOXICITY ASSAY

Ten concentrations of Granola 97 ranging from 6.7 to 5000 µg/plate were tested, with and without S9-mix, in all five tester strains in a preliminary cytotoxicity assay. One plate per test point was used. A reduction in the number of revertants per plate and/or a thinning or absence of the background lawn of bacteria was the measure of cytotoxicity. The background lawn of all five tester strains was normal at all concentrations of Granola 97 studied, with or without S9-mix. Some reduced revertant counts, compared to the solvent controls, were seen but no systematic pattern was evident except for WP2(uvrA). The mean number of revertants per plate at 1000, 3333 and 5000 µg/plate in this strain was reduced, compared to the solvent control value of 21 both with and without S9-mix, to 8, 5, and 3, respectively, with S9-mix and to 7, 8 and 4, respectively, without S9-mix. No test material precipitation was observed. The limit concentration of 5000 µg/plate was selected as the highest concentration used in the mutagenicity assay.

B. MUTAGENICITY ASSAY

Five concentrations of Granola 97 ranging from 75 to 5000 µg/plate were tested, with and without S9-mix in all five bacterial strains. An additional concentration of 25 µg/plate was used with strain WP2(uvrA). All plating was in triplicate. Granola 97 was not mutagenic to any of the five tester strains at any concentration tested, with or without S9-mix. Solvent and positive control values were appropriate for the respective strains. Results of the mutagenicity study are summarized in Appendix Table 1 (MRID 44487801, p. 30).

III. REVIEWER'S DISCUSSION/CONCLUSIONS

A. This is an acceptable study. Granola 97 was tested to a limit concentration of 5000 µg/plate, the experimental protocol was acceptable and the solvent and positive control values were appropriate for the respective strains.

B. STUDY DEFICIENCIES

No study deficiencies were identified.

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Salmonella/E. Coli Mutagenicity Assay
Summary of Results

Table 1

Test Article Id : Granola 97 (SCJ NB # 14735R108)
Study Number : G97BF49.502 Experiment No : B1

Average Revertants Per Plate ± Standard Deviation

Liver Microsomes: None

Dose (μg)	TA98	TA100	TA1535	TA1537	WP2 uvrA
0.0	13 ± 1	152 ± 14	8 ± 3	8 ± 1	15 ± 3
25					14 ± 3
75	21 ± 5	154 ± 4	10 ± 1	7 ± 2	17 ± 2
200	11 ± 2	153 ± 11	7 ± 2	8 ± 2	17 ± 2
600	13 ± 3	147 ± 6	9 ± 2	8 ± 2	15 ± 4
1800	13 ± 1	117 ± 11	9 ± 2	7 ± 2	9 ± 7
5000	12 ± 1	144 ± 7	7 ± 2	6 ± 3	11 ± 1
Pos	206 ± 60	729 ± 48	502 ± 15	403 ± 140	134 ± 9

Liver Microsomes: Rat liver S9

Dose (μg)	TA98	TA100	TA1535	TA1537	WP2 uvrA
0.0	17 ± 3	147 ± 7	14 ± 4	14 ± 5	17 ± 2
25					14 ± 3
75	13 ± 4	163 ± 12	10 ± 3	10 ± 2	18 ± 3
200	16 ± 3	137 ± 6	11 ± 3	7 ± 3	17 ± 1
600	12 ± 3	174 ± 22	10 ± 1	7 ± 3	15 ± 3
1800	12 ± 2	164 ± 9	6 ± 2	6 ± 1	13 ± 4
5000	14 ± 1	142 ± 15	11 ± 3	4 ± 4	10 ± 3
Pos	938 ± 186	976 ± 102	127 ± 35	83 ± 40	264 ± 59

0.0 = Vehicle plating aliquot of 50 μL
Pos = Positive Control concentrations as specified in Materials and Methods section.

DATA EVALUATION REPORT

p-MENTHANE-3,8-DIOL

STUDY TYPE:*In vitro* mammalian cytogenetics (chromosomal aberration) assay in Chinese hamster ovary CHO-K1 cells; OPPTS 870.5375 [§84-2]

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

Chemical Hazard Evaluation Group
Toxicology and Risk Analysis Section
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 22

Primary Reviewer: Bradford L. Whitfield, Ph.D.	Signature:	B L Whitfield
	Date:	9/25/98
Secondary Reviewers: Cheryl B. Bast, Ph.D., D.B.A.T.	Signature:	Cheryl B Bast
	Date:	OCT 02 1998
Robert H. Ross, M.S., Group Leader	Signature:	Robert H. Ross
	Date:	OCT 02 1998
Quality Assurance: Susan Chang, M.S.	Signature:	Robert H. Ross for SS Chang
	Date:	OCT 02 1998

Disclaimer

This Data Evaluation Report may have been altered by the Biopesticides and Pollution Prevention Division subsequent to signing by Oak Ridge National Laboratory personnel.

Oak Ridge National Laboratory, managed by Lockheed Martin Energy Research Corp. for the U.S. Department of Energy under contract number DE-AC05-96OR22464.

EPA Work Assignment Manager: Reilly
Biopesticides & Pollution Prevention Division

Roger Gardner for Date 11/20/98

DATA EVALUATION RECORD

STUDY TYPE: *In vitro* mammalian cytogenetics (chromosomal aberration) assay in Chinese hamster ovary CHO-K1 cells; OPPTS 870.5375 [§84-2]

DP BARCODE: D243976
CASE: 061954

SUBMISSION CODE: S538748
TOX. CHEM. NO.: 011550

TEST MATERIAL (PURITY): Granola 97 (p-methane-3,8-diol)(98.3% a.i.)

