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

OFFICE OF PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

DATE: October 4, 2007

SUBJECT: Science Review and Human Health Risk Assessment in Support of the Registration of the Insect Repellent Refined Oil of *Nepeta cataria* (TGAI), and two lotion end-use products.

Decision Nos.: 371861, 372756, 371862
DP Nos.: 338556, 339493, 339547
EPA File Symbol: 71654-EN (TGAI), 71654-EG (7% a.i.), 71654-ER (15% a.i.)

PC Code: 004801
MRID Nos.: 469773-01 through -06; 469774-01 through -14, -20 & -22; 470031-02 & -05; 470156-01 & -02

FROM: Roger Gardner, Senior Scientist
Biochemical Pesticides Branch
Biopesticides & Pollution Prevention Division (7511P)

TO: Raderrio Wilkins, Regulatory Action Leader
Biochemical Pesticides Branch
Biopesticides & Pollution Prevention Division (7511P)

******** CONTAINS CONFIDENTIAL BUSINESS INFORMATION ********

ACTION REQUESTED: Review of scientific information submitted by E.I. du Pont de Nemours and Company to support registration of Refined Oil of *Nepeta cataria* (71654-EN) as a dermally applied insect repellent in a 7% Lotion (71654-EG) a 15% Lotion (71654-ER).

RECOMMENDATIONS AND CONCLUSIONS

There are adequate data for conducting a risk assessment that supports registration of the insect repellent Refined Oil of *Nepeta casaria* and the 7% and 15% lotion products. Specific conclusions and recommendations are summarized as follows:

1. The active ingredient is classified into Toxicity Category III for oral toxicity and primary eye irritation and Toxicity Category IV for dermal, inhalation and skin irritation. It is not a skin sensitizer.

2. The lotion formulations containing 7 and 15% active ingredient are classified into Toxicity Category IV for oral and dermal toxicity as well as eye and skin irritation and they are not skin sensitizers.
    a. The acute inhalation study for the 15% lotion was waived on the basis of the lack of inhalable particles and viscosity of the formulation.
    b. The acute toxicity data for the 15% formulation are used to support registration of the 7% lotion.

3. In the acute neurotoxicity study, behavioral effects (decreased motor activity) were noted in rats after a single oral dose of 200 mg active ingredient per kg body weight, and effects were temporary with treated rats adapting to the neurological effects after repeated dosing in other studies.

4. The subchronic oral toxicity study in rats demonstrated a no-observed-effect level (NOEL) of 200 mg/kg/day and a lowest-observable-effect level (LOEL) of 1000 mg/kg/day based on the increased incidence of minimal to mild degeneration/regeneration of the olfactory epithelium lining the nasal turbanates of treated male and female rats.

5. No systemic toxicity was observed in the subchronic dermal toxicity study at dose levels up to 1000 mg/kg/day.

6. No adverse effects were observed in a 28-day oral immunotoxicity study or in a developmental toxicity study at oral doses up to 1000 mg/kg/day.

7. No genetic toxicity was observed in bacteria (point mutation assay), an in vitro cytogenetics assay, or in a mouse micronucleus assay. However, a point mutation assay in mouse lymphoma cells reported an increased frequency of point mutations at doses approaching cytotoxic levels without metabolic activation. These results should be confirmed with another assay in a mammalian cell system.

8. An in vitro dermal penetration study indicated that human skin is relatively impermeable (2% of the applied dose) compared to rat skin (78% of the applied dose).

9. Since there were no endpoints indicated in the subchronic dermal toxicity study, and since human skin is relatively impermeable , no endpoints were selected for risk characterizations. The acute neurotoxicity endpoint is appropriate to an incidental oral exposure for children, but because the effect is reversible and pharmacological in nature (reduced activity) and because the label contains instructions to avoid incidental exposure (i.e., licking of fingers and hands), no risk characterization was done for incidental oral scenarios.

10. The only data gap is for a confirmatory gene mutation assay in mammalian cells to determine reproducibility and/or reduce uncertainty associated with the positive results in the mouse lymphoma assay.

I. CHEMICAL AND PRODUCT IDENTITY

A. Background

The active ingredient is a refined, multi-component extract of *Nepeta cataria* which is a member of the mint family of plants (Labiatae). The technical grade active ingredient (TGAI) is identified on proposed product labels as Refined Oil of *Nepeta cateria* and is also referred to as hydrogenated catmint oil (HCO). The plant is commonly known as catnip and is indigenous from eastern Mediterranean to eastern Himalayan regions. The perennial herb can also be grown in North America. Therefore, general information on the nature of the active ingredient is readily available (e.g., http://chemistry.about.com/library/weekly/aa103001a.htm; accessed on October 2, 2007) and is summarized as background below.

Nepetalactone is the major component of the refined oil, but there are other components such as puleganic acid with known insect repellent activity. Nepetalactone is a terpene comprised of two isoprene units, and it has a chemical structure similar to that of the valepotrates (from the herb valerian) which have mild central nervous system effects in humans (sedative or stimulant depending on the individual).

The feline behavioral effects of the nepetalactone in catnip are well known, but not all cats respond to the activity of the oil; their sensitivity is inherited (an autosomal dominant gene). Sensitive kittens do not develop responsiveness until they are 3 months old, and young kittens have been known to exhibit avoidance behavior. The variety of responses includes rubbing of the head, chin, cheek or body as well as head shaking or rolling. Sensitive cats may also lick or chew the plant or other source of nepetalactone. These reactions are temporary and can not be induced for an hour or more after exposure. Individual responses vary among sensitive cats. Since the feline receptors for nepetalactone are located in the vomeronasal organ above the cat's palate, the response is associated with the inhalation route of exposure.

