Pest Management Regulatory Agency
Agence de réglementation de la lutte antiparasitaire

Proposed Acceptability for Continuing Registration

PACR2004-36

Re-evaluation of Citronella Oil and Related Active Compounds for Use as Personal Insect Repellents

In June 1990 (A90-01, *Re-evaluation of Personal Insect Repellents*), it was announced that personal insect repellent active ingredients, including citronella oil and related compounds, were subject to re-evaluation under authority of Section 19 of the Pest Control Products (PCP) Regulations.

Based on the available information, the PMRA was unable to conclude that the human health risks from the use of personal insect repellent products containing citronella oil and related compounds, applied directly to the skin, continues to be acceptable. As a result, the PMRA is proposing that the registrations for personal insect repellent products containing citronella oil and related active compounds applied directly to the skin be phased out unless registrants commit to provide further data that would address the deficiencies identified in this document.

This Proposed Acceptability for Continuing Registration (PACR) document provides a summary of the data reviewed and the rationale for the proposed regulatory decision for citronella oil and related substances. The PMRA will accept written comments on this proposal up to 60 days from the date of publication of this document. Please forward all comments to the Publications Coordinator at the address below.

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Foreword

The re-evaluation of the active ingredient citronella oil and related substances as well as the associated end-use products (EPs) for use as personal insect repellent applied directly to the skin has been completed by the Pest Management Regulatory Agency (PMRA).

In June 1990 (A90-01, *Re-evaluation of Personal Insect Repellents*), it was announced that personal insect repellent active ingredients, including citronella oil and related compounds, were subject to re-evaluation under authority of Section 19 of the PCP Regulations.

Based on the available information, the PMRA was unable to conclude that the human health risks from use of personal insect repellent products containing citronella oil and related compounds, applied directly to the skin, continues to be acceptable. As a result, the PMRA is proposing that the registrations for personal insect repellent products containing citronella oil and related active compounds applied directly to the skin be phased out unless registrants commit to provide further data that would address the deficiencies identified in this document.

The PMRA will accept written comments up to 60 days from the date of publication of this document to allow interested parties an opportunity to provide input into the proposed re-evaluation decision for these products. As part of normal re-evaluation practice, registrants of insect repellant products containing citronella oil and related active compounds may use this consultation period to provide any available data that has not been previously submitted or to indicate their commitment to generate further studies. The PMRA will consider this information before confirming the remaining data requirements and arriving at a final re-evaluation decision.

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1.0 Purpose

In June 1990 (A90-01, *Re-evaluation of Personal Insect Repellents*), it was announced that personal insect repellent active ingredients, including citronella and related substances, applied directly to the skin, were subject to re-evaluation under authority of Section 19 of the PCP Regulations. The purpose of this document is to provide the registrants, pesticide regulatory officials and the Canadian public with the results of an assessment to evaluate the acceptability of the insect repellent citronella oil, its related active compounds and its end-use products. This document includes a human health assessment, efficacy assessment and information on the value of citronella oil to pest management in Canada. By way of this document, the PMRA is soliciting comments from all interested parties on these assessments and the regulatory proposal.

2.0 General background on re-evaluation

In Announcement A90-01, *Re-evaluation of Personal Insect Repellents,* Agriculture Canada requested that the registrants of personal insect repellent products submit, within six months, indices to all known toxicology and efficacy studies on their products, plus copies for re-evaluation of any studies that had not already been submitted.

Since the publication of Announcement A90-01, the PMRA has announced a larger re-evaluation effort, under Section 19 of the Regulations pursuant to the *Pest Control Products Act* (PCPA), of all pesticides, both active ingredients and formulated end-use products (EPs), that were registered prior to 1995 to ensure that their continued acceptability is examined using current scientific approaches. Regulatory Directive DIR2001-03, *PMRA Re-evaluation Program,* outlines the details of the re-evaluation activities.

The re-evaluation of citronella oil is being completed under Program 2 of the PMRA re-evaluation program, as described in Regulatory Directive DIR2001-03, *PMRA Re-evaluation Program*.

3.0 Re-evaluation of citronella oil and related active compounds

At the time of re-evaluation Announcement A90-01, *Re-evaluation of Personal Insect Repellents*, citronella oil and its related active compounds were among eight active ingredients used in insect repellent products.

The focus of the re-evaluation is on the human health effects of this chemical, and its efficacy and value as an insect repellent. The effect of citronella oil on the environment is not included in this assessment because citronella oil is not applied directly to the environment.

The first personal insect repellent containing citronella oil in Canada was registered from 1940 to 1958. Several different, but related, active substances currently used in insect repellent products are affected by the re-evaluation of citronella oil. Most of these active substances are complex mixtures composed principally of chemically/structurally related terpenes. Each of these complex mixtures is considered by the PMRA to be an individual active substance. This group of active substances includes natural citronella oil, artificial citronella oil (blend of natural oils), citronellal (an individual constituent of citronella oil) and natural oil blends (not claimed to be citronella oil) that contain citronella oil, its constituents or related active substances.

Currently, there are 13 registered EPs containing citronella in a variety of formulation types, all intended for direct application to skin for the purpose of repelling biting insects (Appendix I). These formulation types include citronella oil (CIT); an artificial essential oil blend (CTR) containing citronella oil and other essential oils; citronellal (CIL), which is an extract of citronella oil; citronella terpenes (CIR); and a mixture (CIO) of oils of camphor, citronella, citrus, eucalyptus, geranium and pine.

3.1 Information used in re-evaluation

Where available, chemistry data originally submitted for each of these active ingredients (CIT, CTR, CIR, CIL, CIO) were used for this re-evaluation.

The toxicology database supporting citronella oil and related active substances is based on a United States Environmental Protection Agency (USEPA) Reregistration Eligibility Decision (RED) document for citronella oil, National Toxicology Program (NTP) study data, Joint WHO/FAO Expert Committee on Food Additives (JECFA) evaluation documents and the published scientific literature. Little mammalian toxicity data were available for the natural oils and for some of their individual constituents. Registrants did not provide requested mammalian toxicology data.

No chemical-specific consumer exposure or use data were submitted in support of this re-evaluation.

Efficacy data for citronella oil and the related active substances (CTR, CIR, CIL, CIO, other related natural oils) were found in unpublished reports from registrants or applicants for registration as well as books and papers published between 1939 and 1995.

4.0 Identity of the active substance

Five different, but related, ‘active substances’ (i.e., CIT, CTR, CIR, CIL, CIO) currently used in insect repellent products are affected by the re-evaluation of citronella oil and related active substances. Most of these active substances are complex mixtures composed principally of chemically/structurally related terpenes. Each of these complex mixtures is considered by the PMRA to be an individual ‘active substance’. The true

active component(s) in these “active substances” is unknown, however, it is very likely that more than one component possesses insect repellent activity.

