Proposed Regulatory Decision Document PRDD99-02

Soybean Oil

The active ingredient soybean oil and the four associated Blocker™ personal repellent products listed below have been granted time-limited registrations until December 31, 1999.

* Consep Soybean Oil Technical (Registration Number [Reg. No.] 25748): 100% soybean oil
* Blocker™ Insect Repellent Long-Lasting Oil (Reg. No. 25749): 2% soybean oil
* Blocker™ Insect Repellent Lotion (Reg. No. 25750): 2% soybean oil
* Blocker™ Insect Repellent Light Herbal Scent Lotion (Reg. No. 25751): 2% soybean oil
* Blocker™ Insect Repellent Easy-To-Use Spray (Reg. No. 25752): 2% soybean oil

This document provides a summary of data reviewed and the rationale for the regulatory decision concerning these products.

This document has been prepared in keeping with the ongoing efforts of the Pest Management Regulatory Agency (PMRA) to regulate pest control products in an open and transparent manner. The PMRA will accept written comments on this proposal up to 45 days from the date of publication of this document. Please forward all comments to the Publications Coordinator at the address listed below.

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Foreword

Special consideration has been given to the safety profile of this active ingredient (i.e., "food grade" material) and the exemption status that it holds in the United States (U.S.) regulatory system. While appreciating all of these elements, the PMRA is also cognizant of its responsibilities toward consultation, communication, and an open regulatory process.

Under current circumstances, the PMRA believes the "time-limited" registration provides a reasonable balance in the face of conflicting demands (i.e., the PMRA's interests and responsibilities with respect to consultation, communication and an open regulatory process, while at the same time recognizing legitimate concerns of pesticide manufacturers and users). Opportunity is provided for input in association with limited usage inherent in the market introduction year. While the PMRA would not expect any significant public comment or reaction in the case of a compound such as soybean oil, should a substantial issue emerge, appropriate action can be taken prior to the next use season.

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1.0 Introduction

Consep Inc., of Bend, Oregon, submitted applications to register the technical active ingredient soybean oil and four personal repellents containing soybean oil to repel mosquitoes and black flies. Soybean oil is a new active ingredient.

The five submissions reviewed are as follows:

1) Consep Soybean Oil Technical (Submission No. [Sub. No.] 96-1526): 100% soybean oil

2) Blocker™ Insect Repellent Long-Lasting Oil (Sub. No. 96-1527): 2% soybean oil (previously called Bite Blocker™ Light Country Scent Oil)

3) *Blocker™ Insect Repellent Lotion (Sub. No. 96-1528): 2% soybean oil (previously called Bite Blocker™ Light Country Scent Lotion and Blocker™ Insect Repellent Moisturizing Lotion)

4) Blocker™ Insect Repellent Light Herbal Scent Lotion (Sub. No. 96-1529): 2% soybean oil

5) *Blocker™ Insect Repellent Easy-To-Use Spray (Sub. No. 98-0170): 2% soybean oil

* Same formulation

1.1 Mode of Action

It is hypothesized by the applicant that soybean oil repellents interfere with both the long and short range host-seeking behaviour of insects by "masking" odours that are given off by the host (e.g., lysine, alanine and carbon dioxide), and by cooling the temperature above the skin surface. No data have been submitted to support these theories on mode of action.

1.2 Verification of Active Ingredient Status

To support the claim that soybean oil contributes to the insect repellent activity of the end-use formulations and can be designated as an active ingredient, the applicant submitted a laboratory efficacy trial entitled *Laboratory evaluation of the efficacy of eight Bite Blocker™ formulations to repel Aedes aegypti mosquitoes* (Lindsay, Surgeoner, and Heal, University of Guelph, 1997), which tested the proposed Blocker™ formulations with and without the soybean oil component.

In the submitted study, subjects inserted their hand (protected with a latex glove) and treated forearm into a sleeve cage containing approximately 100 adult female *Aedes aegypti* L. mosquitoes for 20 seconds, and then counted the number of mosquitoes biting the exposed forearm. Subjects also used their non-treated forearm to conduct 20-second biting counts as a control before inserting the treated forearm. The difference in biting counts between the treated and non-treated forearms was used to calculate percent repellency. Percent repellency values of test formulations without the soybean oil component (i.e., Formulations #1001 and #1003) were lower than the percent repellency values obtained for a similar formulation containing soybean oil (e.g., Light Country Scent Oil, Sub. No. 96-1527) (see Appendix I). When the data for all assessment times were combined, the percent repellency obtained with Formulation #1003 (no soybean oil or coconut oil) was significantly less than that of Formulation #1001 (no soybean oil), which was significantly lower than that of the other formulations. The researchers point out in their study that because there was no statistically significant difference in percent repellency between Formulation #1002 (containing soybean oil but no coconut oil) and all other formulations containing soybean oil and coconut oil (e.g., Light Country Scent Oil, Light Herbal Scent Lotion, Light Country Scent Lotion), this supports the claim that soybean oil has active properties.

1.3 Status in the United States

Soybean oil is exempted from *Federal Insecticide, Fungicide and Rodenticide Act* (FIFRA) requirements for registration based on its status as a common food or constituent of a common food. The final rule for exempting minimum risk pesticides from regulation under FIFRA 25(b) was published in the *Federal Register* of March 6, 1996 (61 FR 8876) and is codified at 40 CFR 152.25(g).