Historically, catnip has been used in herbal medicine to treat fever, head and tooth aches, colds, colic and spasms in humans. In some individuals catnip can be used to induce sleep, but it can also act as a stimulant in others. At high doses it is emetic in cats and humans. Other historical uses included rubbing meat with catnip leaves, adding it to salads or making tea with it.

Refined Oil of *Nepeta cataria* is being formulated into two lotion products for direct application to human skin to repel biting flies, mosquitoes and other insects. The two concentrations of the active ingredient proposed for these uses are 7% and 15%.

B. Physical and Chemical Properties (Table 1)

The principal insect repellent components in Refined Oil of *Nepeta cataria* are dihydronepetalactone (69.99% w/w) and puleganic acid (6.77% w/w).

Table1: Physical and Chemical Properties for Refined Oil of *Nepeta cataria*[a]

Guideline Reference No./Property		Description of Result	Methods
830.6302	Color	Yellow @ 21°C	CCL SOP 10.11
830.6303	Physical State	Liquid @ 21°C	CCL SOP 10.12
830.6304	Odor	Minty	CCL SOP 10.13
830.6313	Stability	Stable @ room and elevated temperatures and in the presence of metals and ions	OPPTS 830.6313
830.6314	Oxidation/Reduction: Chemical Incompatibility	Dihydronepetalactone was relatively stable in solution with metals and metal salts after 14 days at 25°C, with slight decreases at 54°C after 14 days.	
830.6315	Flammability	>99°C	CCL SOP 10.18
830.6316	Explodability	**Not addressed**	
830.6317	Storage Stability	In short-term testing at 25 and 54°C, dihydronepetalactone content was relatively stable. Guideline study is in progress.	
830.6319	Miscibility	Not applicable, product is not to be diluted in petroleum solvents	
830.6320	Corrosion Characteristics	Guideline study is in progress	
830.6321	Dielectric Breakdown Voltage	Not applicable, product is not for use around electrical equipment	
830.7000	pH	3.97 @ 25°C (1% w/w in deionized water)	CCL SOP 10.17
830.7050	UV/Visible Absorption	Not applicable,	
830.7100	Viscosity	18.09 mm^2/s (cSt) @ 22°C	ASTM D 445 and D446
830.7200	Melting Range	Not applicable, product is a liquid	
830.7220	Boiling Range	266.0 ± 12.0°C	Mettler FP900 Thermosystem
830.7300	Density/Relative Density/Bulk Density	1.0334 @ 20.7°C	**Not provided**
830.7370	Dissociation Constant in Water	Not applicable, required only for pure active ingredient	
830.7550	Partition Coefficient	Not applicable, required only for pure active ingredient	
830.7840	Water Solubility	0.254 ± 0.013 g/L @ 30°C	OPPTS 7840
830.7950	Vapor Pressure	591, 707, 907. 1100, 1320, and 1630 Pa @ 20, 25, 30, 35, and 40°C, respectively	Terranova 722A diaphragm gauge controller

[a]Data from MRIDs 46977420, 46977422, 47003102, 47003105

C. Use Pattern

End-use product labels include the following instructions for use:

> Dispense a small amount of lotion directly onto skin. Spread uniformly to completely cover any exposed skin surface. Reapplication after six hours may be necessary. When applying to children, dispense into an adult's hand and then spread evenly and completely over the child's exposed skin taking care not to contact the child's fingers and hands.
>
> Do not apply over cuts or damaged skin.

The signal word on the label is CAUTION, and other precautionary statements regarding hazards to humans and domestic animals include:

- Keep out of reach of children.
- Avoid contact with eyes.

First aid statements on the label are as follows:

Have the product container or label with you when calling a poison control center or doctor, or going for treatment.
If in Eyes: • Hold eye open and rinse slowlyand gently with water for 15-20 minutes. • Remove contact lenses, if present, after five minutes, then continue rinsing eye. • Call a Poison Control Center or doctor for further treatment advice.
If a reaction to this product is suspected: • Discontinue use. • Take off contaminated clothing. • Wash skin thoroughly with plenty of water. • Call a Poison Control Center or doctor for further treatment advice.
If Swallowed: • Call Poison Control Center or doctor immediately for treatment advice. • Do not induce vomiting unless told to do so by the poison control center or doctor • Do not give anything by mouth to an unconscious person

II. TOXICITY OF THE TGAI

A. Acute Toxicity

1. Active ingredient (Table 2)

In the acute oral toxicity study (MRID 46977401), one rat dosed at 1750 mg/kg and two dosed at 5000 mg/kg died or were sacrificed for humane reasons on the day of dosing. A surviving rat given 550 mg/kg exhibited no clinical signs of toxicity. Wet fur, lethargy, ataxia, partially closed

or dark eyes, slow or labored breathing, prostrate posture, lacrimation, stained fur/skin, dark extremities, and/or moribundity were noted on the other rats with recovery of the survivors by day 3 of observation.

Ataxia was noted during exposure or immediately after test material removal in the acute dermal toxicity study (MRID 46977402). Wet fur of the inguenal region, leaning, high carriage, absent feces, labored breathing, lethargy, lacrimation, not eating, and/or stained fur around face, perineum, inguen, or abdomen were noted with recovery by day 6 post-dosing.