This group of active substances includes natural citronella oil, artificial citronella oil (blend of natural oils), an individual constituent of citronella oil, citronellal, and natural oil blends (not claimed to be citronella oil) that contain citronella oil, it constituents or related active substances. As these are extracted from natural sources, lot to lot variation in the composition of these oils occurs.

Natural citronella oil is a volatile oil, obtained by steam distillation of freshly cut or partially dried cultivated grasses: *Cymbopogon nardus* (Rendale) or *Cymbopogon winteranus* (Jowitt). It is made of more than 80 compounds of closely related terpenic hydrocarbons, alcohols and aldehydes, and, depending on the growing conditions and time of harvest, the levels of these components will vary. A study submitted by the Citronella Joint Venture provided information on the identity of most of the components found in oil of citronella. Two principal varieties of citronella oil are available commercially: the “Ceylon type” (extracted from *Cymbopogon nardus*) and “Java type” (extracted from *Cymbopogon winteranus*). The “Java type” oil is produced in larger quantities and contains a higher concentration of the aldehyde citronellal than the “Ceylon type” oil.

Normally, the PMRA requires that technical grade active ingredients be analysed for all components present at or above 0.1%. Given the complexity in chemical composition of citronella oil, this requirement has been waived. Instead, the PMRA has accepted characterization of the oil based on criteria such as the range of total aldehydes and total alcohols present. However, components of toxicological concern must be analyzed to the method limit of quantitation.

Impurities of toxicological concern as identified in Regulatory Directive DIR98-04 or TSMP Track 1 substances as identified in Regulatory Directive DIR99-03, Appendix II, are not expected to be present in the technical grade active ingredient (TGAI).

5.0 Products containing citronella oil and related compounds

A list of registered EPs, their classification and labelling, as of February 2003, is presented in Appendix I.

The EPs are either solutions, emulsifiable concentrates or pastes applied to skin either directly or as a pump spray. Products generally contain less than 15% of total “active ingredient”. The labels of six products claim to repel only mosquitoes, two claim to repel only black flies, and five claim to repel both mosquitos and black flies. Most labels have general application directions and instruct users to apply a thin film to exposed skin. However, of the thirteen products registered, only eight products specify a re-application interval and only four products suggest a maximum number of applications. Three products additionally note that users should not apply the product to damaged skin

(sunburn, abrasion, cut) and to wash off the product after returning inside. All product labels suggest avoiding contact with eyes and/or mouth and also direct users not to use the products on infants or toddlers.

6.0 Effects having relevance to human health

6.1 Metabolism and toxicology

The metabolism/toxicokinetic profiles of natural citronella oil have not been described as such because they contain many individual constituents. However, the majority of citronella is composed of a group of structurally related monoterpenes. As a result of the close structural relationship of the monoterpene compounds in this group, these compounds have similar metabolic pathways. The metabolic profiles of individual compounds within citronella oil have been established. The metabolism of these compounds is rapid and occurs predominantly via high capacity enzyme systems. In general, monoterpenes are rapidly and extensively absorbed orally and dermally, and are rapidly metabolized and excreted, primarily in the urine (Dilberto et al., 1988; WHO TRS. No. 828; WHO Food Additive Series 40, 1998, and 42, 1999; Chadha and Madyastha, 1982; Ishida et al., 1989).

Based on their closely related, well characterized chemical structures and metabolic pathways, rapid metabolism and excretion, and the fact that citronella oil is composed primarily of monoterpenes, the toxicity profiles of individual monoterpenes can reasonably be expected to approximate the potential toxicity of natural citronella oil. Since there was very little mammalian toxicity data specific to natural citronella oil, the mammalian toxicity data available for several components of citronella oil were used to assess the potential toxicity of natural citronella oil (Appendix II).

Acute toxicity studies (in USEPA, 1997) indicated that natural citronella oil was of low acute toxicity by the oral and inhalation routes in rats, and by the dermal route in the rabbit, and was mildly irritating to eyes and skin of rabbits. “Ceylon type” natural citronella oil was a dermal sensitizer in guinea pigs; “Java type” oil was not. Acute toxicity clinical signs following a single oral gavage dose included lethargy, piloerection and prostration. Lesions at lethal doses indicated toxic effects in the brain, spleen, kidneys, small intestine, stomach, liver and urinary bladder.

No mammalian toxicity studies describing the subchronic and/or chronic toxicity of natural citronella oil were available. However, published studies were available for some individual natural constituents of natural citronella oil (e.g., α-terpinene, methyleugenol or *d*-limonene)[1] and a structurally related compound, citral (a metabolite of geraniol, which is a constituent of citronella; citral is contained in other oils used in formulated

[1] The PMRA has not received data to confirm the presence/absence of each of these constituents in each source of citronella oil found in registered products. Source-specific chemistry data would be required to confirm the presence and levels of each constituent.

products). Subchronic oral gavage of dogs with *d*-limonene did not cause clinical signs of toxicity or histopathological changes, but did cause increased absolute and relative kidney weights at the highest dose. Subchronic oral gavage or feeding studies with citral using rats were associated with decreases in food intake and weight gain, liver hypertrophy, peroxisome proliferation, cytochrome P-450 induction, hyperkeratosis, bone marrow atrophy and haemorrhage, nephrotoxicity or forestomach epithelial hyperplasia. In mice, subchronic oral treatment with citral resulted in mortality, forestomach necrosis, hepatocyte vacuolization, lethargy and ovarian atrophy. Chronic exposure of rats and mice to citral caused decreased body weight in both rats and mice (NTP draft technical report TR-505, 2001).

No mammalian toxicity data describing the oncogenic potential of natural citronella oil were available. However, published long-term toxicity/oncogenicity studies were available for two constituents of citronella oil: *d*-limonene and methyleugenol and the structurally related compound, citral. Chronic oral dosing with *d*-limonene caused renal tumours in male rats. However, numerous studies have demonstrated that this response is specific to male rats; the mechanism of tumour development does not exist in humans and this response is not considered relevant to humans (Flamm and Lechman-Meckeeman, 1991; Hard and Whysner, 1994). Following chronic exposure of rats and mice to citral, an observed increase in malignant lymphoma incidence in female mice was considered incidental since the incidence was within historical control values (NTP draft technical report TR-505, 2001). The NTP concluded that there was no evidence of carcinogenic activity in rats or male mice and equivocal evidence for carcinogenic activity in female mice. Methyleugenol demonstrated evidence of carcinogenicity in both sexes of rats and mice following chronic oral dosing (Johnson, J.D. et al., 2000; NTP TRS 491, 1998), and has been classified as “reasonably anticipated to be a human carcinogen” by NTP (NTP TRS 491, 1998). Methyleugenol was genotoxic in several in vitro mutagenicity tests including chromosomal recombination assay in yeast, unscheduled DNA synthesis in rat primary hepatocytes, DNA adduct formation in rat and human liver microsomes, and in vivo liver DNA adduct formation in mice. Natural citronella oil (“Java type”), eugenol, *d*-limonene and citral were negative in a number of mutagenicity tests.