SYNONYMS: p-Menthane-3,8-diol;
SCJ NB # 14735R108

CITATION: Gudi, R. and E.H. Schadly (1997) *In vitro* mammalian cytogenetic test using Chinese hamster ovary (CHO) cells with Granola 97 (SCJ NB # 14735R108). MA BioServices, Inc., 9630 Medical Center Drive, Rockville, MD 20850. Laboratory study number G97BF49.335. November 20, 1997. MRID 44438708. Unpublished

SPONSOR: S.C. Johnson & Son, Inc., 1525 Howe Street, Racine, WI 53403-2236

EXECUTIVE SUMMARY: In a mammalian cell chromosomal aberration cytogenetics assay (MRID 44438708), Chinese hamster ovary CHO-K1 cell cultures were exposed to Granola 97 (98.3% a.i., batch No. 703001) in DMSO in two independent assays. In the initial assay, concentrations of 50, 150, 500 and 1500 µg/mL, with and without metabolic activation (S9-mix), were evaluated following a 6 hour treatment and a 14 hour recovery period. In the repeat assay without S9-mix, concentrations of 250, 500, 1000 and 1500 µg/mL were evaluated after 20 hours continuous treatment and concentrations of 125, 250, 500 and 1000 µg/mL were evaluated after 44 hours continuous treatment. In the repeat assay with S9-mix, concentrations of 250, 500, 1000 and 1500 µg/mL were evaluated after 6 hours treatment and either a 14 hour or 38 hour recovery period. The S9-fraction was obtained from Aroclor 1254 induced male Sprague-Dawley rat liver.

Granola 97 was tested up to cytotoxic concentrations. In the initial assay without S9-mix, cell growth was essentially uneffected at Granola 97 concentrations up to and including 500 µg/mL, growth was reduced to 72% compared to the solvent control at 1500 µg/mL and reduced to 0% at 5000 µg/mL. In the presence of S9-mix, cell growth was 55% of the solvent control at 1500 µg/mL and 0% at 5000 µg/mL. In the repeat assay after 20 hours exposure to Granola 97 without S9-mix, cell growth remained above 50% of the solvent control value at all concentrations except 3000 µg/mL where growth was 4% of the solvent control. After 44 hours exposure, cell growth remained above 50% of the solvent control value at concentrations up to and including 500 µg/mL and was reduced to 42%, 18% and 0% at 1000, 1500 and 3000 µg/mL, respectively. In the presence of S9-mix (6 hour exposure), cell growth remained above 50% of the solvent

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control value at all concentrations except 3000 µg/mL where growth was 3% of the solvent control. The test material precipitated in treatment medium at 5000 µg/mL with S9-mix but not in the absence of S9-mix or at any other concentration. In the initial assay without S9-mix, no statistically significant increase ($p \leq 0.05$) over the solvent control was seen in the percentage of cells with structural or numerical aberrations at any tested concentrations of Granola 97. In the presence of S9-mix, there was no significant increase in the percentage of cells with numerical aberrations at any concentration of Granola 97 tested; however, there was a statistically significant increase in the percentage of cells with structural aberrations relative to the solvent control at 150 and 1500 µg/mL. The percentages of aberrant cells found at these two concentrations (3.5% each) were within the historical solvent control range (0 - 6%) and were not statistically increased compared to untreated controls. Also the Cochran-Armitage trend test was negative for a dose response ($p \geq 0.05$). Therefore, the percentage increase in cells with structural aberrations is unlikely to be biologically significant. Positive and solvent control values were appropriate in the initial assay. In the repeat assay without S9-mix, there was no statistically significant increase in structural or numerical aberrations at any test material concentration at the 20 hour harvest time. At the 44 hour harvest time, a significant increase was seen in the percentage of cells with numerical aberrations at 1000 µg/mL (6.5% vs 2.5% for the solvent control) and in the percentage of cells with structural aberrations at 125 µg/mL (10.5% vs 4.0% for the solvent control). No statistically significant increase in structrual aberrations was seen at 250, 500 or 1000 µg/mL although a dose-related increase in cytotoxicity was seen. A positive response at the lowest dose tested with no supporting evidence at higher concentrations makes it unlikely that the increase in percentage of cells with structural aberrations at 125 µg/mL is biologically relevant. Likewise, the statistically significant increase in numerical aberrations at 1000 µg/mL was not considered biologically significant because the percentage of aberrant cells (6.5%) was only 1% above the historical solvent control range of 0 to 5.5% and within the historical untreated control range of 0 to 6.5%. Positive and solvent control values were appropriate. **There was no evidence of chromosomal aberrations induced over background.**

This study is classified as acceptable (guideline). It satisfies the requirement for FIFRA Test Guideline 84-2 for in vitro cytogenetic mutagenicity data.

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Granola 97

Description: thick, clear, colorless liquid
Lot/Batch #: 703001
Purity: 98.3% a.i.
Stability of compound: responsibility of sponsor
CAS #: 42822-86-6
Structure: not provided

Solvent used: DMSO
Other comments: store at 37±2°C, protected from light

2. Control materials

Negative: untreated
Solvent/final concentration: DMSO /
Positive:(concentrations/solvent)
Nonactivation: mitomycin C / 0.08 and 0.15 µg/mL / H_2O
Activation: cyclophosphamide / 5 and 10 µg/mL / H_2O

3. Activation: S9 derived from male Sprague-Dawley rats

x Aroclor 1254	x induced	x rat	x liver
_ phenobarbital	__ non-induced	_ mouse	__ lung
_ none		_ hamster	__ other
_ other		__other	

S9 mix composition:
2 mM $MgCl_2$, 6 mM KCl, 1 mM glucose-6-phosphate, 1 mM NADP and 20 µL S9 fraction per mL of medium (McCoy's 5A serum-free medium supplemented with 100 units penicillin and 100 µg streptomycin/mL and 2 mM L-glutamine)