Male rats in the inhalation study (MRID 44677406) exhibited lethargy, labored breathing and/or hunched posture immediately following exposure. Colored nasal discharge was noted form three males one day post-exposure with recovery by day 3. Lethargy, labored breathing, gasping, hunched posture, incoordination, and/or prostration were noted from two female rats immediately following exposure with recovery by day 4. Colored nasal, oral, or ocular discharge was noted from two females one day post-exposure with recovery by day 7 of observation.

Table 2: Acute Toxicity Profile – Hydrogenated Catmint Oil

Study Type (Guideline)	Species	Results	Toxicity Category	MRID
Acute oral (870.1100)	Rat	LD_{50} = 1750 (95% C.L. 455.5-9230) mg/kg (females using the Up-and Down Method)	III	46977401
Acute dermal (870.1200)	Rat	LD_{50} > 5000 mg/kg for males, females, and for both sexes combined.	IV	46977402
Acute inhalation (870.1300)	Rat	LC_{50} > 5.5 mg/L (males, females, and both sexes combined; 4 hour nose-only exposure)	IV	46977406
Primary eye irritation (870.2400)	Rabbit	Corneal opacity persisted for 24 to 48 hours after treatment with clearance by 72 hours. Iritis was noted at 1 and 24 hours after treatment and cleared by the 48 hour observation. Conjunctival irritation was noted on one rabbit one hour throughout 48 hours after treatment with clearance by 72 hours. The maximum average score was 24.0 at 24 hours after test material instillation. Hydrogenated Catmint Oil was mildly irritating.	III	46977403
Primary dermal Irritation (870.2500)	Rabbit	No dermal irritation or clinical signs of toxicity were observed during the study. The primary irritation index was 0.0.	IV	46977404
Dermal sensitization (870.2600)	Mouse	A local lymph node assay (LLNA) indicated that hydrogenated catmint oil is not a dermal sensitizer.	---	46977405

2. Acute toxicity of the lotion products (Table 3)

A battery of six acute toxicity studies on the 15% lotion indicated the following profile:

Table 3: Acute Toxicity Profile – 15% Hydrogenated Catmint Oil Lotion

Study Type (Guideline)	Species	Results	Toxicity Category	MRID
Acute oral (870.1100)	Rat	LD_{50} > 5000 mg/kg (females using the Up-and Down Method)	IV	46977301
Acute dermal (870.1200)	Rat	LD_{50} > 5000 mg/kg for males, females, and for both sexes combined.	IV	46977302
Acute inhalation (870.1300)	Rat	The registrant is seeking to waive the requirement for an acute inhalation test. The rationales are: 1) its intended use as an insect repellent lotion for direct application is to the skin, 2) its high viscosity as an oil-water emulsion, and 3) the low vapor pressure and low toxicity of the active ingredient	---*	46977303
Primary eye irritation (870.2400)	Rabbit	Corneal opacity, iritis, or positive conjunctival irritation were not noted on any rabbit during the study. The maximum average score was 4.7 at one hour after test material instillation..	IV	46977303
Primary dermal Irritation (870.2500)	Rabbit	Well defined erythema was noted on 2/3 rabbits one hour after patch removal with reduction to very slight erythema by 24 and 48 hours that cleared by 72 hours. Well defined erythema was noted on another rabbit one hour after patch removal with persistence through 24 hours, reduction to very slight erythema by 48 hours, and clearance by 72 hours.	IV	46977304
Dermal sensitization (870.2600)	Mouse	A local lymph node assay (LLNA) indicated that lotion I is not a dermal sensitizer.	---	46977305
*This data requirement has been waived on the basis of the rationale presented by the Registrant.				

A second product containing 7% active ingredient is also being proposed for registration. No data on that product have been submitted, but the data summarized above will support the second product because the composition of both products is substantially similar (i.e., both products contain the same inert ingredients) based on review of confidential statements of formula (CSF).

3. Acute Neurotoxicity (OPPTS 870.6200)

In an acceptable acute neurotoxicity study (MRID 46977409), groups 12 male or 12 female rats were given a single oral dose of hydrogenated catmint oil (>99$ by weight) in corn oil at 0, 40, 200 or 1000 mg/kg body weight. Neurobehavioral assessment (functional observational battery [FOB] and motor activity testing) was performed on all animals pre-dosing and on the day of dosing as well as 7 and 14 days after dosing. Body weight and food consumption were measured weekly throughout the study. At study termination, 6 animals/sex/group were euthanized and

perfused *in situ* for neuropathological examination. Those animals from the control and high dose groups were subjected to histopathological evaluation of central and peripheral nervous system tissues.

No deaths or clinical signs of toxicity were observed and body weight, body weight gain and food consumption were unaffected by treatment. Selected functional observational battery (FOB) results are summarized in Table 4 as follows:

Table 4: Selected FOB results

Observation	Incidence (number affected/number evaluated)	
	Controls	1000 mg/kg
Males		
Unbalanced swaying and/or uncoordinated gait		
In home cage	0/12	2/12
In open field	0/12	3/12
Abnormal posture	0/12	11/12
Low Arousal	0/12	4/12
No reaction to auditory stimulus	0/12	2/12
Females		
Curled-up posture	1/12	9/12
Appeared to be sleeping	0/12	3/12
Unbalanced swaying and/or uncoordinated gait		
In home cage	0/12	4/12
In open field	0/12	3/12
Slow righting reflex	0/12	11/12
Lacrimation	0/12	2/12
Ataxic gait	0/12	4/12
Low arousal	0/12	2/12
No reaction to auditory stimulus	0/12	8/12
Walking on toes	0/12	2/12

Following the motor activity evaluation, 1/12 males and 1/12 females at 1000 mg/kg vs. none of the controls had slow and/or no pupillary response. Mean hindlimb foot splay was significantly increased in males (33% higher) and females (30% higher) at 1000 mg/kg. Mean body temperature was decreased in males (4% lower) and females (7% lower) at 1000 mg/kg.