No reproductive or developmental toxicity studies for natural citronella oil were available. Data were available for α-terpinene, a natural constituent of citronella oil and a structurally related compound, citral. Although limited, a non-guideline one-generation dermal rat reproductive toxicity study dosed with citral prior to mating only caused decreases in implantation sites, reduced litter size, decreased pup viability, increased postimplantation loss, decreased number of corpora lutea and degeneration of ovarian follicles (Toaff, 1979). All pups from dams treated with citral for 100 days prior to mating died. A rat developmental toxicity study with citral demonstrated similar effects (decreased pregnancy rate, implantation sites, number of live fetuses, and delayed ossification in fetuses at maternally toxic doses) but did not demonstrate any evidence of teratogenicity at the doses tested. In a rat developmental toxicity study, α-terpinene caused decreased fetal weights at the highest dose; incomplete ossification of some

skeletal elements and increased incidences of irregularly shaped skull bones were observed below a maternally toxic dose, indicating increased sensitivity of the fetus.

No neurotoxicity studies on citronella oil were available. Constituents of citronella oil and structurally related compounds (citral, beta-mycerene, pinene, eugenol, citronellol, citronellal, linalool and linalyl acetate) have been reported in the public literature to have sedative properties manifested by a similar mode of action (Aoshima, 1999; de Barros 2000; Buchbauer, 1993, 1991; Gheldardini, 1999; Lis-Balchin, 1999; Re, 2000; Elisabetsky, 1995).

Incident cases of poisoning with citronella oil were available from published literature. Overall, these data suggest that systemic poisoning with citronella oil is very rare and is not a major concern in relation to other pesticides. One fatal case of poisoning with citronella oil was reported involving a 21-month-old child, but it was difficult to determine if the fatal outcome of the case was solely due to citronella oil. Five non-fatal cases of poisoning with citronella oil have been reported to the New Zealand National Poisons and Hazardous Chemicals Information Centre, Dunedin (Wayne et al., 1991).

6.2 Consumer non-cancer risk assessment

No chemical-specific exposure or use data were submitted in support of this re-evaluation; therefore exposure was estimated using default assumptions for body surface area, body weight, the percent of the body area to which citronella oil was applied and the rate of application (mg product/cm^2 of skin). Given the difference in surface area and body weight, it was considered appropriate to perform separate assessments for adults and children. It was assumed that an individual wearing a short-sleeved shirt, shorts and shoes has approximately 25% of their body surface area exposed (i.e., head, hands, forearms, and lower legs); this exposed body surface area was assumed to represent the area where citronella oil would typically be applied.

Assumptions: body weight and surface area, application area and exposure

Assumption	Adult	Child (3 years)
Body weight (kg)	70	15
Body surface area (cm^2)[a]	18 440	6565
Application area (cm^2)	4610	1641

[a] Based on draft NAFTA Harmonization Paper (1999)

One application per day was estimated to represent a typical use scenario. The rate of application for citronella oil was assumed to be 1 mg/cm^2 of skin area. This method produced exposure estimates similar to those calculated for other repellents evaluated by the PMRA. In the absence of data, it was assumed that the 1 mg/cm^2 would be representative of all formulation types (spray, lotion and paste). In addition to the lack of

exposure data, no dermal absorption data was submitted. However, physical–chemical characteristics of citronella oil, including a high octanol/water partition coefficient and slight solubility in water, suggest that it is likely to be well absorbed via the dermal route. One published study examining citral absorption in male rats suggests that citral may be rapidly absorbed through the skin (Diliberto et al., 1988). A dermal absorption of 100% was assumed in calculations.

Daily dermal exposure is calculated using the following equation:

$$\textit{mg a.i./kg bw} = \frac{\textit{application area (cm}^2\textit{)} \times \textit{1 mg formulation/cm}^2 \times \textit{X\% a.i.}}{\textit{body weight}}$$

Exposure to citronella oil may be either acute (one day) or intermediate-term (i.e., daily over several weeks). Separate exposure estimates were not derived for the acute and intermediate term risk assessments as the exposure assumptions are the same. The calculated risks are expressed in terms of a margin of exposure (MOE) which are derived using the following formula:

$$\textbf{MOE} = \frac{\textbf{NOAEL (dermal or oral)}}{\textbf{human dermal exposure}}$$

The PMRA used a no observed adverse effect level (NOAEL) of 30 mg/kg bw/day from a rat teratology study with α-terpinene for acute-term dermal risk assessment. An MOE of 1000 was selected to include 10× for interspecies extrapolation, 10× for intraspecies variability and an extra safety factor of 10×. This additional safety factor was selected to account for the data gaps in the citronella oil database and indications of fetal/offspring sensitivity in the α-terpinene rat teratology study.

The PMRA used a NOAEL of 30 mg/kg bw/day determined in the α-terpinene rat teratology study for intermediate-term dermal risk assessment. An MOE of 3000 was selected to include 10× for interspecies extrapolation, 10× for intraspecies variability and an additional safety factor of 30×. This additional safety factor was selected to account for data gaps in the citronella oil database, uncertainty of surrogate data use, observed/potential differences in potency of individual constituents of whole natural citronella oil, indications of fetal/offspring sensitivity (10×) and for the use of a short-term study for a longer term exposure period (3×).

The achieved MOE values following a single application of citronella and/or related active-containing products were in the range of 3 to 36 for children and adults, with the majority of values below 10. Achieved MOE values for repeated applications of these products were lower. Thus, the achieved MOE values for a child or adult for either acute- or intermediate-term use scenarios following 1, 2 or 5 applications of products containing citronella oil or related active compound(s) did not meet the target MOE values.

6.3 Cancer risk assessment

Although available data for whole natural citronella oil (in vitro) or citral (in vitro and in vivo mutagenicity tests, two-year rat and mouse chronic/oncogenicity bioassays) did not indicate oncogenic hazard, a qualitative cancer risk assessment was conducted. The oncogenicity concern stems from the natural presence of methyleugenol in whole natural citronella oil. Methyleugenol has been demonstrated to be mutagenic, to induce tumour formation in rats and mice, and is "reasonably anticipated to be a human carcinogen" (NTP, 2002).