4. Test compound concentrations used

Initial assay: (6 hour treatment, 14 hour recovery)
Toxicity test:
Nonactivated conditions: 1.5, 5, 15, 50, 150, 500, 1500, 5000 µg/mL
Activated conditions: 1.5, 5, 15, 50, 150, 500, 1500, 5000 µg/mL
Cytogenetic test:
Nonactivated conditions: 50, 150, 500, 1500 µg/mL
Activated conditions: 50, 150, 500, 1500 µg/mL
Repeat assay:
Toxicity test:
Nonactivated conditions: 62.5, 125, 250, 500, 1000, 1500, 3000 µg/mL (20 and 44 hour continuous treatment)
Activated conditions: 62.5, 125, 250, 500, 1000, 1500, 3000 µg/mL (6 hour treatment, 14 hour recovery and 6 hour treatment, 38 hour recovery)
Cytogenetic test:
Nonactivated conditions: 250, 500, 1000, 1500 µg/mL (20 hour continuous treatment); 125, 250, 500, 1000 µg/mL (44 hour continuous treatment)
Activated conditions: 250, 500, 1000, 1500 µg/mL (6 hour treatment, 14 hour recovery and 6 hour treatment, 38 hour recovery)

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5. Test cells: mammalian cells in culture - Chinese hamster ovary CHO-K1 cells

Properly maintained? Y

Cell line or strain periodically checked for Mycoplasma contamination? Y

Cell line or strain periodically checked for karyotype stability? Y (not specifically checked but not used beyond passage 20 to assure karyotypic stability)

B. TEST PERFORMANCE

1. Preliminary cytotoxicity assay

A cytotoxicity evaluation was included as part of the initial and repeat cytogenetic assays with cytotoxicity determinations based upon cell growth inhibition relative to the solvent control.

2. Cytogenetic assay

a. Cell treatment:

Cells exposed to test compound, solvent, or positive control for 6, 20 or 44 hours (nonactivated), 6 hours (activated)

b. Spindle inhibition

Inhibition used/concentration: Colcemid® / 0.1 µg/mL

Administration time: 2 hours (before cell harvest)

c. Cell harvest

Cells exposed to test material, solvent or positive control were harvested 0 or 14 hours after termination of treatment (nonactivated), 14 or 38 hours after termination of treatment (activated)

d. Details of slide preparation

Cells were harvested by trypsinization and collected by centrifugation at approximately 800 rpm for five minutes. The cell pellet was resuspended in two to four mL of 0.075 M KCl and allowed to stand at room temperature for about four to eight minutes. The cells were collected again by centrifugation, the supernatant aspirated and the cells fixed with two washes of approximately two mL fixative (methanol:glacial acetic acid, 3:1, v/v).

The fixed cells were centrifuged at 800 rpm for five minutes, the supernatant decanted and the cells resuspended to opalescence in fresh fixative. A portion of

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this suspension was dropped onto the center of a glass slide and allowed to air dry. The slides were then stained with 5% Giemsa, air dried, permanently mounted and coded.

e. Metaphase analysis

No. of cells examined per dose: 200

Scored for structural: Y

Scored for numerical: Y (polyploid and endoreduplicated cells)

Coded prior to analysis: Y

f. Evaluation criteria

Whenever possible, 200 metaphase spreads (20±2 centromeres), 100 per duplicate flask, were scored per treatment group. The chromatid aberrations scored were chromatid and isochromatid breaks, exchange figures such as quadriradials and triradials and complex rearrangements. The chromosome aberrations scored were breaks and exchange figures such as dicentrics and rings. Fragments observed in the absence of any exchange figure were scored as breaks while those with an exchange figure were not scored as an aberration but were considered part of the incomplete exchange. Pulverized cells, pulverized chromosomes and cells with ≥10 aberrations were recorded. Chromatid and isochromatid gaps were recorded but not included in the analysis. The percentage of polyploid and endoreduplicated cells was recorded per 100 metaphase cells and the mitotic index recorded as the percentage of cells in mitosis per 500 cells counted.

g. Statistical analysis

Data were evaluated for statistical significance at $p \leq 0.05$, using Fisher's exact test

II. REPORTED RESULTS

A. PRELIMINARY CYTOTOXICITY ASSAY

No preliminary cytotoxicity assay was performed; however, cytotoxicity determinations were part of the initial and repeat cytogenetic assays.

B. CYTOGENETIC ASSAY

Initial: Cells were exposed to Granola 97 concentrations ranging from 1.5 to 5000 µg/mL, with and without S9-mix. The test material precipitated in treatment medium at 5000 µg/mL with S9-mix but not in the absence of S9-mix or at any other concentration. In the absence of S9-mix, cell growth was essentially uneffected at Granola 97 concentra-

tions up to and including 500 µg/mL, growth was reduced to 72% compared to the solvent control at 1500 µg/mL and reduced to 0% at 5000 µg/mL. In the presence of S9-mix, cell growth was 55% of the solvent control at 1500 µg/mL and 0% at 5000 µg/mL. Results of the concurrent cytotoxicity test are given in Appendix Table 1 (MRID 44438708, p.19). Four concentrations of Granola 97 were evaluated for cytogenetic effects: 50, 150, 500 and 1500 µg/mL with and without S9-mix. The mitotic index at 1500 µg/mL was not reduced relative to the solvent control in the absence of S9-mix but was reduced by 18% in the presence of S9-mix.