2.0 Chemistry of soybean oil technical

Common name: Soybean oil, edible grade

Chemical name: Soybean oil

Chemical Abstracts Service Registry Number:
8001-22-7

Product purity: 100% soybean oil

Specifications: Soybean oil is composed of refined plant extractives from soybean *(Glycine max* [L.] Merrill) and their physically modified derivatives. It consists primarily of the glycerides of the following fatty acids:

Palmitic acid	(C16:0)	7 - 14%
Stearic acid	(C18:0)	1 - 6%

Oleic acid	(C18:1)	. . . . . 19 - 30%
Linoleic acid	(C18:2)	. . . . . 44 - 62%
%-Linoleic acid	(C18:3)	. . . . . 4 - 11%

Soybean oil must conform to the physical/chemical specifications of a refined, edible soybean oil as found in the U.S. Pharmacopoeia (USP) #23 and/or the British Pharmacopoeia (BP) 1993.

Density (20°C): 0.916–0.922 g/mL

Chemical and physical properties:

The product is a clear, light, yellow oil, with a boiling point of >350°C, and of negligible water solubility. The shelf life is stated as approximately two years. Waivers of the requirements to determine the octanol water partition coefficient, the dissociation constant, and the UV/visible spectrum were requested and accepted.

Conclusion: Soybean oil, as a food-grade material, has been well characterized and its properties are already well documented in the literature. The chemistry data required for the registration of the technical active ingredient are complete.

3.0 Chemistry of end-use products

The formulations of two of the end-use products are identical (i.e., Blocker™ Insect Repellent Lotion [Sub. No. 96-1528] and Blocker™ Insect Repellent Easy-to-Use Spray [Sub. No. 98-0170]).

The guarantee is 2% (nominal) for all four products.

The analytical methods used are standard methods published in the literature for the determination of USP/BP grade soybean oil. The fatty acid distribution is determined by gas chromatography after saponification. The methods are acceptable for use as enforcement methods.

Blocker™ Insect Repellent Long-Lasting Oil (Sub. No. 96-1527) is a clear, pale yellow liquid with a herbaceous odour. The three other products are opaque white liquids with a herbaceous odour.

All are stable at 20°C for one year in high-density polyethylene bottles.

Conclusion: The product chemistry data for the four products are complete.

4.0 Toxicology evaluation

The applicant has requested waivers for all the toxicological studies with soybean oil. The following information on the toxicological profile of soybean oil is obtained from the published literature.

4.1 Metabolism - Technical

The metabolism of soybean oil is similar to all the common fatty acids. In the gastrointestinal tract, 95–100% of most dietary fatty acids are absorbed. Free fatty acids are combined with glycerol to form triglycerides. Triglycerides are stored in the adipose tissue as fat until they are needed as a source of calories. Appreciable amounts of dietary carbohydrate and some protein can also be converted to fat. Fat can be mobilized from the adipose tissue back to free fatty acids. The amount of fat stored/mobilized depends on the caloric balance of the whole organism. Fatty acids are metabolized via different pathways into several important components of the living cells. The $-oxidation of free fatty acids is a major source of energy for the body. Fatty acids are the essential components of the biosynthesis of membrane phospholipids via the formation of 1,2-diacyl (fatty acids)-glycerol-3-phosphate. Some polyunsaturated fatty acids such as linoleic and arachidonic acids are the precursors of the biosynthesis of prostaglandins and thromboxanes via the cyclo-oxygenase pathway. They are also the precursor of the biosynthesis of eicosanoids (leukotrienes, lipoxins, prostacyclin and epoxyeicosatetraenoic acids) via the lipoxygenase pathway (Institute of Shortening and Edible Oils, Washington, D.C., 1994).

4.2 Acute Toxicity - Technical

According to the Environmental Protection Agency (EPA) Reregistration Eligibility Decision (1993), vegetable oils (including soybean oil) are of very low acute toxicity by the oral and dermal routes of exposure. The physico-chemical characteristics of soybean oil (slightly viscous liquid, low vapour pressure) indicate that inhalation is not a potential route of exposure under normal conditions. Soybean oil is not known to be irritating to skin and eyes. Soybean oil does not appear to be an allergenic or dermal-sensitizing compound.

4.3 Short- and Long-term Toxicity - Technical

4.3.1 Mice

Soybean oil was used as solvent for the test substance %-tocopherol in a long-term oncogenicity study. Animals from the vehicle control group (22 Balbc mice/sex/group) were injected sub-cutaneously with 0.1 mL of pure soya oil (Golden Harvest brand) once a week for 10 months (approximately 5000 mg of soybean oil/kg body weight [bw]/week [wk]). Long-term injection with soybean oil did not produce any tumours or adverse effects in mice (Constatinides P., and Harkey M., 1985).

Inbred C3H/OUJ female mice carrying the mammary tumour virus type MMTV-S (females from this strain typically show a high incidence of mammary tumours) were fed with diets containing either 5% or 20% of soybean oil (equivalent to 1500 mg of oil/kg bw/day [d] and 6250 mg of oil/kg bw/d, respectively) for 40 wk. Animals receiving normal diet were used as controls. Results indicate that females fed 5% of soybean oil in diets had similar incidence of virus-induced mammary tumours as control animals (60–65% of mice with tumours). Females fed 20% of soybean oil in diets had higher incidence of mammary tumours (89% of mice with tumours) than mice fed 5% of soybean oil in diets (Olson L. *et al*., 1987).