In a document dated 26 September 2001, the European Commission's Scientific Committee on Food "recommended that absence of methyleugenol in food products be ensured and checked with the most effective available analytical method". At the 14th Plenary Meeting of the Committee for Cosmetic Products, and Non-Food Products Intended for Consumers, 24 October 2000, the Committee concluded that methyleugenol should not be intentionally added as a cosmetic ingredient. The Committee's conclusions also state the following:

> ...for a fragrance compound containing methyleugenol naturally present in essential oils, the concentration of methyleugenol should not exceed 0.05% in the fragrance compound. This means that, based on the highest exposure, the concentration of methyleugenol in the finished cosmetic product may not exceed 0.01% in a fine fragrance, 0.004% in eau de toilette, 0.002% in a fragrance cream, 0.0002% in other leave-on products and in oral hygiene products, and 0.001% in rinse-off products.

Methyleugenol has been shown to be present as a natural constituent of some sources of oil of citronella. A study submitted by the Citronella Joint Venture provided information on the composition of five samples of oil of citronella from different locations. Methyleugenol was found both in samples of the "Ceylon type" and of the "Java type". Most personal insect repellents with citronella oil registered in Canada contain the "Java type" oil. The PMRA has no data to confirm the presence/absence of methyleugenol in those sources of citronella oil used in the registered products and, if present, at what levels.

In consideration of the use pattern that involves frequent dermal application of products containing natural citronella oil and the limited data available, it could not be concluded that the carcinogenic hazard posed by methyleugenol in these products remains acceptable.

7.0 Value assessment

7.1 Biology of pests

The labels of six personal insect repellents that contain citronella oil and/or related substances claim that the products repel mosquitoes (*Culicidae*), the labels of two products claim that the products repel black flies (*Simuliidae*) and the labels of five products claim that they repel both insects. These insects are considered pests because the adult females bite people and feed on their blood.

7.2 Medical importance

Blood feeding can cause annoyance, blood loss or allergic reactions and may infect people with diseases. Although rare, arthropod-borne diseases known to occur in Canada include viral encephalitis (Western Equine, Eastern Equine and St. Louis), transmitted by *Culex* mosquitoes. West Nile virus, a mosquito-borne virus that can cause encephalitis (inflammation of the brain), has recently become a public health concern. Many mosquito species can become infected with West Nile virus. The occurrence or threat of these diseases stimulates the sales of personal insect repellents in Canada.

The Population and Public Health Branch, Health Canada, advises Canadians who travel abroad to use personal insect repellents to protect themselves against tropical diseases such as malaria (http://www.hc-sc.gc.ca/pphb-dgspsp/publicat/ccdr-rmtc/97vol23/23s5/index.html), transmitted by *Anopheles* mosquitoes (not major pests in Canada). Travellers may buy Canadian products for this purpose before departure. Neither the re-evaluation of citronella oil nor the value assessment for new personal insect repellents submitted for registration have included estimates of their potential value in other countries. The effectiveness of personal insect repellents containing citronella oil and related substances in protecting people against arthropod-borne diseases is not well documented for any country.

7.3 Relative importance of pests

The principal bloodsucking arthropod pests in Canada are *Aedes* and *Ochlerotatus* mosquitoes, especially snowmelt mosquitoes of the *Ochlerotatus communis* group in spring and *Aedes vexans* in summer, as well as black flies, especially members of the *Simulium venustum* complex. Mosquito and black fly problems probably account for most of the sales of personal insect repellents in Canada.

7.4 Rationale for reassessment of value

As stated in Announcement A90-01, *Re-evaluation of Personal Insect Repellents*, one of the six factors in the decision to re-evaluate personal insect repellents was that “there is some uncertainty that all registered products are efficacious for the pests, uses and

protection times claimed". This led to the call for efficacy data and to conducting a survey of literature.

7.5 Data review

7.5.1 Methodology

Most of the efficacy data reviewed were generated by treating the forearms or lower legs of test subjects with standard dosages of the repellents (usually 1 mL product per forearm, or 1 mL per 600 cm^2), and exposing the treated areas continuously or intermittently either to unfed insects in a cage, or to biting populations in the field. The usual index of efficacy has been the 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). This is an appropriate index for end-use products, because most users want *complete* protection rather than partial protection for a longer period. Several of the tests with citronella oil products, however, have used percent repellency as the index of efficacy because the complete protection times for such products are often short. Percent repellency is determined by counting the numbers of bites on treated and untreated subjects during the same time period, and using the following formula:

$$\text{Percent repellency} = 100\ (N_u - N_t)/N_u$$

where N_t and N_u are the numbers of bites on treated and untreated subjects, respectively.

Most laboratory tests used the yellow fever mosquito, *Aedes aegypti* (L.), reared under standard conditions and being uniform in age and nutritional state. In these tests, the repellents were applied at standardized dosages to human forearms, which were then exposed to mosquitoes in a test cages to determine the CPT or percent repellency.

In field tests against mosquitoes and black flies, the test surfaces were the bared forearms of each subject, from wrist to elbow, or the legs from ankle to knee.

In general, a new product is not acceptable for registration as a personal insect repellent unless it has shown a CPT of at least 30 minutes, or at least 95% repellency in counts beginning at least 30 minutes after application, in field trials conducted either in Canada itself, or in similar areas (e.g., northern United States [U.S.]) against pest species known to occur in Canada.

7.5.2 Criteria for inclusion of data

The aim was to include only data from trials on human subjects, where the dosages and test arthropods were specified and counts on untreated subjects included, to confirm biting pressure. Field trials were to be restricted to those in Canada or similar areas (e.g., northern U.S. and Russia), involving species found in Canada. However, laboratory

data for *Aedes aegypti* were included, even though it is not found in Canada, because it is the only mosquito against which almost all repellents have been tested.

7.5.3 Summary of results

Seven laboratory and twelve field trials on mosquitoes, and a single field study with black flies, met the criteria for inclusion in the review. The results from these studies show no consistent relationship between active ingredient content (identity and concentration) and efficacy. As a result, only product-specific data were used in the assessment of efficacy of the EPs.

Seven of thirteen personal insect repellents (that contain citronella oil and related active substances) are supported by product-specific efficacy data. The efficacy data support the protection time claims (re-application intervals) shown on the labels. All the studies are recent and were conducted in Canada. The re-application intervals indicated on the labels are 30 minutes (for mosquitoes, Reg. Nos. 22481 and 25517), 45 minutes (for mosquitoes, Reg. Nos. 25446 and 25447), 1 hour (for black flies, Reg. Nos. 26913 and 26914) and 2 hours (for mosquitoes, Reg. No. 25797).

The remaining six products lack product-specific efficacy data. Results from other available data (for mosquitoes only) show inconsistent results between active ingredient content and efficacy. Of the six products, five have no re-application intervals on their labels and claim to repel both mosquitoes and black flies. One product specifies a re-application interval of 30 minutes and is labelled only for the control of mosquitoes. As a result, product-specific efficacy data would be required to confirm the efficacy of these products and to establish the re-application intervals.