In the absence of S9-mix, no statistically significant increase (statistical significance defined as $p \leq 0.05$ unless otherwise specified) over the solvent control was seen in the percentage of cells with structural or numerical aberrations at any tested concentrations of Granola 97. Positive and solvent control values were appropriate. **In the presence of S9-mix**, there was no significant increase in the percentage of cells with numerical aberrations at any concentration of Granola 97 tested; however, there was a statistically significant increase in the percentage of cells with structural aberrations relative to the solvent control at 150 and 1500 µg/mL. The percentages of aberrant cells found at these two concentrations (3.5% each) were within the historical solvent control range (0 - 6%) and were not statistically increased compared to untreated controls. Historical control values are given as an attachment to the Appendix (from MRID 44438708, pp. 34 and 35). Also the Cochran-Armitage trend test was negative for a dose response ($p \geq 0.05$). Therefore, the percentage increase in cells with structural aberrations is unlikely to be biologically significant. The solvent and positive control values were appropriate. Results of the initial cytogenetics assay are summarized in Appendix Table 2 (MRID 44438708, p. 23).

Repeat: Seven concentrations of Granola 97 ranging from 62.5 to 3000 µg/mL were tested with and without S9-mix in the repeat cytogenetic assay. Exposure times were 20 and 44 hours without S9-mix and 6 hours with S9-mix. In the absence of S9-mix, after 20 hours exposure to Granola 97 cell growth remained above 50% of the solvent control value at all concentrations except 3000 µg/mL where growth was 4% of the solvent control. After 44 hours exposure, cell growth remained above 50% of the solvent control value at concentrations up to and including 500 µg/mL and was reduced to 42%, 18% and 0% at 1000, 1500 and 3000 µg/mL, respectively. In the presence of S9-mix (6 hour exposure), cell growth remained above 50% of the solvent control value at all concentrations except 3000 µg/mL where growth was 3% of the solvent control. Results of the concurrent cytotoxicity test are given in Appendix Table 3 (MRID 44438708, p. 24).

In the absence of S9-mix, four Granola 97 concentrations ranging from 250 to 1500 µg/mL were evaluated for cytogenetic effects at the 20 hour harvest time and four concentrations ranging from 125 to 1000 µg/mL were evaluated at the 44 hour harvest time. No dose-related effect on the mitotic index was seen at the 20 hour harvest time; however, at 44 hours, the mitotic index was reduced to 50% of the concurrent solvent control at 1000 µg/mL. There was no statistically significant increase in structural or numerical aberrations at any test material concentration at the 20 hour harvest time. At the 44 hour harvest time, a significant increase was seen in the percentage of cells with numerical aberrations at 1000 µg/mL (6.5% vs 2.5% for the solvent control) and in the

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percentage of cells with structural aberrations at 125 µg/mL (10.5% vs 4.0% for the solvent control). Because of the apparent positive effect at 125 µg/mL, extra slides from this dose level were evaluated and a statistical analysis, performed on the pooled data from all slides at this dose, showed significance at $p \leq 0.01$. No statistically significant increase in structrual aberrations was seen at 250, 500 or 1000 µg/mL although a dose-related increase in cytotoxicity was seen. A positive response at the lowest dose tested with no supporting evidence at higher concentrations led the authors to conclude that the increase at 125 µg/mL was not scientifically relevant. Likewise, the statistically significant increase in numerical aberrations at 1000 µg/mL was not considered biological significant because the percentage of aberrant cells (6.5%) was only 1% above the historical solvent control range of 0 to 5.5% and within the historical untreated control range of 0 to 6.5%. Positive and solvent control values were appropriate. Results from the 20 hour harvest time are summarized in the top half of Appendix Table 4 (MRID 44438708, p.32).

In the presence of S9-mix, the mitotic index varied somewhat compared to the solvent control at both the 20 and 44 hours harvest times but not in a dose-related manner. No statistically significant increase in the percentage of cells with numerical or structural aberrations was seen at any tested dose at the 20 hour harvest time. At the 44 hour harvest time, no significant increase in percentage of cells with numerical aberrations was seen at any tested dose; however, a statistically significant, dose-related increase in the percentage of cells with structural aberrations (2.5 to 3.5%) was seen. The percentage of aberrant cells at all doses was within the historical solvent control range of 0 to 6.0% and, therefore, not considered biologically significant by the authors. Positive and solvent control values were appropriate. Results from the 44 hour harvest time are summarized in the bottom half of Appendix Table 4 (MRID 44438708, p.32).

III. REVIEWER'S DISCUSSION/CONCLUSIONS

A. This is an acceptable study. Granola 97 was tested to cytotoxic concentrations, acceptable experimental protocol was followed and the positive and solvent control values were appropriate. As discussed in section II.B., a statistically significant increase in the percentage of cells with structural aberrations compared with the solvent control was seen at a number of experimental points in this study. Although statistically significant, the results at 150 and 1500 µg/mL with S9-mix in the initial assay and at 250, 500, 1000 and 1500 µg/mL with S9-mix in the repeat assay (44 hour harvest) were not considered biologically significant because the values were within the historical solvent control or untreated control ranges. The increase in percentage of cells with structural aberrations after a 44 hour exposure to 125 µg/mL without S9-mix in the repeat assay was outside the historical solvent or untreated control ranges but was considered not relevant because the increase occurred only at this dose (the lowest dose tested) and not at three higher doses. One incidence of a statistically significant increase in percentage of cells with numerical aberrations occurred. This increase, seen after a 44 hour exposure to 1000 µg/mL Granola 97 without S9-mix in the repeat assay, was not considered biologically significant because the increase was only 1% above the historical solvent control range and within the untreated control range. The reviewers concur with the

authors' conclusions that none of increases represent a test material induced increase in percentage of cells with structural or numerical aberrations.

B. STUDY DEFICIENCIES

No study deficiencies were identified.