On day 1, the cumulative (total) duration of movement was decreased in males and females at 200 mg/kg (19-20%) and 1000 mg/kg (48-52%); the changes were statistically significant in males and females at 1000 mg/kg. The cumulative number of movements on day 1 was decreased in males and females at 200 mg/kg (9-24%) and 1000 mg/kg (35-41%); only the difference in females at 1000 mg/kg was statistically significant.

The LOAEL for acute neurotoxicity of hydrogenated catmint oil in rats was 200 mg/kg based on deceased motor activity on the day of dosing in males and females. The NOAEL was 40 mg/kg.

B. Subchronic Toxicity

1. Oral Toxicity (OPPTS 870.3100)

In an acceptable oral toxicity study (MRID 46977407), hydrogenated catmint oil (HCO) was administered by gavage daily to groups of ten rats/sex at doses of 0, 40, 200, or 1000 mg/kg body weight for 93 days Hematological, clinical chemistry, urinalysis, opthalmoscopic, neurological, and microscopic tissue and organ effects were determined only in the subchronic studies.

All rats in the study survived until scheduled sacrifice. The only persistent clinical observation reported was perineal staining throughout the study on three female high-dose rats. No neurological or opthalmoscopic effects were noted. Total body weight gain was decreased 12% and food efficiency decreased 14% in male rats treated with 1000 mg/kg dose during the study; but no treatment-related effects were found in the remaining groups.

No treatment-related hematological effects were found during the subchronic study. Total bilirubin was slightly increased in high-dose male rats and cholesterol was slightly increased in high-dose male and female rats on study days 48/49 and 92/93; consistent with slight hepatic congestion. Total urine protein was increased on days 48 and 92 and granular casts were observed on day 92 in all male treatment groups. No increase in urine protein or cast formation was found in female rats.

Centrilobular hepatocellular hypertrophy was statistically significantly increased in male and female rats at the 200 and 1000 mg/kg/day dose level.

A dose-related increase in the incidence and severity of hyaline droplet formation within the epithelium of the proximal convoluted tubule was found in all treatment groups of male rats. In addition, a minimal to mild increase in the incidence of eosinophilic granular casts concomitant with the hyaline droplet formation was found. The casts consisted of multiple focal accumulations of granular material in the tubular lumen near the junction of the inner and outer stripes of the renal medulla. An associated increase in the incidence and severity of minimal to moderate chronic progressive nephropathy was also observed in high-dose male rats.

Minimal to mild degeneration/regeneration of the olfactory epithelium lining the nasal turbinates was observed in high-dose male and female rats. This lesion was characterized by multifocal hypercellularity in the olfactory epithelium at nose levels III and IV due to regeneration of sensory cell nuclei and degeneration of sustentacular cells. In some areas, the olfactory epithelium was thinner than normal but sensory cell nuclei predominated. Sensory or sustentacular

cell necrosis was not apparent and there was no exfoliation of the epithelium or associated inflammation.

The LOAEL for refined oil of *Nepetea cataria*, hydrogenated catnip oil, for male and female rats is 1000 mg/kg/day based on treatment-related effects to the olfactory epithelium. The NOAEL is 200 mg/kg/day for male and female rats.

2. Dermal Toxicity (OPPTS 870.3220)

In an acceptable 28-day dermal toxicity study (MRID 46977415), HCO (purity >99%), was applied to the shaved skin of groups of 10 male and 10 female rats at doses of 0, 100, 500, or 1000 mg/kg/day six hours/day for 29 days.

All rats survived until scheduled sacrifice and no treatment-related effects were found on body weight, body weight gain, food intake, food efficiency, hematology, or clinical chemistry of treated male and female rats. No neurotoxicity was observed.

Treatment-related effects were found only in male rats of all groups and were consistent with hyaline droplet formation. Urine protein excretion was increased 80, 80, and 131% in the low- to high-dose male rats, respectively, and male rats had an increase in urine white blood cells (2/10, 6/10, 9/10, and 9/10, in the control through high-dose group, respectively) and in finely granular casts (0/10, 1/10, 3/10, and 8/10, respectively).

The absolute and relative liver weights of male rats treated with ≥500 mg/kg/day were increased 10 – 20% and absolute and relative kidney weights were increased 7-15% in male rats treated with ≥100 mg/kg/day. A dose-related increase in minimal to mild hyaline droplet formation within the epithelium of the proximal convoluted tubule was observed microscopically in all groups of treated male rats (0/10, 3/10, 9/10, and 10/10 for the control through high-dose groups, respectively). No treatment-related effects were observed microscopically in the liver; however, the increased absolute and relative liver weights were consistent with hypertrophy.

Very slight to moderate erythema was noted on some animals at the treatment site early in the study, but resolved on all by Day 12. No edema was observed. Epidermal scaling, hyperkeratosis, and epidermal sloughing were also observed at necropsy, but the effects were unrelated to dose.