7.6 Current pest management strategies

Products registered under the PCPA for personal protection against mosquitoes and other biting flies include personal repellents, space sprays and mosquito coils and yard foggers. Non-chemical methods of personal protection include clothing (e.g., head nets), screens and timing one's activities to avoid exposure to bloodsucking arthropods.

Almost all the personal insect repellents registered in Canada contain diethyl toluamide (DEET) as the sole active ingredient. The re-evaluation of DEET was recently completed [RRD2002-01, *Personal insect repellents containing DEET (N,N-diethyl-m-toluamide and related compounds)*, 15 April 2002]. Although products with 30% or less DEET alone are acceptable, products containing DEET in combination with one or both of two other active ingredients, di–n-propyl isocinchomeronate (MGK Repellent 326) and n-octyl bicycloheptene dicarboximide (MGK Synergist 264), are being phased out as a result of their re-evaluations [RRD2001-01, *Di-n-propyl isocinchomeronate (MGK Repellent 326)*, and RRD2001-02, *n-Octyl bicycloheptene dicarboximide (MGK Synergist 264)*, 12 December 2001]. There are four products containing soybean oil as the

active ingredient, which was first registered in August 2000. A dermally applied insect repellent product containing p-menthane 3,8-diol is also registered.

Registered products for community protection against biting flies include larvicides (e.g., organophosphates, insect growth regulators and *Bacillus thuringiensis*) for use against mosquito and black fly larvae, space sprays, fogs and residual premise sprays (e.g., pyrethrum and synthetic pyrethroids) for use primarily against adult mosquitoes. Non-chemical methods for community protection include habitat modification to control mosquito and black fly larvae.

8.0 Proposed regulatory action

As part of the re-evaluation of citronella oil, registrants were asked to submit safety data to allow for an assessment of the risk to human health. The Citronella Joint Venture submitted a package of data that included only acute toxicology studies and mutagenicity data. The PMRA has conducted a risk assessment based on the submitted data and any other available data. This assessment is based on substandard mammalian toxicity data and surrogate data. As a result, the level of uncertainty present in the risk assessment is high. To compensate for uncertainty related to data gaps, a conservative approach was taken in the risk assessment. This included conservative exposure assumptions and application of additional uncertainty factors.

The current assessment identifies concerns regarding the potential risks to human health from use of personal insect products containing citronella oil and related compounds. Endpoints of concern include reproductive effects and teratogenicity. In addition, natural citronella contains a variable amount of the known multi-site, multi-species carcinogen, methyleugenol. Methyleugenol content in cosmetic products, which have use patterns similar to insect repellents, is currently regulated in the European Union as well as by the International Fragrance Association. Additional high-quality safety data addressing all required endpoints would contribute to the refinement of the risk assessment.

Based on the available information, the PMRA was unable to conclude that the human health risks from use of personal insect repellent products containing citronella oil and related compounds applied directly to the skin continues to be acceptable. As a result, the PMRA is proposing that the registrations for personal insect repellent products containing citronella oil and related active compounds applied directly to the skin be phased out unless registrants commit to provide further data that would address the deficiencies identified in this document.

The PMRA will accept written comments up to 60 days from the date of publication of this document to allow interested parties an opportunity to provide input into the proposed re-evaluation decision for these products. As part of normal re-evaluation practice, registrants of insect repellant products containing citronella oil and related active compounds may use this consultation period to provide any available data that has not been previously submitted or to indicate their commitment to generate further studies. The PMRA will consider this information before confirming the remaining data requirements and arriving at a final re-evaluation decision.

List of abbreviations

ADI	acceptable daily intake
a.i.	active ingredient
bw	body weight
CAS	Chemical Abstracts Service
CHO	Chinese hamster ovary
CIL	citronellal
CIO	blend of natural oils
CIR	citronella terpenes
CIT	citronella oil
CTR	artificial essential oil (citronella) blend
CPT	complete protection time
d	day(s)
D	domestic class
DEET	N,N-diethyl-m-toluamide and related compounds
DNA	deoxyribonucleic acid
EC	emulsifiable concentrate
EP	end-use product
F_1	1st generation offspring
FAO	Food and Agriculture Organization (United Nations)
GC/ECD	gas chromatography/electron capture detection
GC/MS	gas chromatography/mass selective
i.p.	interperitoneal
JECFA	Joint FAO/WHO Expert Committee on Food Additives
K_d	adsorption quotient
kg	kilogram
K_{oc}	adsorption quotient normalized to organic carbon
K_{ow}	octanol/water partition coefficient
L	litre
LC_{50}	lethal concentration 50%
LD_{50}	lethal dose 50%
LI	liquid
LOAEL	lowest observed adverse effect level
mg	milligram
mL	millilitre
MOE	margin of exposure
NAFTA	North American Free Trade Agreement
NOAEL	no observed adverse effect level
NTP	National Toxicology Program
PA	paste
PACR	Proposed Acceptability for Continuing Registration
PCP	pest control product(s)
PCPA	*Pest Control Products Act*
PMRA	Pest Management Regulatory Agency
ppm	parts per million

RED	Reregistration Eligibility Decision
S9	mammalian metabolic activation system
SN	solution
TGAI	technical grade active ingredient
TSMP	Toxic Substances Management Policy
UDS	unscheduled deoxyribonucleic acid synthesis
µg	micrograms
µL	micro litre
USEPA	United States Environmental Protection Agency
WHO	World Health Organization
wt	weight

Appendix I Classification and labelling of end-use products

Reg. no.	Active, % active on label	Class	Formulation type	Insect controlled	Label directions
21424	CTR 10	D	SN	Black fly Mosquito	Apply thin uniform film to exposed skin. For added protection, apply to clothing. Avoid eyes. Do not use on infants and toddlers. [Application interval not specified.]
21425	CTR 10	D	SN	Black fly Mosquito	Apply thin uniform film to exposed skin. Spray on hands to treat face/neck. For added protection, apply to clothing. Avoid eyes and mouth. Do not use on infants and toddlers. [Application interval not specified.]
22427	CTR 10	D	SN	Black fly Mosquito	Apply thin uniform film to exposed skin. Spray on hands to treat face/neck. For added protection, apply to clothing. Avoid eyes and mouth. Do not use on infants and toddlers. [Application interval not specified.]
22481	CIT 1.5	D	EC	Mosquito	Apply thin uniform film to exposed skin. Spray on hands to treat face/neck. Avoid eyes and mouth. Do not use on infants and toddlers. 45 minute intervals if bugs continue.
24443	CIT 5	D	EC	Mosquito	Shake before using. For best results, apply in a thin uniform film to exposed skin, avoiding eyes and mouth. Reapply at 30 minute intervals if exposure to mosquitoes continues. For added protection, apply to clothing.
24537	CIT 15	D	PA	Black fly Mosquito	Apply thin uniform film to exposed skin. Avoid eyes and mouth. Do not use on infants and toddlers. [Application interval not specified.]
25446	CIR/CIT 5/10	D	SN	Mosquito	Spray exposed skin to provide thin uniform film. Spray on hands to treat face/neck. Avoid eyes, mouth, sensitive skin, cuts, wounds and sunburned skin. Wash hands after applying. Do not use on infants and toddlers. Do not apply under clothes. 45 minute intervals if bugs continue. Do not exceed 5 applications/day.