4.3.2 Rat

In a long-term/oncogenicity study, young Wistar rats (100/sex/group) were fed with diets containing 16% weight per weight (w/w) of fish oil, soya oil or rapeseed oil (which is equivalent to approximately 4.8 g of oil/kg bw/d) for 110 wk. At the end of 110 wk, moderate to marked presence of fine lipid droplets were noted in hearts of animals treated with fish oil. Low grade lipidosis was noted in hearts of animals treated with soybean oil or rapeseed oil. Total serum cholesterol levels were increased in all treated groups. General histopathological examination showed some non-neoplastic changes in all treated groups, including adrenal cortical fatty vacuolation, nephrocalcinosis and transitional cell hyperplasia, hepatic basophilic foci, bile duct hyperplasia, pulmonary vascular mineralization and perivascular lymphocytic infiltration. The neoplastic changes in all treated groups were at similar incidences as compared to age-matched animals from the same testing laboratory (Duthie I. *et al.*, 1988).

4.4 Reproductive Toxicity - Technical

4.4.1 Rat

Wistar rats (40/sex/group) were fed diets containing 16% of soybean oil (equivalent to 4.8 g of oil/kg bw/d) during the 10-wk pre-mating and throughout the mating, gestation and lactation periods. Some reproductive parameters, including mating index, fertility index, litter size, litter weight, and number of live pups at 0, 4, 12, and 21 d post-partum, were measured. The results indicate that all the measured reproductive parameters were comparable to historical control values (Duthie I. *et al.*, 1988).

4.5 Teratogenicity - Technical

4.5.1 Rat

Soybean oil was used as a negative control in a combined reproduction and teratology study (with ethanol). Female Wistar rats received 7.5% soya oil emulsion in drinking fluid during the four-week pre-mating period and throughout the pregnancy. The actual daily intake (mg of soybean oil/kg bw/d) was not calculated. Some dams were sacrificed one day prior to

delivery and the fetuses were examined for malformations (external, visceral and skeletal examinations). The remaining dams were allowed to deliver, and pups were examined up to 24 d of age. No malformations or adverse effects were found in soybean-oil treated (control) fetuses/pups (Oisund I. *et al.*, 1978).

4.6 Genotoxicity - Technical

The applicant has requested waivers for all the genotoxicity studies with soybean oil. No published studies were found/submitted.

4.7 Neurotoxicity - Technical

The applicant has requested waivers for all the neurotoxicity studies with soybean oil. No published studies were found/submitted.

4.7.1 Acute Toxicity of the End-Use Formulations

The applicant has requested that data generated from a product identified as Pery Cut Chemie AG Insect Repellent (with all ingredients at relatively comparable concentrations to those found in the four proposed Blocker™ formulations) be used in support of the registrations of Sub. Nos. 96-1527, 96-1528, 96-1529 and 98-0170. Based on the submitted bridging data, these products are expected to be of low acute toxicity, minimally irritating to the eyes, and not irritating to the skin. Blocker™ insect repellent formulations are considered to be potential dermal sensitizers on some hypersensitive people.

4.7.2 Toxicology Summary

Similar to most dietary fatty acids, soybean oil is completely absorbed (95–100%) following oral dosing. Free fatty acids are combined with glycerol to form triglycerides. Triglycerides are stored in the adipose tissue as fat until they are needed as a source of calories. Appreciable amounts of dietary carbohydrate and some protein can also be converted to fat. Fat can be mobilized from the adipose tissue back to free fatty acids. Fatty acids are metabolized via different pathways into several important components of the living cells. The $-oxidation of free fatty acids is a major source of energy for the body. Fatty acids are the essential components of the biosynthesis of membrane phospholipids.

Soybean oil appears to be of low acute toxicity by the oral, dermal and inhalation routes of exposure. Soybean oil is not known to be irritating to skin and eyes and does not appear to be an allergenic or dermal-sensitizing compound.

According to the available information, long-term exposure to soybean oil does not appear to cause cancer in rats and mice. Although an increased incidence of virus-induced mammary tumours was seen in female CH3/OUJ mice (animals carrying tumour virus) fed

extremely high levels of soybean oil in the dietary mixture (6250 mg/kg bw/d), this effect was not reported in other strains of mice. In addition, soybean oil is widely used for human consumption, and long-term human use has not indicated any carcinogenic potential of this compound.

Results from published literature indicate that soybean oil is not a reproductive toxicant nor a teratogen in tested animals. Soybean oil is consumed daily as a food source without any reproductive or teratogenic effects reported in humans. In contrast, it is well known that fatty acids are required for the normal development of fetuses during the pregnancy.

It is well known that unsaturated fatty acids of the lipid membrane can react with many free radicals or alkylating agents (a reaction known as lipid peroxidation), resulting in formation of reactive intermediates such as 4-hydroxyalkenal and fatty acid free radicals. These reactive products are genotoxic and can react with proteins or DNA, causing cell damage (Amdur *et al*., 1991). Direct genotoxic effects of soybean oil or fatty acids are not reported in the literature, however, and this compound is not considered to be a carcinogen. Soybean oil, therefore, is not considered to be genotoxic or mutagenic.

It is well known that fatty acids are important constituents in the formation/development of the central nervous system (CNS). The white matter of the brain consists of myelinated fibres, which are composed principally of lipidic materials such as cholesterol, cerebroside and phospholipids. The total amount of myelin in the CNS increases from birth to maturity, and dietary fatty acids are the principal source of myelin synthesis (Ham A., 1963). Soybean oil, which contains several common fatty acids, is not likely, therefore, to be a neurotoxicant.

The four Blocker™ end-use formulations (each containing 2% soybean oil) are expected to be of similar acute toxicity. Based on the submitted bridging data, these products are expected to be of low acute toxicity, minimally irritating to the eyes, and not irritating to the skin. Blocker™ insect repellent formulations are considered to be potential dermal sensitizers on some hypersensitive people.

5.0 Food Exposure

No food uses are proposed at this time.