The dermal LOAEL for male and rats treated with HCO for 29 days was not established in this study. The NOAEL is the highest dose tested, 1000 mg/kg bw/day.

C. Immunotoxicity (OPPTS 870.7800)

In an acceptable immunotoxicity study (MRID 46977407), groups of ten rats/sex were treated by gavage with daily doses of 0, 40, 200, or 1000 mg HCO/kg body weight for 28 days. An additional groups of ten rats/sex received saline (negative control) or 20 mg/kg cyclophosphamide (positive control) daily. On the 22nd day of treatment, all test animals were given an intravenous injection containing sheep red blood cells (SRBC), and at the end of the study, sera were collected and subjected to an enzyme-linked immunosorbent assay (ELISA) to determine if the test material suppressed an immune response.

No effects on humoral immune function were found in HCO-treated male and female rats. All test animals survived until scheduled sacrifice. No neurological, opthalmoscopic, or other toxicity was noted.

The LOAEL for HCO in male and rats was not established for effects on humoral immune function. The NOAEL for male and female rats was the highest dose tested, 1000 mg/kg/day.

D. Developmental Toxicity (OPPTS 870.3700)

In an acceptable developmental toxicity study (MRID 46977408), HCO (>99%) was administered by gavage to groups of 22 time-mated female rats at doses of 0, 200, 500 or 1000 mg/kg/day in corn oil on gestation days 6 through 20. On gestation day 21 (GD 21), all dams were euthanized and a gross external and visceral examination was performed. The uterus of each pregnant female was removed and the uterine contents were examined and described. All fetuses were removed and individually identified, weighed, sexed, and examined for external and skeletal alterations; approximately one half of the fetuses were examined for visceral and head abnormalities. The total number of fetuses examined (number of litters) was 259 (21), 275 (22), 285 (22), and 256 (21) for the 0, 200, 500, and 1000 mg/kg/day groups, respectively.

There were no treatment-related adverse effects in survival, clinical signs, body weight, or cesarean parameters. Maternal toxicity was limited to reductions in body weight gain (27% and 54%, respectively) and food consumption (~10%) during the first two days of dosing at 500 and 1000 mg/kg/day. These reductions were not considered adverse since they were transient and had no significant impact on overall body weight gain or food consumption for the entire gestation period. Stained fur was observed in the 1000 mg/kg group and was considered possibly test substance-related but not adverse.

Based on the results of this study, the oral maternal toxicity LOAEL for hydrogenated catmint oil in rats was not identified. The maternal NOAEL is 1000 mg/kg bw/day.

There were no treatment-related adverse effects in developmental parameters (deaths/resorptions, fetal weight, developmental alterations) at any dose level tested. Developmental variations common to this strain of rat were observed in the treated and control groups at a similar incidence. No treatment-related malformations were seen.

The oral developmental toxicity LOAEL for hydrogenated catmint oil in rats was not identified. The developmental NOAEL is 1000 mg/kg bw/day.

E. Genetic Toxicity

1. Point mutation assay – bacteria (OPPTS 870.5100)

In an acceptable reverse gene mutation assay in bacteria (MRID 46977410), strains TA98, TA100, TA1535 and TA1537 of *Salmonella typhimurium* and strain WP2 *uvr*A *of Escherichia coli* were exposed to HCO (>99% a.i. by weight) dissolved in DMSO in two independent assays using a standard plate incorporation procedure and duplicate and triplicate plating in the first and second assays respectively. In the first mutagenicity assay, which was called the toxicity-mutation test, concentrations of 0, 33.3, 66.7, 100, 333, 667, 1000, 3333 or 5000 µg/plate were tested with and without S9-mix. In the second assay, which was called the mutagenicity test, concentrations of 0, 333, 667, 1000, 3333 or 5000 µg/plate were tested with and without S9-mix. The S9 fraction was obtained from Aroclor 1254-induced male Sprague-Dawley rat liver.

In the first assay, cytotoxicity was observed at the limit concentration in strain TA1537 both in the presence and absence of S9 mix as well as in strain TA1535 in the presence of S9 mix. In the second assay, cytotoxicity was observed at the limit concentration, and also at 3333 µg/plate, in strain TA1537 both in the presence and absence of S9 mix. Cytotoxicity never caused any more than a slight reduction in the background lawn. No precipitation was observed at any concentration level in either assay. The number of revertants per plate was not increased over the concurrent solvent control value at any test material concentration, with or without S9-mix, in any tester strain. The solvent and positive controls induced the appropriate responses in the corresponding strains. **There was no evidence of induced mutant colonies over background.**

2. *In vitro* mammalian cell point mutation assay (OPPTS 870.5300)

In an acceptable mammalian cell gene mutation assay (MRID 46977413), L5178Y/TK+/- mouse lymphoma cells cultured *in vitro* were exposed for 4 hours to hydrogenated catmint oil (>99% a.i. by weight) dissolved in dimethyl sulfoxide at concentrations of 0, 100, 150, 200, 250, 300 or 350 µg/mL in the absence of mammalian metabolic activation and at concentrations of 0, 300, 350, 425, 500 or 600 µg/mL in the presence of mammalian metabolic activation (S9-mix with S9 fraction from livers of Aroclor 1254 induced male rats).