Reg. no.	Active, % active on label	Class	Formulation type	Insect controlled	Label directions
25447	CIR/CIT 5/10	D	EC	Mosquito	Apply thin uniform film to exposed skin. Dab on hands to treat face/neck. Avoid eyes, mouth, sensitive skin, cuts, wounds and sunburned skin. Wash hands after applying. Do not use on infants and toddlers. Do not apply under clothes. 45 minute intervals if bugs continue. Do not exceed 5 applications/day
25517	CIL 2.55	D	SN	Mosquito	Apply thin uniform film to exposed skin. Avoid eyes, mouth, sensitive skin, cuts, wounds and sunburned skin. Wash hands after applying. Do not use on infants and toddlers. Do not apply under clothes. 30 minute intervals if bugs continue. Do not exceed 2 applications/day.
25797	CIO 3	D	SN	Mosquito	Apply thin uniform film to exposed skin. Avoid eyes, mouth and sensitive skin. After returning inside, wash treated skin with soap and water. Do not use on infants and toddlers. 2 hour intervals if bugs continue. Do not exceed 2 applications/day.
26913	CIT 0.05	D	EC	Black fly	Use only when protection from sunburn and black flies is required (not to be used solely as a sunscreen or moisturizer). Apply liberally 20 minutes before exposure to sun and black flies. To maintain black fly repellency, reapply at 1-hour intervals if exposure to black flies continues. Reapply after swimming or excessive perspiration and if exposure to black flies continues. Avoid contact with eyes or lips. May irritate eyes and cause skin reactions. Discontinue use if irritation occurs. Do not use on infants or toddlers.

Reg. no.	Active, % active on label	Class	Formulation type	Insect controlled	Label directions
26914	CIT 0.05	D	EC	Black fly	Use only when protection from sunburn and black flies is required (not to be used solely as a sunscreen or moisturizer). Apply liberally 20 minutes before exposure to sun and black flies. To maintain black fly repellency, reapply at 1-hour intervals if exposure to black flies continues. Reapply after swimming or excessive perspiration and if exposure to black flies continues. Avoid contact with eyes or lips. May irritate eyes and cause skin reactions. Discontinue use if irritation occurs. Do not use on infants or toddlers.
27188	CIT 10	D	SN	Mosquito Black fly	Gently press applicator tip to exposed skin. Spread a thin, even film to exposed skin. May be harmful if swallowed. Avoid eye contact. Do not allow use by small children without adult supervision. Do not use on infants or toddlers.

D = domestic class
SN = solution
EC = emulsifiable concentrate
PA = paste

Appendix II Data used for toxicity risk assessment

Table 1 Toxicology profile for citronella oil

NOTE: Effects noted below are known or assumed to occur in both sexes unless otherwise specified.

Study/species/ # of animals per group	Dose levels/purity of test material	NOAEL (mg/kg bw/day)	Results/effects	Reference
METABOLISM/TOXICOKINETIC STUDIES:				
No studies submitted/available				
ACUTE TOXICITY STUDIES:				
Acute oral toxicity—rat	"Ceylon type" oil	LD_{50} > 5000 mg/kg bw	**(low toxicity)**	USEPA RED 1997
Acute oral toxicity—rat	"Java type" oil	LD_{50} > 4380 mg/kg bw	**(low toxicity)**	USEPA RED 1997
Acute dermal toxicity—rabbit	"Ceylon type" oil	LD_{50} > 2000 mg/kg bw	**(low toxicity)**	USEPA RED 1997
Acute dermal toxicity—rabbit	"Java type" oil	LD_{50} > 2000 mg/kg bw	**(low toxicity)**	USEPA RED 1997
Acute inhalation toxicity—rat	"Ceylon type" oil	LC_{50} > 5000 mg/kg bw	**(low toxicity)**	USEPA RED 1997
Acute inhalation toxicity—rat	"Java type" oil	LC_{50} = 3.1 mg/L	**(low toxicity)**	USEPA RED 1997
Eye irritation—rabbit	"Ceylon type" oil	mild irritant		USEPA RED 1997
Eye irritation—rabbit	"Java type" oil	mild irritant		USEPA RED 1997
Dermal irritation—rabbit	"Ceylon type" oil	mild irritant		USEPA RED 1997
Dermal irritation—rabbit	"Java type" oil	mild irritant		USEPA RED 1997
Dermal sensitization—guinea pig	"Ceylon type" oil	skin sensitizer		USEPA RED 1997
Dermal sensitization—guinea pig	"Java type" oil	not a skin sensitizer		USEPA RED 1997
SUBCHRONIC TOXICITY STUDIES:				
No studies submitted/available				
NEUROTOXICITY STUDIES:				
No studies submitted/available				

Study/species/ # of animals per group	Dose levels/purity of test material	NOAEL (mg/kg bw/day)	Results/effects	Reference
CHRONIC TOXICITY/ONCOGENICITY STUDIES:				
No studies submitted/available				
REPRODUCTIVE AND DEVELOPMENTAL TOXICITY STUDIES:				
No studies submitted/available				
GENOTOXICITY STUDIES: ***In vitro studies***				
Ames *Salmonella* test TA98, TA100, TA1537, TA1535 (USEPA)	1–1000 µg/plate ± S9 ("Java type" oil)		negative	USEPA RED 1997
Ames *Salmonella* test; TA98, TA100, TA1537, TA1535, TA1538	up to 0.2 µL/disc ± S9		negative	Hachiya, N. et al. 1985
DNA Repair (rec) assay; *Bacillus subtilis* H17 (wild type) 7 M45 (rec-)	5 µL/disc ± s9		negative: + S9 fraction positive: - S9 fraction	Hachiya, N. et al. 1985
Chromosomal aberration in Chinese hamster ovary (CHO) cell line (USEPA)	75.5 µg/plate - s9; 150 µg/plate ± s9		negative	USEPA RED 1997
Unscheduled DNA synthesis (UDS)—rat primary hepatocytes (USEPA)	30–50 µg/mL		negative	USEPA RED 1997
GENOTOXICITY STUDIES: ***In vivo studies***				
No studies submitted/available				

Table 2 Toxicology profile for some individual constituents of citronella oil or structurally related compounds from published literature