6.0 Drinking water exposure and risk assessment

The PMRA does not conduct environmental assessments for Use Site Category #26, *Human Skin Clothing and Proximal Sites*, and a drinking water exposure and risk assessment would not be applicable for the above-mentioned proposed products.

7.0 Occupational and bystander exposure

7.1 Qualitative Exposure Assessment

All proposed end-use formulations contain 2% soybean oil as the active ingredient to repel insects. They are to be applied to exposed skin when biting insects may be a nuisance, avoiding contact with lips and eyes. They are to be applied as needed, with effectiveness lasting one to three and one-half hours (h) for protection against mosquitoes, and three to eight hours for protection against black flies. The proposed labels also instruct the user not to apply on hands of young children. These products are all formulated as oils or lotions for application by hand except for Blocker™ Insect Repellent Easy-to-Use Spray, packaged in a spray pump.

Soybean oil conforms to specifications found in the USP #23 and the BP 1993. It is "generally recognized as safe" by the U.S. Food and Drug Administration and allowed for human consumption as a food and as a component that is allowed in contact with human food.

Soybean oil has a non-toxic mode of action for the target pests. It is a commonly used food, widely distributed in commerce, and available to the general public throughout Canada for non-pesticidal uses (i.e., daily consumption of soybean oil by adults and children [one year and older] of the Canadian population varies between 57 and 307 mg/kg bw, based on information collected by the PMRA).

As mentioned previously in the introduction, in March 1996, the EPA published in the *Federal Register*, a list of substances that are exempted from FIFRA requirements for registration. Soybean oil is exempted based on its status as a common food or constituent of a common food.

7.2 Risk Assessment

Based on information on toxicological profile and use of soybean oil as a common food, it is concluded that use of the proposed products is not likely to result in adverse human health effects. The risk associated with use of the proposed soybean oil products is acceptable for adults and children.

8.0 Value assessment

8.1 Description of the Pest Problem

In Canada, mosquitoes and black flies are considered to be pests primarily as a result of the annoyance caused by their presence and the discomfort and irritation caused by reactions to their bites. There are also occasional outbreaks of mosquito-borne encephalitides.

8.2 Survey of Alternatives

Diethyl-m-toluamide (DEET) and oil of citronella (and derivatives) are the active ingredients used in most insect repellents currently registered in Canada, with most registered products containing DEET alone. The registration of the Blocker™ series of soybean oil repellents would provide access in Canada to a new type of personal insect repellent.

8.3 Efficacy Evaluation

8.3.1 Criteria for Efficacy Evaluation

According to the PMRA's Draft Efficacy Assessment Guidelines, *Personal Repellents* (1995), the usual index of efficacy is Complete Protection Time (CPT), which is defined as the time from application of the repellent to the first confirmed bite (a bite followed by another within 30 minutes [min]). This is the most appropriate index for end-use products because most users want complete protection, rather than partial protection for a longer period. Where the main aim of the tests is to measure partial protection over a long period rather than complete protection, however, subjects can be exposed intermittently rather than continuously, and the numbers of bites on each subject during each exposure period counted. The index of repellency is, then, the difference in the numbers of bites between treated and untreated subjects. A repellent will normally only be considered "effective" for registration purposes for as long as it reduces biting by 95%. (This criterion must be met for a minimum of 30 min.)

8.3.2 Efficacy Data

A total of six field studies, three in 1996 (one black fly and two mosquito) and three in 1997 (one black fly and two mosquito) were conducted by personnel at the University of Guelph. (Note: Because the names of the test products for the same formulations change from one study to the next, the equivalent product in terms of submission number is provided.)

8.4 Black Fly Studies

Two black fly studies were submitted in support of the proposed products:

a) Study 1 - 1996 (a): *Comparative evaluation of the efficacy of Bite Blocker® and 20% DEET to repel black flies in Ontario, Canada*. L.R. Lindsay, G.A. Surgeoner and J.D. Heal. University of Guelph, July 1996. 9 pp.

b) Study 2 - 1997 (a): *Comparison of the efficacy of four Bite Blocker® formulations, Muskol®, OFF!® and Natrapel® to protect against black flies in Ontario, Canada*. L.R. Lindsay, G.A. Surgeoner, and J.D. Heal. University of Guelph, October 1997. 8 pp.

In Study 1, the repellents tested against black flies were Blocker™ Oil (2% soybean oil, equivalent to the formulation of Sub. No. 96-1527) and a 20% DEET standard. In Study 2, the repellents tested were Bite Blocker™ Light Country Scent Oil (equivalent to Sub. No. 96-1527), Bite Blocker™ Light Country Scent Lotion and Bite Blocker™ Spray (formulations identical; therefore, equivalent to both Sub. Nos. 96-1528 and 98-0170), and Bite Blocker™ Light Herbal Scent Lotion (equivalent to Sub. No. 96-1529).

The surface area of the forearm of each subject was measured, and 0.5 mL (for liquids) or 0.5 g (for lotions) of repellent was applied per 600 cm^2 of each forearm (wrist to elbow) of each subject. The dosage used in these studies was half of what is normally applied in repellent testing (i.e, the standard amount normally applied during personal repellent testing is 1 mg or 1 mL per 600 cm^2). According to J. Heal (University of Guelph, personal communication), only half the standard dosage was used because the standard dosage gave complete protection for the whole day (i.e., until after sunset, so there was no endpoint), and because the applicant also wanted to compare the soybean products to DEET-based products (i.e., by using half the dosage, differences among products could be demonstrated).

8.4.1 Results

Results for Study 1 are presented in Appendix II. Over the four-day study period, the Blocker™ Oil formulation (equivalent to Sub. No. 96-1527) and the 20% DEET formulation provided complete protection from black flies for approximately 9.7 (range of 9.2–10+) and 6.6 (range of 4.1–9.3) h, respectively.