Hydrogenated catmint oil was tested up to concentrations limited by cytotoxicity, which was clearly demonstrated in a preliminary cytotoxicity assay. There was significant concentration-related cytotoxicity of hydrogenated catmint oil. In the preliminary cytotoxicity assay, precipitation of hydrogenated catmint oil in the culture medium was seen at the end of the 4-hour exposure only at the concentration of 4500 µg/mL, which was the highest concentration tested. Mutant frequencies were significantly increased in the absence of metabolic activation only. Two cultures had mutant frequencies of at least 100 mutants per 10^6 clonable cells above that of the solvent control, and that extent of an increase is considered biologically significant. Two other cultures, also in the absence of S9 mix, had mutant frequencies between 55 and 99 mutants per 10^6 clonable cells above that of the solvent control. There was a concentration-related increase in the mutant frequency in the absence of S9 mix. Analysis of colony size distributions showed an increase in the frequency of small colonies in the cultures treated with the test substance. Solvent and positive controls gave appropriate responses. **There was clear evidence of induced mutant colonies over background.**

3. *In vitro* mammalian cell chromosomal aberration assay (OPPTS 870.5375)

In an acceptable mammalian cell cytogenetics assay (MRID 46977411), cultured human peripheral blood lymphocytes were exposed for 4 hours to HCO (>99% a.i. by weight) dissolved in dimethyl sulfoxide (DMSO) at concentrations of 0, 50, 200 or 550 µg/mL without metabolic activation or at concentrations of 0, 210, 420 or 840 µg/mL with metabolic activation, and in both cases the treatment was followed by a 16-hour recovery period so that the total time to harvest was 20 hours after the initiation of treatment. In addition, other cells of this same type were exposed for 20 hours without any recovery period to the same test substance dissolved in DMSO at concentrations of 0, 37.5, 75 or 350 µg/mL without metabolic activation. Metabolic activation was provided by S9 mix with S9 fraction from livers of Aroclor 1254-induced male rats.

HCO was tested up to cytotoxic concentrations based on mitotic indices found in a preliminary cytotoxicity study and concurrently with the cytogenetic assay. At least in the cytogenetic assay, the mitotic index at the highest test concentration was reduced to less than half of that in the solvent control. There were no statistically significant increases over the solvent control values in the percentages of cells with structural aberrations including or excluding gaps at any test material concentration with or without S9-mix. Also there were no increases in numerical aberrations. There was no precipitation of the test substance. Solvent and positive control values were appropriate and within the testing laboratory's historical control ranges for structural chromosomal aberrations and numerical aberrations. **There was no evidence of chromosome aberrations induced over background.**

4. *In vivo* mammalian chromosomal aberration test (OPPTS 870.5395)

In an acceptable mouse bone marrow micronucleus assay (MRID 446977412), groups of 10 mice/sex were given HCO (purity >99% by weight) in a single dose by gavage at 0, 500, 1000, or 2000 mg/kg body weight. Bone marrow cells were harvested from 5 mice/group at approximately 24 or 48 hours after the treatment. Two additional mice/sex/sacrifice time were treated at the highest dose level to observe toxicity and to be in reserve should some of the animals die before bone marrow could be harvested. Because no effect was seen on the frequency of micronucleated polychromatic erythrocytes at any dose of the test substance at 24 hours or at the highest dose at 48 hours, slides were not evaluated for the two lower doses at the 48-hour harvest time. The vehicle was corn oil.

At least one animal treated with HCO at every dose level showed symptoms of toxicity after administration in both the rangefinder experiment and the main experiment. At five and 15 minutes after treatment in the rangefinder experiment on males, all three animals showed ataxia, and two of them showed low posture 15 minutes after treatment. In the rangefinder experiment, no clinical signs of toxicity were observed at 30 or more minutes after treatment. In the main experiment at 2000 mg/kg bw, ataxia was seen in all 14 males and all 14 females, and prostration was observed in two females. At 1000 mg/kg bw, ataxia was seen in seven of the 10 animals treated of each sex, and at 500 mg/kg bw, ataxia was seen in one of the 10 animals treated of each sex. The only sign of moribundity or mortality of the test substance was the death of one female in the high dose group. Polychromatic erythrocytes (PCEs) were examined for micronuclei in five animals/sex/dose level. PCEs were similarly examined in the vehicle control and in the positive control, cyclophosphamide. The vehicle and positive control treatments were also made by oral intubation, and the positive control was examined only at the 24-hour harvest time. Hydrogenated catmint oil was tested at an adequate dose, which was the limit dose for the assay. The positive control induced the appropriate response. There were no statistically significant changes seen in the PCE:NCE ratio. **There was not a significant increase in the frequency of micronucleated polychromatic erythrocytes in bone marrow after any treatment time.** It was concluded that the test chemical was negative in this *in vivo* study.

III. DOSE-RESPONSE ASSESSMENT (Table 5)

Table 5: Toxicity Profile for Hydrogenated Catmint Oil

Study Type (Guideline)	Species	Dose-Response Information	Effects	MRID
Acute Neurotoxicity (870.6200)	Rat	**Doses tested:** 0, 40, 200 & 1000 mg/kg **NOAEL** = 40 mg/kg **LOAEL** = 200 mg/kg	Deceased motor activity on the day of dosing in males and females	45977409