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DATA EVALUATION REPORT

p-MENTHANE-3,8-DIOL

STUDY TYPE: ***in Vivo*** **Mammalian Cytogenetics - Micronucleus Assay in Mouse Bone Marrow; OPPTS 870.5395 [§84-2]**

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

Chemical Hazard Evaluation Group
Toxicology and Risk Analysis Section
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 22

Primary Reviewer: Bradford L. Whitfield, Ph.D.	Signature:	BL Whitfield
	Date:	9/25/98
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	Date:	OCT 0 2 1998
Robert H. Ross, M.S., Group Leader	Signature:	Robert H. Ross
	Date:	OCT 0 2 1998
Quality Assurance: Susan Chang, M.S.	Signature:	Robert H. Ross for S.S. Chang
	Date:	OCT 0 2 1998

Disclaimer

This Data Evaluation Report may have been altered by the Biopesticides and Pollution Prevention Division subsequent to signing by Oak Ridge National Laboratory personnel.

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Oak Ridge National Laboratory, managed by Lockheed Martin Energy Research Corp. for the U.S. Department of Energy under contract number DE-AC05-96OR22464.

EPA Work Assignment Manager: Reilly
Biopesticides & Pollution Prevention Division

Roger Gardner for, Date 11/20/98

DATA EVALUATION RECORD

STUDY TYPE: *In vivo* mammalian cytogenetics - micronucleus assay in mouse bone marrow; OPPTS 870.5395 [§84-2]

DP BARCODE: D243976
CASE: 061954

SUBMISSION CODE: S538748
TOX. CHEM. NO.: 011550

TEST MATERIAL (PURITY): Granola 97 (p-methane-3,8-diol)(98.3% a.i.)

SYNONYMS: p-Menthane-3,8-diol;
SCJ NB # 14735R108

CITATION: Gudi, R. and P. Ritter (1997) Mammalian erythrocyte micronucleus test with granola 97 (SCJ NB # 14735R108). MA BioServices, Inc., 9630 Medical Center Drive, Rockville, MD 20850. Laboratory study number G97BF49.123001. November 20, 1997. MRID 44438707. Unpublished

SPONSOR: S.C. Johnson & Son, Inc., 1525 Howe Street, Racine, WI 53403-2236

EXECUTIVE SUMMARY: In an ICR mouse bone marrow micronucleus assay (MRID 44438707), five mice/sex/dose were treated once i.p. with Granola 97 in corn oil (98.3% a.i., batch No. 703001) at doses of 104, 208, 416 mg/kg or dermally over four days with 3 mL/kg total of neat agent. Bone marrow cells were harvested at 24 hours (all doses) and at 48 hours (416 mg/kg only) post-treatment.

Granola 97 was tested at an adequate dose. All mice in the 208 and 416 mg/kg groups were lethargic following treatment. Convulsions and prostration were also seen in all mice in the 416 mg/kg group. Seven of 15 males and 7/15 females in the 416 mg/kg group displayed pilo-erection. All mice in the dermal application group showed both hyperactivity and lethargy after treatment. There was no statistically significant increase in the number of micronucleated PCEs at any dose, harvest time or route of exposure tested in this study. The solvent and positive control values were appropriate (The mean number of micronucleated PCEs per 1000 PCEs was, for the corn oil control, 0.7 ± 1.10 in males and 0.5 ± 0.61 in females at 24 hours and 0.7 ± 0.84 in males and 0.5 ± 0.35 in females at 48 hours. Positive control values were 19.4 ± 3.13 and 17.1 ± 4.60 for males and females, respectively, at 24 hours). **There was no significant increase in the frequency of micronucleated polychromatic erythrocytes in bone marrow at any dose, harvest time or route of exposure.**

This study is classified as acceptable (guideline). It satisfies the requirement for FIFRA Test Guideline 84-2 for *in vivo* cytogenetic mutagenicity data.

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Granola 97

Description: thick, clear, colorless liquid
Lot/Batch #: 703001
Purity: 98.3% a.i.
Stability of compound: responsibility of sponsor
CAS #: 42822-86-6
Structure: not provided
Solvent used: DMSO
Other comments: store at 37±2°C, protected from light

2. Control materials

Vehicle/Final volume/Route of administration:
corn oil / 20 mL/kg body weight / i.p.;
corn oil / 20 mL/kg body weight / dermal

Positive/Final dose(s)/Route of administration:
cyclophosphamide / 60 mg/kg body weight / i.p.

3. Test compound administration

Volume of test substance administered:
20 mL/kg body weight i.p.; 3 mL/kg body weight dermal
Route of administration: i.p. and dermal
Dose levels used:
Pilot assay:1, 10, 100, 1000 mg/kg (males only); 2000 mg/kg (males and females)
Cytotoxicity assay: 100, 300, 600, 800 mg/kg (males and females)
Micronucleus assay:
i.p. - 104, 208, 416 mg/kg (males and females); dermal -3 mL/kg total of neat agent over four days (male and female)

4. Test animals

a. Species mouse Strain ICR Age 6-8 wks
Weight:
Pilot study - male 30.3-33.7 g female 25.2-27.5 g
Cytotoxicity study - male 32.0-35.3 g female 24.9-27.8 g
Micronucleus assay - males 30.2-34.7 g female 23.7-30.0 g

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Source: Harlan Sprague Dawley, Inc., Frederick, MD

b. No. animals used per dose: _5_ males _5_ females

c. Properly maintained? Y

B. TEST PERFORMANCE

1. Treatment and sampling times

a. Test compound and vehicle control

i.p. dosing: _x_ once ___ twice (24 hr apart)
___ other (describe):

Dermal dosing: ___ once ___ twice (24 hr apart)
_x_ other (describe): daily for four days