Study/species/ # animals per group	Dose levels/purity of test material	NOAEL (mg/kg bw/day)	Results/effects	Reference
SUBCHRONIC TOXICITY STUDIES:				
CITRAL (published studies)				
14-day oral (gavage) toxicity—mouse 5/sex/dose	0, 534, 1068, 2137 mg/kg	534 mg/kg/day	↑ mortality and necrosis of fore stomach in both sexes at 2137 mg/kg, ↑ mortality in ♂ at 1068 mg/kg; ↑ liver wt, vacuolization of hepatocytes in ♀ at 1068 mg/kg and in ♂ at 2137 mg/kg	Dieter 1993
14-week dietary toxicity—F344/N rat 10/sex/dose	0, 3900, 7800, 15 600, 31 000 ppm (0, 345/335, 820/675, 1785/1330 mg/kg/d, ♂/♀)	NOAEL not set LOAEL = 345/335 mg/kg/d (♂/♀)	≥ 335 mg/kg: ↓ mean bw ≥ 675 mg/kg: ↓ food intake during first week of the study ≥ 1330 mg/kg: All rats killed moribund in second week of the study, and showed signs of listlessness, hunched posture, absent or slow paw reflex, dull eyes, fore stomach epithelial hyperplasia, hyperkeratosis, bone marrow atrophy and haemorrhage, nephrotoxicity	NTP TR505 2001
14-week dietary toxicity—B6C3F_1 mice 10/sex/dose	0, 3900, 7800, 15 600, 31 000 ppm (= 0, 745/790, 1840/1820, 3915/3870, 8110/7550 mg/kg/d, ♂/♀)	NOAEL not set LOAEL = 745 mg/kg/d	≥ 745 mg/kg: ↓ mean bw; ↑ food consumption by the end of the study ≥ 1820 mg/kg: ↑ food intake during first week (♀); a few males thin ≥ 3870 mg/kg: thinness and lethargy; ↑ incidence of ovarian atrophy; mild forestomach hyperkeratosis and epithelial hyperplasia (♀) 8110 mg/kg: 4 males killed moribund by second week	NTP TR505 2001

Study/species/ # animals per group	Dose levels/purity of test material	NOAEL (mg/kg bw/day)	Results/effects	Reference
CHRONIC TOXICITY/ONCOGENICITY STUDIES:				
METHYLEUGENOL (published studies)				
2-year oral (gavage) oncogenicity— $B6C3F_1$ mouse 50/sex/dose 5 days/week	0, 37, 75, 150 mg/kg	NOAEL not set LOAEL = 37 mg/kg/d	≥ 37 mg/kg: ↓ survival in ♀, ↓ bw, ectasia of glandular stomach, liver oval cell hyperplasia, liver adenoma and carcinoma in both sexes; liver haematopoietic cell proliferation in ♀ ≥ 75 mg/kg: atrophy of glandular stomach in both sexes, active inflammation of glandular stomach and liver necrosis in ♀ **Evidence of oncogenicity in mice**	Johnson 2000
2-year oral (gavage) oncogenicity— F344/N rat 50/sex/dose 5 days/week	0, 37, 75, 150, 300 mg/kg <u>6 or 12 month interim</u> 0, 300 mg/kg 60/sex/time 5/rat/sex/time of sacrifice	NOAEL not set LOAEL = 37 mg/kg/d LOAEL = 37 mg/kg/d	≥ 37 mg/kg: bile duct hyperplasia, liver hypertrophy, atrophy of glandular stomach, liver adenoma in both sexes; liver oval cell hyperplasia in ♀; skin fibroma and fibrosarcoma in ♂ ≥ 75 mg/kg: ↓ bw in both sexes, focal liver degeneration in liver oval cell proliferation, focal renal tubule hyperplasia, multiple liver carcinoma, benign and malignant neuro-endocrine tumour of glandular stomach and mammary gland fibroadenoma in ♂ ≥ 150 mg/kg: all ♂ died by week 89; ↓ survival in ♀; multiple liver carcinoma in ♀ malignant neuroendocrine tumours of glandular stomach in ♂ 300 mg/kg: cholangioma and cholangiocarcinoma in ♂	Johnson 2000

Study/species/ # animals per group	Dose levels/purity of test material	NOAEL (mg/kg bw/day)	Results/effects	Reference
			Interim sacrifice: atrophy of glandular stomach, liver atrophy, liver oval cell hyperplasia and multiple liver adenomas in both sexes at 300 mg/kg at 6 and 12 months **Evidence of carcinogenicity in rats**	
***d*-LIMONENE** (published studies)				
2-year oral (gavage) oncogenicity— F344/N rat 50/sex/dose	0, 75, 150 mg/kg (♂) 0, 300, 600 mg/kg (♀)	NOAEL not set LOAEL = 75 mg/kg/d	≥ 75 and 150 mg/kg: renal tubular cell hyperplasia, renal adenomas and adenocarcinomas in ♂ ≥ 300 or 600 mg/kg: no renal lesions or tumours in ♀ **Evidence of carcinogenicity in male rats, but alpha-2 µ globulin-related, and therefore of no biological relevance to humans**	NTP 347 1990
2-year oral (gavage) oncogenicity— $B63CF_1$ mouse 50/sex/dose	0, 250, 500 mg/kg (♂) 0, 500, 1000 mg/kg (♀)	NOAEL = 1000 mg/kg/d	No renal lesions or tumours in ♂ and ♀ **No evidence of carcinogenicity in mice**	NTP 347 1990
6-month oral (gavage)— beagle dog 5/sex/dose	0, 100, 1000 mg/kg	NOAEL = 100 mg/kg	1000 mg/kg: ↑ absolute and relative kidney weights	Web 1990
CITRAL (published studies)				
2-year bioassay— F344/N rat 50/sex/dose	0, 1000, 2000, 4000 ppm (0, 50, 100, 210 mg/kg/d)	NOAEL = 100 mg/kg/d	≥ 50 mg/kg: ↑ survival (♂) 210 mg/kg: ↓ mean bw from ≥ 49 weeks (♂) or ≥ 25 weeks (♀) **No evidence of carcinogenicity**	NTP TR505 2001