Results for Study 2 are also presented in Appendix II. The Bite Blocker™ Light Country Scent Oil formulation (equivalent to Sub. No. 96-1527) had the longest CPT (5.6 h) from black flies of the formulations tested. The 25% DEET formulation had a CPT of 3.7 h, and the CPTs of the other tested formulations were similar (i.e., 2.8, 2.8, 2.5, and 2.9 h of CPT from black flies for 15% DEET, Bite Blocker™ Light Country Scent Lotion (equivalent to both Sub. Nos. 96-1528 and 98-0170), Bite Blocker™ Spray (also equivalent to both Sub.

Nos. 96-1528 and 98-0170), and Bite Blocker™ Light Herbal Scent Lotion (equivalent to Sub. No. 96-1529), respectively).

The test subjects had no negative comments concerning any of the products.

Simulium venustum Say was the only species found in sub-samples of black flies collected during the biting counts.

8.5 Mosquito Studies

Four mosquito studies were submitted in support of the proposed products:

a) Study 3 - 1996 (b): *Evaluation of Bite Blocker® as a repellent against spring Aedes spp. mosquitoes*. L.R. Lindsay, G.A. Surgeoner, and J.D. Heal. University of Guelph. July 1996. 5 pp.

b) Study 4 - 1996 (c): *Comparative evaluation of the efficacy of Bite Blocker®, OFF!® Skintastic, and Avon® Skin-So-Soft to protect against Aedes species mosquitoes in Ontario*. L.R. Lindsay, G.A. Surgeoner, and J.D. Heal. University of Guelph, August 1996. 5 pp.

c) Study 5 - 1997 (b): *Comparative field evaluation of the efficacy of Bite Blocker® Light Country Scent Oil, OFF!® insect repellent and Muskol insect repellent to repel Aedes mosquitoes in southern Ontario*. J.D. Heal, G.A. Surgeoner, and S.M. Butler. University of Guelph, October 1997. 11 pp.

d) Study 6 - 1997 (c): *Comparative field evaluation of the efficacy of two Bite Blocker™ lotion formulations, one Bite Blocker™ spray formulation and Natrapel® to repel Aedes mosquitoes in southern Ontario*. J.D. Heal, G.A. Surgeoner, and S.M. Butler. University of Guelph, October 1997. 15 pp.

In Studies 3, 4, and 5, the repellent tested against mosquitoes was Bite Blocker™ Light Country Scent Oil, also known as Bite Blocker™ (equivalent to Sub. No. 96-1527). Also tested were 6.7% DEET (Study 4), and 15% and 25% DEET (Study 5). In Study 6, the repellents tested were Bite Blocker™ Light Country Scent Lotion and Bite Blocker™ Spray (formulations identical; therefore, equivalent to both Sub. Nos. 96-1528 and 98-0170) and Bite Blocker™ Light Herbal Scent Lotion (equivalent to Sub. No. 96-1529).

Subjects were randomly assigned to a particular treatment, and repellent was applied to subjects at various pre-set intervals ranging from 0.5 to 7.5 h before the start of the biting counts. Biting counts were started approximately 30 min before sunset to correspond with peak mosquito biting activity. Biting counts were performed over a 30-min period so that the

protection provided by the repellents during a particular 30-min interval after product application could be determined.

8.5.1 Results

Percent repellency provided by all products at the various time intervals following application was calculated for the entire 30-min interval (see Appendix III).

In Study 3 (see Appendix III), with respect to percent repellency, regardless of when Bite Blocker™ (Sub. No. 96-1527) was applied before exposure to mosquitoes, it provided $99% reduction in bites versus (vs.) control subjects (i.e., when the product was applied at 30, 90, 150, and 210 min before exposure to mosquitoes, the average (ave.) percent repellency provided was greater than 99%). With respect to CPT, over the five-night evaluation period, when the soybean oil repellent was applied 30 and 90 min before the start of the biting counts, no bites and one bite, respectively, were received during the 30 min of exposure to mosquitoes, which meant that complete protection times could not be determined (i.e., no confirmed bite). When the repellent was applied at 150 and 210 min before the start of the biting counts, however, complete protection times could be determined on three of five and four of five nights, respectively. Considering only nights when two or more bites were received, the Bite Blocker™ formulation provided a CPT of 199.4 ± 29.7 min (3.3 h) from mosquitoes. This would be a conservative estimate of CPT, as there were no endpoints for some of the replicates.

In Study 4 (see Appendix III), the percent repellency provided by Bite Blocker™ Light Country Scent Oil (Sub. No. 96-1527) was 99.2%, 99% and 97% during the intervals of 0.5–1.0, 1.5–2.0 and 3.5–4.0 h, respectively. As a comparison, the percent protection provided by the 6.7% DEET formulation was 100%, 99.4% and 86% during the intervals of 0.5–1.0, 1.5–2.0 and 3.5–4.0 h, respectively.

In Study 5 (see Appendix III), Bite Blocker™ Light Country Scent Oil (Sub. No. 96-1527) provided an 89.4%, 83.1% and 77.6% reduction in bites vs. control subjects during the intervals of 3.5–4.0, 5.5–6.0 and 7.5–8.0 h, respectively. The 25% DEET formulation provided a 98.0%, 86.3% and 73% reduction in bites vs. control subjects during the intervals of 3.5–4.0, 5.5–6.0 and 7.5–8.0 h, respectively. The 15% DEET formulation provided an 88.1%, 67.4% and 44.9% reduction in bites vs. control subjects during the intervals of 3.5–4.0, 5.5–6.0 and 7.5–8.0 h, respectively.