Sampling (after last dose): ___ 6 hr ___ 12 hr
_x_ 24 hr _x_ 48 hr ___ 72 hr (mark all that are appropriate), ___ other (describe):

b. Positive control
Dosing: _x_ once ___ twice (24 hr apart)
___ other (describe):

Sampling (after last dose): ___ 6 hr ___ 12 hr
_x_ 24 hr ___ 48 hr ___ 72 hr (mark all that are appropriate), other (describe):

2. Tissues and cells examined:

_x_ bone marrow ___ other (list):

No. of polychromatic erythrocytes (PCE) examined per animal: 2000
No. of normochromatic erythrocytes (NCE; more mature RBCs) examined per animal:
the number found while screening 2000 PCEs

3. Details of slide preparation

Mice were killed by CO_2 inhalation at the designated sacrifice time, the femurs were exposed and cut just above the knee and the bone marrow aspirated into a syringe containing fetal bovine serum. The bone marrow cells were transferred to a centrifuge tube containing 1 mL of fetal bovine serum, the tube capped and the cells pelleted by centrifugation at approximately 100 x g for five minutes. Most of the supernatant was removed and the cells resuspended in the remaining serum. A small drop of the cell suspension was spread onto a clean glass slide (two to four slides per mouse),

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the slides were fixed in methanol, stained with May-Gruenwald-Giemsa and permanently mounted. Slides were independently coded using a random number table.

4. Statistical methods

Statistical significance ($p \leq 0.05$) was determined using the Kastenbaum-Bowman tables. All analyses were performed separately for each sex and sampling time.

5. Evaluation criteria

Micronuclei were defined as "round, darkly staining nuclear fragments, having a sharp contour with diameters usually from 1/20 to 1/5 of the erythrocyte". Results were considered positive if there was a positive dose-responsive increase in micronucleated PCEs and the increase in micronucleated PCEs at one or more doses was statistically elevated relative to the vehicle control ($p \leq 0.05$) at any sampling time. A significant increase at one sacrifice time in a single treatment group with no dose-response was considered a suspect or unconfirmed positive. The test article was considered negative if no statistically significant increase in micronucleated PCEs above the concurrent solvent control was seen at any sampling time. To be considered a valid test, the mean incidence of micronucleated PCEs must not exceed 5 per 1000 PCEs in the solvent control and the incidence of micronucleated PCEs in the positive control group must be significantly increased relative to the solvent control group ($p \leq 0.05$).

II. REPORTED RESULTS

A. Preliminary toxicity assay: In a pilot study, two male mice were treated once i.p. with one of four concentrations of Granola 97 ranging from 1 to 1000 mg/kg. Five males and five female mice were treated once with 2000 mg/kg. All mice treated with 2000 mg/kg Granola 97 and both males treated with 1000 mg/kg had convulsions and died within four hours of treatment. All other mice appeared clinically normal throughout the three day observation period.

A preliminary toxicity assay was also conducted using Granola 97 concentrations of 100, 300, 600 and 800 mg/kg. Groups of five males and five females were treated i.p. at each dose level. One of five females at 600 mg/kg and three of five males and two of five females at 800 mg/kg died within one day of dosing. Clinical signs seen after dosing were lethargy, convulsions and prostration in both males and females at 300, 600 and 800 mg/kg. Ataxia was seen in males and females and piloerection in males at 600 mg/kg. Irregular breathing was seen in males and females at 800 mg/kg. An $LD_{50/3}$ of 831.6 mg/kg for both male and female mice was calculated by probit analysis and 416 mg/kg (50% of the $LD_{50/3}$) selected as the upper dose for the micronucleus assay. Details of the preliminary toxicity assay are given in Appendix Table 1 (MRID 44438707, p. 16).

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B. MICRONUCLEUS ASSAY

Five males and five females per dose group were treated i.p. once with 104, 208 or 416 mg/kg Granola 97 in a volume of 20 mL/kg body weight. Another group of five mice of each sex was treated dermally with neat Granola 97 for four consecutive days at a total volume of 3 mL/kg. No deaths occurred in any of the dose groups. All mice in the 208 and 416 mg/kg groups were lethargic following treatment. Convulsions and prostration were also seen in all mice in the 416 mg/kg group. Seven of 15 males and 7/15 females in the 416 mg/kg group displayed piloerection. All mice in the dermal application group showed both hyperactivity and lethargy after treatment.

There was no statistically significant increase ($p \leq 0.05$) in the incidence of micronucleated PCEs over solvent control values in either sex at any Granola 97 concentration, route of exposure or sacrifice time. Slightly reduced PCE/total erythrocyte ratios were reported in males at 104 and 208 mg/kg i.p. at 24 hours(11% decrease compared to solvent control values at both concentrations). No reduction in the PCE/total erythrocyte ratio was seen in either sex at the 24 hour harvest time at 416 mg/kg but at 48 hours the ratios at this dose were reduced by 4% in males and 2% in females. The reductions are unlikely to be biologically significant. The 11% reduction at 104 mg/kg was due to a very low value in one mouse (0.20 compared to the solvent control value of 0.54, also only 980 PCEs were found on the slides from this mouse compared to at least 2000 from other mice). When this mouse is excluded from the calculation, the PCE/total erythrocyte ratio is the same as the solvent control value. Solvent and positive control values were appropriate (the testing laboratory's historical control data are given in an attachment to the Appendix (from MRID 44438707, p. 25)).

Results from the i.p. and dermal routes of exposure are summarized in Appendix Table 2 (MRID 44438707, p. 19) and Appendix Table 3, (MRID 44438707, p. 22), respectively.