Study/species/ # animals per group	Dose levels/purity of test material	NOAEL (mg/kg bw/day)	Results/effects	Reference
2-year bioassay—$B63CF_1$ mice 50/sex/dose	0, 500, 1000, 2000 ppm (0, 60, 120, 260 mg/kg/d)	NOAEL not set LOAEL = 60 mg/kg/d	≥ 60 mg/kg: ↓ mean bw from 30 weeks (♀); a positive trend for incidence of malignant lymphoma (spleen, mesenteric lymph node, thymus) in females (3/49, 5/50, 9/50 and 12/50 at 0, 0, 60, 120, 260 mg/kg/d, respectively) ≥ 120 mg/kg: ↓ mean bw throughout the study (♂) 260 mg/kg: ↑ incidence of malignant lymphoma (♀) (24% versus 6% in controls), statistically significant in high dose, but within historical control range for NTP **No evidence of carcinogenicity in male mice; equivocal evidence of carcinogenicity in female mice**	NTP TR505 2001
REPRODUCTIVE AND DEVELOPMENTAL TOXICITY STUDIES:				
α-**Terpinene** (published studies)				
Oral (gavage) teratology study —Wistar rat 15–28 sperm positive ♀/group	0, 30, 60, 125, 250 mg/kg/day on gestation days 6–15; 98% pure	Maternal NOAEL = 60 mg/kg Developmental NOAEL = 30 mg/kg	Maternal effects: ↓ wt gain at ≥ 125 mg/kg Reproductive effects: no effects Developmental effects: delayed ossification of vertebral column and sternum, ↑ incidences of irregularly shaped os squamosum and os basisphenoid at ≥ 60 mg/kg; ↓ fetal wt at 250 mg/kg **Evidence of fetal sensitivity**	Araujo 1996

Study/species/ # animals per group	Dose levels/purity of test material	NOAEL (mg/kg bw/day)	Results/effects	Reference
REPRODUCTIVE AND DEVELOPMENTAL TOXICITY STUDIES:				
CITRAL (published studies)				
One-generation—1 litter dermal reproductive toxicity —virgin Wistar rat 19–20 ♀/dose	0, 460 mg/kg for 60 or 100 days pre-mating (i.e., animals not treated during pregnancy and the 21-day nursing period); dams and pups killed 21 days postpartum	NOAEL for maternal toxicity > 460 mg/kg/d NOAEL for reproductive toxicity not set (LOAEL = 460 mg/kg/d) NOAEL for offspring toxicity not set (LOAEL = 460 mg/kg/d)	Maternal toxicity: No apparent toxic effects Reproductive toxicity: 28% postimplantation loss (60 days) and 31.8% postimplantation loss (100 days) versus 7.4% postimplantation loss in the control group; ↓ number of implantation sites, ↓ litter size, ↓ number of primordial and primary follicles, degeneration of ovarian follicles and ↓ number corpora lutea in dams treated prior to mating only for 100 days Offspring toxicity: All offspring from dams treated topically for 100 days died in first week of birth (↓ offspring survival) **Evidence of (delayed) reproductive and embryofetal toxicity, and increased fetal/offspring sensitivity**	Toaff 1979

Study/species/ # animals per group	Dose levels/purity of test material	NOAEL (mg/kg bw/day)	Results/effects	Reference
Oral (gavage) teratogenicity—Wistar rat 19–20 ♀/group	0, 60, 125, 250, 500, 1000 mg/kg; gestation days 6–15	Maternal NOAEL not set Developmental NOAEL = 60 mg/kg	Maternal effects: ↓ food intake and wt gain at ≥ 60 mg/kg Reproductive effects: ↓implantation, live fetuses/dam at ≥ 125 mg/kg Developmental effects: ↓ live fetuses at 125 and 1000 mg/kg bw/d, ↑ spleen wt at 125 mg/kg bw/d. Delayed ossification at ≥ 125 mg/kg (incidence not dose-related) **No evidence of teratogenicity or fetal sensitivity in rats**	Nogueria 1995
GENOTOXICITY STUDIES:				
CITRONELLAL				
Ames *Salmonella* test (his-)	0.05–500 µg/plate ± S9	negative		Kasamaki 1982
METHYLEUGENOL				
Ames *Salmonella* test; TA100, TA1535, TA98, TA1537, TA1538	30–300 µg/plate ± S9	negative		Sekizawa 1982
Ames *Salmonella* test; TA97, TA98, TA100, TA102,	0.25–6.0 µM/plate ± S9	negative: TA97 and TA100 weak positive: TA98 and TA102 ± S9		Schiestl 1989
Escherichia coli WP2 uvrAgene reversion test	30–300 µg/plate ± S9	negative		Sekizawa 1982
Chromosomal recombination assay —*Saccharomyces cervisiae* strain RS9	0.68–2.04 µM ± S9	positive		Schiestl 1989
Chromosomal recombination assay —RS112 diploid yeast strain	up to 1 mg/mL ± S9	positive		Brennan 1996

Study/species/ # animals per group	Dose levels/purity of test material	NOAEL (mg/kg bw/day)	Results/effects	Reference
Unscheduled DNA synthesis (UDS)—rat primary hepatocytes	10^{-6}–10^{-3} M	positive		Howes 1990
Unscheduled DNA synthesis (UDS)—rat and mouse primary hepatocytes	10–500 µM	positive		Burkey 2000
DNA adduct test—rat and human liver microsomes	1–2000 µM	positive		Gardner 1997
In vivo liver DNA adduct (32P-postlabelling assay)—♀ CD-1 mice	2 or 10 mg/mouse (i.p.)	positive		Randerath 1984
EUGENOL				
Ames *Salmonella* test; TA97, TA98, TA100, TA102,	0.25–9.0 mM ± S9	negative: TA97 and TA100 weak positive: TA98 and TA102 ± S9		Schiestl 1989
Ames *Salmonella* test; TA100, TA1535, TA98, TA1537, TA1538	60–600 µg/plate ± S9	negative		Sekizawa 1982
Chromosomal recombination assay—*Saccharomyces cervisiae* strain RS9	0.65–2.27 mM ± S9	positive		Schiestl 1989
Unscheduled DNA synthesis (UDS)—rat primary hepatocytes	10^{-6}–10^{-3} M	negative		Howes 1990
***d*-LIMONENE**				
Ames Salmonella test; TA98, TA100, TA1535, TA137	0.3–3333 µg/plate ± S9	negative		NTP 347 1990
Sister chromatid exchange test—Chinese hamster ovary cells	16.2–162 µg/mL ± S9	negative		NTP 347 1990

Study/species/ # animals per group	Dose levels/purity of test material	NOAEL (mg/kg bw/day)	Results/effects	Reference
Chromosomal aberration test—Chinese hamster ovary cells	10–100 µg/mL ± S9	negative		NTP 347 1990
CITRAL				
Ames *Salmonella* test; TA98, TA100, TA1535, TA1537, TA1538		± S9: negative		NTP TR505 2001
Sister chromatid exchange—Chinese hamster ovary cells		± S9: positive		NTP TR505 2001
Chromosomal aberration—Chinese hamster ovary cells		± S9: negative		NTP TR505 2001
In vivo bone marrow micronucleus—$B6C3F_1$ mice	0, 250 to 750 mg/kg/d for 3 days (i.p.)	negative		NTP TR505 2001

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