In Study 6 (see Appendix III), Bite Blocker™ Light Country Scent Lotion (equivalent to Sub. Nos. 96-1528 and 98-0170) provided an 87.6%, 86.4% and 77.6% reduction in bites vs. control subjects during the intervals of 1.5–2.0, 3.5–4.0 and 5.5–6.0 h, respectively. Bite Blocker™ Spray (same formulation as Bite Blocker™ Light Country Scent Lotion) provided a 95.1%, 91.4% and 83.8% reduction in bites vs. control subjects during the intervals of 1.5–2.0, 3.5–4.0 and 5.5–6.0 h, respectively. Because the formulations of these two

products are identical, if results are pooled, the resulting mean percent repellency for the shortest post-application interval (i.e., 1.5–2.0 h) is 91.4% over the 30-min interval. Bite Blocker™ Light Herbal Scent Lotion (Sub. No. 96-1529) provided a 91.0%, 87.6% and 73.5% reduction in bites vs. control subjects during the intervals of 1.5–2.0, 3.5–4.0 and 5.5–6.0 h, respectively.

The average percent repellency value obtained against mosquitoes for the lotion and spray products during the first interval tested (1.5–2.0 h) was approximately 91% (as stated above), and it is reasonable to assume that the proposed products would have performed even better if tested sooner after product application. The applicant submitted additional information from the University of Guelph, which predicted the percent repellency that could be expected after shorter intervals post-treatment. A multiple linear regression equation using three points for each line (i.e., using the data presented in Study 6 for the three tested post-application time intervals), was used to solve for the variable "time" and to predict the percent repellency of Bite Blocker™ Light Country Scent Lotion (Sub. No. 96-1528), Bite Blocker™ Spray (Sub. No. 98-0170), and Bite Blocker™ Light Herbal Scent Lotion (Sub. No. 96-1529) at 0.5 and 1.0 h after product application (see Appendix IV). After 0.5 h, expected percent repellency values of 96.2% for Bite Blocker™ Light Country Scent Lotion/Bite Blocker™ Spray (pooled ave. of 92.5% and 99.8% because of identical formulations) and 99.1% for Bite Blocker™ Light Herbal Scent Lotion were derived. After 1.0 h, predicted percent repellency values of 95% for Bite Blocker™ Light Country Scent Lotion/Bite Blocker™ Spray (pooled ave. of 91.4% and 98.6% because of identical formulations) and 97.1% for Bite Blocker™ Light Herbal Scent Lotion were derived.

During Studies 3, 4 and 5, none of the subjects noted any adverse effects after the product was applied. During Study 6, only one subject complained of an adverse reaction to a Bite Blocker™ product. After a male subject applied Bite Blocker™ Light Country Scent Lotion, his forearms itched for approximately 10 min while redness and welts persisted for approximately two hours. A few days later, he applied the same product and had no reaction. It was inconclusive as to whether or not the lotion caused his initial response.

Although captured mosquitoes were not identified for this study, the authors report that in early June 1993, *Aedes stimulans* (Walker), *Ae. canadensis* (Theobald), *Ae. euedes* Howard, Dyar, and Knab, and *Ae. fitchii* (Felt and Young) accounted for more than 88% of the mosquitoes collected at this site.

8.6 Overall conclusions

Based on the submitted efficacy data, the supportable pest and protection time claims are as follows:

Product	Black Flies	Mosquitoes
Blocker™ Long-Lasting Oil (Sub. No. 96-1527)	8 hours Based on two studies with CPTs of 9.7 and 5.6 h (ave. = 7.65) (Appendix II, Studies 1 and 2) (The CPT demonstrated can be rounded off to eight hours considering that only half the normal dosage was used.)	3.5 hours Based on three studies where percent repellency was 99%, 97% and 89.4% from 3.5 to 4.0 h, and a CPT in one study of 3.3 h (Appendix III, Studies 3, 4 and 5).
Blocker™ Moisturizing Lotion (Sub. No. 96-1528) Blocker™ Easy-To-Use Spray (Sub. No. 98-0170) (Because formulations are identical, data are combined.)	3 hours Based on two studies with CPTs of 2.5 and 2.8 h (ave. = 2.65) (Appendix II, Study 2) (The CPT demonstrated can be rounded off to three hours considering that only half the normal dosage was used.)	1 hour The percent repellency at 1.5–2 h after treatment was approximately 91.4% (pooled ave. of 87.6% and 95.1%) for the two products (Appendix III, Study 6). The expected percent repellency value at one hour post-application was 95% (pooled ave. of 91.4% and 98.6%) (Appendix IV).
Blocker™ Light Herbal Scent Lotion (Sub. No. 96-1529)	3 hours Based on one study with a 2.9-h CPT (Appendix II, Study 2)	1 hour The percent repellency at 1.5–2 h after treatment was 90.9% (Appendix III, Study 6). The expected percent repellency value at one hour post-application was 97.1% (Appendix IV).

9.0 Regulatory proposal

The Agency has established, pursuant to Section 13 of the Pest Control Products Regulations, interim registrations (time-limited to December 31, 1999) of the technical grade active ingredient and the associated end-use formulations, and is open to comments on their future regulatory status.

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List of Abbreviations

ave.	average
BP	British Pharmacopoeia
bw	body weight
CNS	central nervous system
CPT	complete protection time
d	day
DEET	diethyl-m-toluamide
EPA	Environmental Protection Agency
FIFRA	*Federal Insecticide, Fungicide and Rodenticide Act*
h	hour
min	minute
PMRA	Pest Management Regulatory Agency
Reg. No.	Registration Number
SD	standard deviation
SE	standard error
Sub. No.	Submission Number
USP	United States Pharmacopoeia
vs.	versus
wk	week
w/w	weight per weight

Appendix I Percent repellency provided over a four-hour evaluation period by Blocker™ formulations against laboratory-reared *Aedes aegypti* mosquitoes.