III. REVIEWER'S DISCUSSION/CONCLUSIONS

A. This is an acceptable study. Granola 97 was tested to toxic levels and appropriate experimental protocol was followed. Positive and solvent control values were acceptable. Although the authors report slight reductions in the PCE/total erythrocyte ratio, as discussed in section II.B., this is not likely to represent bone marrow toxicity. No evidence was found in this study for a micronuclei inducing potential of Granola 97.

B. STUDY DEFICIENCIES

No study deficiencies were identified.

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DATA EVALUATION REPORT

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STUDY TYPE: **Mammalian Cells in Culture Gene Mutation Assay in Mouse Lymphoma L5178Y cells; OPPTS 870.5300 [§84-2]**

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

Chemical Hazard Evaluation Group
Toxicology and Risk Analysis Section
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Task Order No. 22

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Date: OCT 02 1998

Disclaimer

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Oak Ridge National Laboratory, managed by Lockheed Martin Energy Research Corp. for the U.S. Department of Energy under contract number DE-AC05-96OR22464.

EPA Work Assignment Manager: Reilly
Biopesticides & Pollution Prevention Division

Roger Gardner for, Date 11/20/98

DATA EVALUATION RECORD

STUDY TYPE: Mammalian cells in culture gene mutation assay in mouse lymphoma L5178Y cells; OPPTS 870.5300 [§84-2]

DP BARCODE: D243976
CASE: 061954

SUBMISSION CODE: S538748
TOX. CHEM. NO.: 011550

TEST MATERIAL (PURITY): Granola 97 (p-methane-3,8-diol)(98.3% a.i.)

SYNONYMS: p-Menthane-3,8-diol;
SCJ NB # 14735R108

CITATION: San, R.H. and J.J. Clarke (1997) *In vitro* mammalian cell gene mutation test with Granola 97 (SCJ NB # 14735R108). MA BioServices, Inc., 9630 Medical Center Drive, Rockville, MD 20850. Laboratory study number G97BF49.702. November 18, 1997. MRID 44438706. Unpublished

SPONSOR: S.C. Johnson & Son, Inc., 1525 Howe Street, Racine, WI 53403-2236

EXECUTIVE SUMMARY: In a mammalian cell gene mutation assay at the thymidine kinase locus (MRID 44438706), L5178Y/TK$^{\pm}$ cells cultured in vitro were exposed to Granola 97 (98.3% a.i., batch No. 703001) in DMSO at concentrations of 600, 800, 1000, 1250, 1500 and 2000 µg/mL in the absence of mammalian metabolic activation (S9-mix) and to concentrations of 500, 600, 800, 1000, 1250 and 1500 µg/mL in the presence of S9-mix. The S9-fraction was obtained from Aroclor 1254 induced male Sprague-Dawley rat liver.

Granola 97 was tested up to cytotoxic concentrations. In the preliminary cytotoxicity assay, little or no cell growth occurred at 1500 or 5000 µg/mL, with or without S9-mix. Cell growth was unaffected at 500 µg/mL and lower concentrations in the presence of S9-mix or at concentrations of 150 µg/mL and lower in the absence of S9-mix. Cell growth was 70% of the solvent control at 500 µg/mL without S9-mix. In the main mutagenicity assay, the 2000 µg/mL and 1500 µg/mL doses were too toxic to clone in the absence and presence of S9-mix, respectively. Cultures were cloned for five Granola 97 concentrations ranging from 600 to 1500 µg/mL without S9-mix and five concentrations ranging from 500 to 1250 µg/mL with S9-mix. All plating was in triplicate with duplicate cultures at each concentration. No visible precipitate was seen in the treatment medium at any dose level. There was no evidence of a mutagenic effect at any concentration of Granola 97 tested, with or without S9-mix. One of the duplicate cultures at 500 µg/mL with S9-mix did have a mutant frequency of 56 per 10^6 clonable cells over the solvent control value (a mutant frequency of 55 to 99 mutants per 10^6 clonable cells was the criterium for an equivocal response). The other duplicate culture at this concentration had a mutant frequency of 8 mutants

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appropriate responses, within the laboratory's historical control ranges. **There was no evidence of induced mutant colonies over background.**

This study is classified as acceptable (guideline). It satisfies the requirement for FIFRA Test Guideline 84-2 for in vitro mutagenicity (mammalian forward gene mutation) data.

COMPLIANCE: Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided. A Flagging statement was not provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Granola 97

   Description: thick, clear, colorless liquid
   Lot/Batch #: 703001
   Purity: 98.3% a.i.
   Stability of compound: responsibility of sponsor
   CAS #: 42822-86-6
   Structure: not provided
   Solvent used: DMSO
   Other comments: store at 37±2°C, protected from light

2. Control materials

   Solvent/final concentration: DMSO / 100 µL

   Positive:

   Nonactivation (concentrations/solvent): methyl methanesulfonate / 10 and 20 µg /mL / treatment medium

   Activation (concentrations/solvent): 7,12-dimethyl-benz(a)anthracene / 2.5 and 4.0 µg/mL / treatment medium

3. Activation: S9 derived from male Sprague-Dawley rats

x Aroclor 1254	x induced	x rat	x liver
_ phenobarbital	_ non-induced	_ mouse	_ lung
_ none		_ hamster	_ other
_ other			_ other

S9 mix composition:
11.25 mg DL-isocitric acid, 6 mg NADP and 0.25 mL S9 homogenate per mL in F_0P (Fischer's Medium for Leukemic Cells of Mice with 0.1% Pluronics). The cofactor mixture was adjusted to pH 7.0 prior to the addition of S9 homogenate.