Table 5: Toxicity Profile for Hydrogenated Catmint Oil

Study Type (Guideline)	Species	Dose-Response Information	Effects	MRID
Subchronic Oral Toxicity (870.3100)	Rat	**Doses tested:** 0, 40, 200 & 1000 mg/kg/day **NOAEL** = 200 mg/kg **LOAEL** = 1000 mg/kg	Minimal to mild degeneration / regeneration of the olfactory epithelium lining the nasal turbinates of males and females	46977407
Subchronic Dermal Toxicity (870.3200)	Rat	**Doses tested:** 0, 100, 500 & 1000 mg/kg **NOAEL** = 1000 mg/kg **LOAEL** > 1000 mg/kg	No adverse effects were reported.	46977415
Oral Immunotoxicity (870.7800)	Rat	**Doses tested:** 0, 40, 200 & 1000 mg/kg/day **NOAEL** = 200 mg/kg **LOAEL** = 1000 mg/kg	No effects reported	46977407
Oral Developmental Toxicity (870.3700)	Rat	**Doses tested:** 0, 100, 500 & 1000 mg/kg/day Maternal Toxicity **NOAEL** = 1000 mg/kg **LOAEL** > 1000 mg/kg Developmental Toxicity **NOAEL** = 1000 mg/kg **LOAEL** > 1000 mg/kg	Maternal Toxicity No adverse effects were reported. Developmental Toxicity No adverse effects were reported.	46977408
Reverse Mutation Assay (870.5100)	Bacteria	**Doses tested:**0 to 5000 µg/plate with or without metabolic activation (S9 mix)	Negative	46977410
Mammalian cell gene mutation assay (870.5300)	Mouse lymphoma cells	**Doses tested:**0 to 3500 µg/mL without metabolic activation (S9) or 0 to 600 µg/mL with metabolic activation (S9 mix)	Mutagenic at doses approaching or at cytotoxic levels without metabolic avtivation (250 to 350 µg/mL	46977413
In vitro cytogenetics assay (870.5375)	Human peripheral blood lymphocytes	**Doses tested**: 0, 50, 200 or 550 µg/mL without metabolic activation or 0, 210, 420 or 840 µg/mL with metabolic activation	Negative	46977411
Bone marrow micronucleus assay (870.5395)	Mice	**Doses tested:** 0, 500, 1000, or 2000 mg/kg (single oral gavage doses)	Negative	4677412

A. Endpoint Discussion

1. Acute Endpoints

Results of the acute oral toxicity study with the active ingredient (MRID 46977401) characterized effects at higher single oral doses as follows:

> Death occurred on the day of dosing in one of the three rats dosed at 1750 mg/kg and one of two rats dosed at 5000 mg/kg. The remaining rat dosed at 5000 mg/kg was sacrificed for humane reasons on the day of dosing. No clinical signs were observed in the rat at 550 mg/kg. Clinical signs observed in the remaining rats included wet fur, lethargy, ataxia, partially closed or dark eyes, slow or labored breathing, prostrate posture, lacrimation, stained fur/skin, dark extremities, and/or moribundity. No clinical signs were observed by test day 3 (in surviving rats). No body weight losses occurred after dosing. No gross lesions were present in the rats at necropsy.

The only clinical sign noted in an acute oral toxicity study with the 15% lotion (MRID 46977301) was "high carriage" in one of three rats given the 5000 mg/kg dose. No other effects on body weight or incidence of gross lesions were noted in the study.

Dermal application of 5000 mg/kg to a group of 5 male rats had no effects, but the same dose applied to skin of 5 female rats had effects described in the study report (MRID 46977402) as follows:

> The female rats exhibited lethargy, ataxia, absent feces, labored breathing, lacrimation, stained fur/skin, wet fur, not eating, high carriage, and/or leaning. Ataxia was observed only during the exposure period or immediately after test substance removal. The remaining clinical signs cleared by test day 6.

No clinical signs of toxicity were observed in male and female rats dermally exposed to 5000 mg of the 15% lotion per kg body weight (MRID 46977302).

After a 4-hour nose only exposure of 5 male and 5 female rats to air containing 5.5 mg HCO/L, clinical signs were described (MRID 46977406) as follows:

> All animals...survived the exposure and the subsequent recovery period...
>
> Notable clinical signs of toxicity...included lethargy, labored breathing, gasping, hunched or prostrate posture, and incoordination immediatelyfollowing exposure which lasted for 1 to 3 days postexposure for males and females, respectively...

It should be noted that clinical signs similar to those described in acute toxicity studies were reported in the 90-day subchronic oral toxicity study (MRID 46977407). These effects were described as follows:

> At 200 mg/kg/day, two males (of 10) were lethargic on the second day of dosing. At 1000 mg/kg/day, nine males and s8x females were lethargic and four males and one female were ataxic during this period. All of these post-dosing observations were transient in that they resolved prior to the next dose and were not observed beginning on dosing day 3 through the end of the study.

At similar low doses, an acute neurotoxicity endpoint of 40 mg/kg was characterized by decreased motor activity on the day of dosing. These effects were not observed after repeated oral doses at similar levels, and no histopathology was found in nervous tissues from treated animals in the acute or subchronic neurotoxicity studies. In addition, the subchronic dermal toxicity study did not present histopathological effects in the nasal cavity or changes in neurological parameters after repeated dermal exposures up to 1000 mg/kg/day.

These studies indicate:

- The clinical signs observed at lethal oral doses (1750-5000 mg/kg) are not seen at single doses that are 3 to 10-fold lower (550 mg/kg) which suggest a steep dose-response curve.
- Acute oral toxicity study results with the 15% lotion appears to reduce the likelihood that neurological clinical signs will occur
- Acute studies by dermal or inhalation routes also appear to reduce the chances of seeing the clinical signs of concern.
- Lower non-lethal doses (200-1000 mg/kg) decreased motor activity, and results from subchronic studies suggest the effects are reversible and that rats can adapt to these effects even when dosing is continued.