Formulation	Time After Application (minutes)					Over All Of The Time Intervals
	0	60	120	180	240	
Light Country Scent Oil (Sub. No. 96-1527) (re-named Blocker™ Insect Repellent Long-Lasting Oil)	100% a**	100% a	98.3% a	98.1% a	97.1% a	98.7% a
Light Herbal Scent Lotion (Sub. No. 96-1529) (re-named Blocker™ Insect Repellent Light Herbal Scent Lotion)	100% a	98.1% a	98.1% a	96.3% a	92.9% a	97.1% a
Light Country Scent Lotion (Sub. No. 96-1528) (re-named Blocker™ Insect Repellent Lotion)	99.7% a	98.5% a	95.6% a	83.7% ab	88.0% a	93.1% a
#1001 (same % w/w of formulants, except for water, as Sub. No. 96-1527 but with **no soybean oil**)*	99.2% a	80.2% ab	73.2% b	75.2% b	71.0% a	79.8% b
#1002 (same % w/w of formulants, except for water, as Sub. No. 96-1527 but with no coconut oil)*	100% a	97.3% a	93.5% a	92.3% ab	88.2% a	94.2% a
#1003 (same % w/w of formulants, except for water, as Sub. No. 96-1527 but with **no soybean oil** or coconut oil)*	91.9% b	57.6% b	46.3% c	38.5% c	30.0% b	52.9% c

* Remaining percentage of each formulation made up with water.

** Percent repellency values within columns followed by the same letter are not significantly different ($p \# 0.05$).

Appendix II Black fly data submitted for Blocker™ insect repellents (Sub. Nos. 96-1527, 96-1528, 96-1529, 98-0170): 2.0% soybean oil

Treatment: Dermal application
Name of Pest: Black flies (*Simulium venustum*)

Ref. No.	Author, Year, Test Location	Treatment	Dosage per Forearm (600 cm²)	Time of Application	Mean Complete Protection Time (Range) in Hours	Comments
Study 1 1996 (a)	Lindsay, Surgeoner, and Heal (University of Guelph, 1996) Test Location: Montreal River, New Liskeard, Ontario	Blocker™ Oil (2% soybean oil) (Sub. No. 96-1527)	0.5 mL	10:00 a.m.	9.7 ± 0.7 Standard Deviation (SD) (9.2–10+)	- no. of subjects = 4 - no. of days = 4 (June 15–18, 1996) - duration of exposure/day: 10 h (if confirmed bite not received, CPT was considered to be 10+ h) - area exposed: two forearms/person - untreated: approximately 5.7 bites per five min
		20% DEET	0.5 mL	10:00 a.m.	6.6 ± 2.7 (SD) (4.1–9.3)	
Study 2 1997 (a)	Lindsay, Surgeoner, and Heal (University of Guelph, 1997) Test Location: Petawawa, Ontario	Bite Blocker™ Light Country Scent Oil (2% soybean oil) (Sub. No. 96-1527)	0.5 mL	8:00 a.m.	5.6 ± 0.8 Standard Error (SE) (1.9–10+)	- no. of subjects = 7 - no. of days = 7 (June 2–8, 1997) - duration of exposure/day: 10 h (if confirmed bite not received, CPT considered 10+ h) - area exposed: two forearms/person - untreated: approximately two bites per five min
		Bite Blocker™ Light Country Scent* Lotion (2% soybean oil) (= Sub. Nos. 96-1528 and 98-0170)	0.5 mL (for liquids) or 0.5 g (for lotions)	8:00 a.m.	2.8 ± 0.4 (SE) (0.9–4.6)	
		Bite Blocker™ Spray* (2% soybean oil) (= Sub. Nos. 96-1528 and 98-0170)	0.5 mL (for liquids) or 0.5 g (for lotions)	8:00 a.m.	2.5 ± 0.5 (SE) (0.6–6.5)	
		Bite Blocker™ Light Herbal Scent Lotion (2% soybean oil) (Sub. No. 96-1529)	0.5 mL (for liquids) or 0.5 g (for lotions)	8:00 a.m.	2.9 ± 0.6 (SE) (0.9–9)	

Ref. No.	Author, Year, Test Location	Treatment	Dosage per Forearm (600 cm^2)	Time of Application	Mean Complete Protection Time (Range) in Hours	Comments
		25% DEET	0.5 mL	8:00 a.m.	3.7 ± 0.3 (SE) (2.3–5.6)	
		15% DEET	0.5 mL	8:00 a.m.	2.8 ± 0.3 (SE) (1.5–5.4)	

* Identical formulations

Appendix III Mosquito data submitted for Blocker™ insect repellents (Sub. Nos. 96-1527, 96-1528, 96-1529, 98-0170): 2.0% soybean oil

Treatment: Dermal application
Name of Pest: Mosquitoes (primarily *Aedes stimulans*, *Ae. canadensis*, *Ae. euedes* and *Ae. fitchii*)