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4. Test cells: mammalian cells in culture

x mouse lymphoma L5178Y cells
_ Chinese hamster ovary (CHO) cells
_ V79 cells (Chinese hamster lung fibroblasts)
_ other (list):

Properly maintained? Y
Periodically checked for Mycoplasma contamination? Y
Periodically checked for karyotype stability? Y
Periodically "cleansed" against high spontaneous background? Y

Media: Treatment medium was Fischer's Medium for Leukemic Cells of Mice with 0.1% Pluronics (F_0P). Expression medium was F_0P supplemented with 10% horse serum and 2 mM L-glutamine ($F_{10}P$). Selection medium was "cloning medium" (undefined) containing 0.23% granulated agar and 3 µg/mL trifluorothymidine.

5. Locus examined

x thymidine kinase (TK)
Selection agent: _____ bromodeoxyuridine (BrdU)
(give concentr.) _____ fluorodeoxyuridine (FdU)
3 µg/mL trifluorothymidine (TFT)

_ hypoxanthine-guanine-phosphoribosyl transferase (HPRT)
Selection agent: _____ 8-azaguanine (8-AG)
(give concentr. _____ 6-thioguanine (6-TG)

_ Na^+/K^+ ATPase
Selection agent: _____ ouabain
(give concentration)

_ other (locus and/or selection agent; give details):

6. Test compound concentrations used:

Preliminary Cytotoxicity assay:
Nonactivated and activated conditions: 0.5, 1.5, 5, 15, 50, 150, 500, 1500, 5000 µg/mL

Mutagenicity assay:
Nonactivated conditions: 600, 800, 1000, 1250, 1500, 2000 µg/mL
Activated conditions: 500, 600, 800, 1000, 1250, 1500 µg/mL

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B. TEST PERFORMANCE

1. Cell treatment:

a. Cells exposed to test compound, negative/solvent or positive controls for: _4_ hours (nonactivated) _4_ hours (activated)

b. After washing, cells cultured for _2_ days (expression period) before cell selection:

c. After expression, _1 x 10^6_ cells/dish (_3_ dishes/ group) were cultured for _10-14_ days in selection medium to determine numbers of mutants and _200_ cells/dish (_3_ dishes/group) were cultured for _10-14_ days without selective agent to determine cloning efficiency.

2. Statistical methods: none used

3. Evaluation criteria: Criteria for a valid test were:

a. The mutants frequency of the solvent control must be within 20 to 100 TFT-resistant mutants per 10^6 surviving cells with a cloning efficiency greater than 50%.

b. At least one concentration of each positive control must exhibit mutant frequencies of $\geq$100 mutants per 10^6 clonable cells over the background level. The colony size distribution for the MMS positive control must show an increase in both small and large colonies.

c. At least four test material concentrations with analyzable mutant frequency data are required.

The following criteria were considered when interpreting the data:

a. The results were considered positive if a dose related increase in mutant frequency was seen and one or more dose levels (with 10% or greater total growth) exhibited mutant frequencies of $\geq$100 mutants per 10^6 clonable cells over the background level.

b. The results were considered equivocal if the mutant frequency in treated cultures was between 55 and 99 mutants per 10^6 clonable cells over background level.

c. Results were considered negative if fewer than 55 mutants per 10^6 clonable cells over the background level were seen in test material treated cultures.

Mutations occurring only at highly toxic concentration of test material (less than 10% total growth) were not considered biologically relevant. Colony size was

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determined for positive and solvent controls and in test material treated cultures showing a positive response.

II. REPORTED RESULTS

A. PRELIMINARY CYTOTOXICITY ASSAY

Nine concentrations of Granola 97 ranging from 0.5 to 5000 µg/mL were tested with and without S9-mix in the preliminary cytotoxicity assay. In the absence of S9-mix, Granola 97 did not reduce cell growth relative to the solvent control at concentrations up to and including 150 µg/mL. The relative grow was 70%, 5% and 0% at 500, 1500 and 5000 µg/mL, respectively. In the presence of S9-mix, Granola 97 did not reduce the relative growth at concentrations up to and including 500 µg/mL but completely eliminated growth at 1500 and 5000 µg/mL. Results of the preliminary cytotoxicity assay are presented in Appendix Table 1 (MRID 44438706, p.15).

B. MUTAGENICITY ASSAY

All plating was in triplicate with duplicate cultures at each concentration. The authors stated that concentrations of Granola 97 ranging from 100 to 2000 µg/mL were used for the mutagenesis assay; however, cultures were cloned only for five Granola 97 concentrations ranging from 600 to 1500 µg/mL without S9-mix and five concentrations ranging from 500 to 1250 µg/mL with S9-mix. In the absence of S9-mix, the 5000 µg/mL concentration was too toxic to clone while in the presence of S9-mix, 1500 µg/mL was too toxic to clone. No visible precipitate was seen in the treatment medium at any dose level. There was no evidence of a mutagenic effect at any concentration of Granola 97 tested, with or without S9-mix. One of the duplicate cultures at 500 µg/mL with S9-mix did have a mutant frequency of 56 per 10^6 clonable cell over the solvent control value (a mutant frequency of 55 to 99 mutants per 10^6 clonable cells was the criterium for an equivocal response). The other duplicate culture at this concentration had a mutant frequency of 8 mutants per 10^6 clonable cells over the solvent control. The two positive controls and the solvent control gave appropriate responses within the testing laboratory's historical ranges. Results of the mutagenesis assay are presented in Appendix Tables 2-5 (MRID 44438706, pp. 16-19).

III. REVIEWER'S DISCUSSION/CONCLUSIONS:

A. This is an acceptable study. Granola 97 was tested to cytotoxic concentrations, the experimental protocol was acceptable and the solvent and positive control values were appropriate. There was no evidence of a mutagenic effect at any tested concentration, with or without S9-mix.

B. STUDY DEFICIENCIES

No study deficiencies were identified.

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