Therefore, the acute neurotoxicity NOEL is appropriate only in the assessment of incidental oral exposure scenarios for the insect repellent products considered in this assessment.

2. Subchronic Endpoints

Efects noted in the subchronic oral toxicity study showed adaptive changes in the liver (centrilobular hepatocellular hypertrophy) and a sex-related (males only) species specific (rats) kidney effects (hyaline droplet formation and associated nephropathy) at the highest dose tested (1000 mg/kg/day). The 1000 mg/kg/day dose level was associated with significant degenerative/regenerative changes in the nasal cavity of treated rats, and the NOEL was200 mg/kg/day. **Because no similar toxicity was observed in the 280day dermal toxicity study, the 1000 mg/kg/day NOEL will be used to assess short and intermediate-term dermal exposures to the insect repellent products.**

No developmental toxicity or immunotoxicity was noted in rat studies using the same dose levels as those used in subchronic toxicity studies.

IV. Exposure Assessment

A. Use Patterns and Appropriate Endpoints

As indicated previously, the two lotion product labels include the following instructions for use:

> Dispense a small amount of lotion directly onto skin. Spread uniformly to completely cover any exposed skin surface. Reapplication after six hours may be necessary. When applying to children, dispense into an adult's hand and then spread evenly and completely over the child's exposed skin taking care not to contact the child's fingers and hands.
>
> Do not apply over cuts or damaged skin.

The products contains 7 or 15% active ingredient, and the application rate is based on dosimetry information reported in product performance studies (MRID 47015602). The application rates are determined as follows:

$$0.63 \text{ g product}/250 \text{ cm}^2 \text{ for } 15\% \text{ lotion} = 2.52 \text{ mg product/cm}^2$$

$$(2.52 \text{ mg/cm}^2)(0.15) = 0.378 \text{ mg active ingredient/cm}^2$$

The endpoints appropriate for this type of insect repellent use are as follows:

Endpoint Summary

Scenario	Study Type	NOEL/LOEL	Effects	MRID
Acute (Incidental Oral)	Acute Neurotoxicity	40/200 mg/kg	Deceased motor activity on the day of dosing in males and females	45977409
Short- & Intermediate Term	28-Day Dermal Toxicity	≥1000 mg/kg/day	No adverse effects noted.	46977415

NOEL = no-observed-effect level; LOEL = lowest-observed-effect level.

No uncertainty factors are specified because:

- Labeling cautions against application of products to the hands of children or allowing children to apply the lotions themselves, and
- Subchronic dermal toxicity studies did not indicate systemic toxicity after repeated exposure to a limit dose of 1000 mg/kg/day.

B. Dermal Penetration Study (OPPTS 870.7600)

In an *in vitro* dermal penetration study (MRID 47015601), HCO (purity 99%) was applied to twelve 0.64 cm^2 sections of male rat skin and twelve 0.64 cm^2 sections of human cadaver skin for eight hours at a rate of 30,000 $\mu g/cm^2$. The skins specimens were contained in dual-chambered

diffusion cell assemblies. Receptor fluid samples were collected 0.5, 1, 2, 4, 6, 8, and 24 hours after the start of dosing. After eight hours of exposure, the skin specimens were washed with ethanol and tape stripped. All wash, receptor fluid samples, tape strip samples, and skin specimens were analyzed for HCO.

Penetration rates of HCO through rat skin were ~105 – 110-fold greater than through human skin during the initial *in vitro* eight hour exposure. The penetration rates declined approximately 18-fold for rat skin during the 16-hour post-exposure period, but was still approximately five-times greater than the rates reported for human skin. At the end of the study, the total absorbable dose was ~78% for rat skin and ~2% for human skin. While penetration rates through rat skin declined following removal of the test material, penetration rates through human skin were comparable during and after exposure. Total recovery of the test material for skin from both species and all time intervals was ≥89%.

Results from the oral and dermal subchronic toxicity studies (incidence of microscopic changes noted in the kidneys of male rats) suggest that dermal absorption is likely to be >20% based on comparison of the LOELs from the two studies ([oral LOEL/dermal LOEL] x 100). The *in vitro* dermal penetration study with rat and human skin indicated a high degree of penetration in rat skin (78% of the dose after an eight-hour exposure) while human skin was relatively impermeable (2% of the dose was absorbed during the same exposure period) to hydrogenated catmint oil. It should be noted that the application rate for the active ingredient in the dermal penetration study is similar to that determined from the 15% lotion's product performance study (approximately 80% of the product application rate).

B. Occupational and Residential Exposure

No occupational estimates are made in this assessment since HCO is to be used by individuals as an insect repellent that they apply directly to their own skin. Non-occupational dermal exposure estimates were not determined because the subchronic dermal toxicity study did not demonstrate an endpoint for use in risk characterization, and the label indicates that advice from a physician or Poison Control Center should be sought when reactions to exposure from use of the products are suspected. Again, the directions for use on the two product labels indicated that application of the lotions to children's fingers and hands was to be avoided. Therefore, no exposure estimates were determined for incidental oral exposure.

V. RISK CHARACTERIZATION

Based on the absence of short- and intermediate-term toxicity endpoints and precautionary labeling to avoid the likelihood of incidental oral exposure for small children, no risk characterizations are needed in this assessment.