Ref. No.	Author, Year, Test Location	Treatment	Dosage per Forearm (600 cm^2)	Time Post-application	Mean Percent Repellency	Comments
Study 3 1996 (b)	Lindsay, Surgeoner, and Heal (University of Guelph, 1996) University of Guelph Arboretum	Bite Blocker™ (2% soybean oil) (Sub. No. 96-1527)	1 mL	0.5–1 h 1.5–2 h 2.5–3 h 3.5–4 h	100% 99.8% 99.0% 99.0% Note: Mean CPT of 3.3 h also obtained at 2.5+ h post-application.	- no. of subjects = 5 - no. of evenings = 5 (June 3–11, 1996) - duration of exposure/evening = 30 min (five 5-min biting counts/interval) - area exposed: two forearms/person - initiation time: 20:30 - 252 bites received, on average, by non-treated subjects per 30 min
Study 4 1996 (c)	Lindsay, Surgeoner, and Heal (University of Guelph, 1996) University of Guelph Arboretum	Bite Blocker™ Light Country Scent Oil (2% soybean oil) (Sub. No. 96-1527)	1 mL	0.5–1 h 1.5–2 h 3.5–4 h	99.2% 99.0% 97.0%	- no. of subjects = 10 - no. of evenings = 10 (July 10–23, 1996) - duration of exposure/evening = 30 min (ten 2.5-min biting counts per interval) - area exposed: two forearms/person - initiation time: 20:15 - although untreated counts not presented, they were used to calculate percent repellency
		6.65% DEET		0.5–1 h 1.5–2 h 3.5–4 h	100% 99.4% 85.8%	

Ref. No.	Author, Year, Test Location	Treatment	Dosage per Forearm (600 cm^2)	Time Post-application	Mean Percent Repellency	Comments
Study 5 1997 (b)	Heal, Surgeoner, and Butler (University of Guelph, 1997) University of Guelph Arboretum	Bite Blocker™ Light Country Scent Oil (2% soybean oil) (Sub. No. 96-1527)	1 mL	3.5–4 h 5.5–6 h 7.5–8 h	89.4% 83.1% 77.6%	- no. of subjects = 11 - no. of evenings = 11 (June 19–27, July 2–9, 1997) - duration of exposure/evening = 30 min (eleven 2-min biting counts/interval) - initiation time: 20:15 - the number of mosquitoes biting the controls (N = 2) per two-min biting counts was 7.95 ± 4.96 (SD)
		25% DEET	1 mL	3.5–4 h 5.5–6 h 7.5–8 h	98.0% 86.3% 73.0%	
		15% DEET	1 mL	3.5–4 h 5.5–6 h 7.5–8 h	88.1% 67.4% 44.9%	

Appendix III (cont'd) **Mosquito data submitted for Blocker™ insect repellents (Sub. Nos. 96-1527, 96-1528, 96-1529, 98-0170): 2.0% soybean oil**

Treatment: Dermal application
Name of Pest: Mosquitoes (primarily *Aedes stimulans, Ae. canadensis, Ae. euedes* and *Ae. fitchii*)

Ref. No.	Author, Year, Test Location	Treatment	Dosage per Forearm (600 cm^2)	Time Post-application	Mean Percent Repellency	Comments
Study 6 1997 (c)	Heal, Surgeoner, and Butler (University of Guelph, 1997) University of Guelph Arboretum	Bite Blocker™ Light Country Scent Lotion* (2% soybean oil) (= Sub. Nos. 96-1528 and 98-0170)	1 g	1.5–2 h 3.5–4 h 5.5–6 h	87.6% 86.4% 77.6%	- no. of subjects = 14 - no. of evenings = 14 (June 23–27, July 2–16, 1997) - duration of exposure/evening = 30 min (fourteen 1.7 min biting counts/interval) - initiation time: 20:15 - the number of mosquitoes biting the controls (N = 2) per 1.7 min was 4.41 ± 3.75 (SD)
		Bite Blocker™ Spray* (2% soybean oil) (= Sub. Nos. 96-1528 and 98-0170)	1 g	1.5–2 h 3.5–4 h 5.5–6 h	95.1% 91.4% 83.8%	
		Bite Blocker™ Light Herbal Scent Lotion (2% soybean oil) (Sub. No. 96-1529)	1 g	1.5–2 h 3.5–4 h 5.5–6 h	90.9% 87.6% 73.5%	

* Identical formulations

Appendix IV Predicted number of mosquitoes biting subjects per 1.7-minute biting counts and percent repellency versus control subjects calculated[1] from regression equation and regression values for Blocker™ lotion insect repellents (Sub. Nos. 96-1528, 96-1529, 98-0170): 2.0% soybean oil

Treatment: Dermal application
Name of Pest: Mosquitoes (primarily *Aedes stimulans, Ae. canadensis, Ae. euedes* and *Ae. fitchii*)

Ref. No.	Author, Year,	Product	Hours Post-application (*x*)	Predicted Number of Mosquitoes (*y*)[2]	Predicted Percent Repellency
N/A	Heal (University of Guelph. 1998) (Based on data in Heal, Surgeoner, and Butler study, University of Guelph, 1997, reported in Appendix III)	Bite Blocker™ Light Country Scent Lotion* (2% soybean oil) (= Sub. Nos. 96-1528 and 98-0170)	0 0.5 1.0	0.27 0.33 0.38	93.9% 92.5% 91.4%
		Bite Blocker™ Spray* (2% soybean oil) (= Sub. Nos. 96-1528 and 98-0170)	0 0.5 1.0	- 0.07 (0) 0.01 0.06	100.0% 99.8% 98.6%
		Bite Blocker™ Light Herbal Scent Lotion (2% soybean oil) (Sub. No. 96-1529)	0 0.5 1.0	- 0.60 (0) 0.04 0.13	100.0% 99.1% 97.1%

* Identical Formulations

1 Calculated from the linear regression equation $y = a + bx$, where y equals biting rate and x equals time.

2 If value predicted from equation of regression line was less than zero, then the number of bites was considered zero for percent reduction calculation.