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**The Flavor and Fragrance High Production Volume
Consortia**

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The Alicyclic Aldehyde Consortium

Revised Test Plan for HMPCC (2008)

**3 and 4-(4-Hydroxy-4-methylpentyl)-
3-cyclohexene-1-carboxaldehyde (HMPCC)** CAS No. 31906-04-4

**FFHPVC Alicyclic Aldehyde Consortium Registration
Number**

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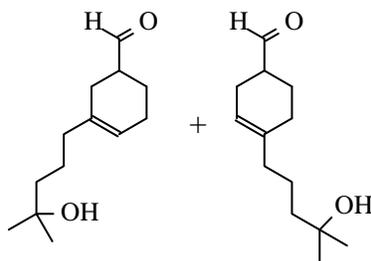
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The Flavor and Fragrance High Production Volume Consortia

Test Plan for HMPCC

1 IDENTITY OF SUBSTANCE



3 and 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde

CAS No. 31906-04-4

Synonyms:

HMPCC
3-Cyclohexen-1-carboxaldehyde, 4-(4-hydroxy-4-methylpentyl)-
Hydroxyisohexyl 3-cyclohexen carboxaldehyde
4-(4-Hydroxy-4-methylpentyl)cyclohex-3-enecarbaldehyde
Kovanol

2 CHEMICAL ANALYSIS

- **INTRODUCTION**

In October of 1999, members of the U.S. flavor and fragrance industries as well as other manufacturers that produce source materials used in flavors and fragrances formed consortia of companies in order to participate in the Chemical Right-to-Know Program. Members of these consortia are committed to assuring the human and environmental safety of substances used in flavor and fragrance products. The consortia are organized as the Flavor and Fragrance High Production Volume Consortia (FFHPVC). The Alicyclic Aldehyde Consortium, as a member of FFHPVC, serves as an industry consortium to coordinate testing activities for alicyclic aldehyde substances under the Chemical Right-to-Know Program. Two (2) companies are current members of the Alicyclic Aldehyde Consortium. The Consortium and its member companies are committed to assembling and reviewing available test data, developing and providing test plans for each of the sponsored chemicals, and where needed, conducting additional testing. The test plan, category analysis and robust summaries presented represent the first phase of the Consortium's commitment to the Chemical Right-to-Know Program.

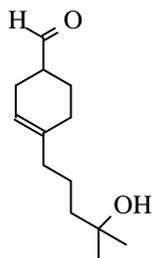
- **BACKGROUND INFORMATION**

This category analysis and test plan provides data for 3- and 4-(4-hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde (from herein referred to as HMPCC) and its structural relatives, hydroxycitronellal, hydroxycitronellol, and perilla aldehyde. HMPCC has not been reported to occur naturally. It is a colorless viscous liquid with a sweet aroma reminiscent of lily of the valley [Bauer and Garbe, 1985]. The functional aroma is similar to that of its naturally occurring counterpart, 7-hydroxycitronellal (*i.e.*, 7-hydroxy-3,7-dimethyloctanal). Therefore, it is not unexpected that HMPCC and 7-hydroxycitronellal contain the same functional groups (*i.e.*, an aldehyde and dimethyl substituted tertiary alcohol) distributed at either end of a carbon chain 8 to 10 carbons in length. Due to the method of preparation (see Section 2.4) HMPCC exists as a 70:30

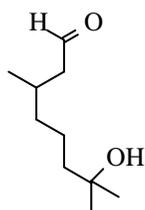
mixture of the 4- and 3-(4-hydroxy-4-methylpentenyl)-3-cyclohexenecarboxyaldehyde isomers. This mixture is the predominant product of commerce. At low concentrations, the mixture has excellent fixative properties, particularly in soap, cosmetics, and perfumes.

- **STRUCTURAL CLASSIFICATION**

The chemical structure of HMPCC and 7-hydroxycitronellal feature an aldehyde function and a dimethyl substituted tertiary alcohol located at either end of a carbon chain 8 to 10 carbons in length. In the case of HMPCC, the aldehyde is bonded to a cyclohexene ring that is substituted at the 4 position with a 4-hydroxy-4-methylpentyl substituent. The aldehyde group is separated from the dimethyl substituted tertiary alcohol moiety by seven carbons. In 7-hydroxycitronellal, the aldehyde is bonded to an eight carbon chain containing a 7-hydroxy-7-methyl substituent. In this case, the aldehyde group is separated from the dimethyl substituted tertiary alcohol by five carbons (see structure below).



HMPCC



7-Hydroxycitronellal

- **INDUSTRIAL PRODUCTION**

HMPCC is synthesized by a Diels-Alder reaction in which the double bond of acrolein (2-propenal) adds to the 1- and 4-positions of myrcenol (6-methylene-2-methyl-7-octen-2-ol). At elevated temperature in the absence of a catalyst, the Diels-Alder orientation of addition of the acrolein unit to the diene of myrcenol yields a 30:70 mixture of 3- and 4-

(4-hydroxy-4-methylpentyl)-3-cyclohexenecarboxaldehyde. The mixture is the typical product of commerce.

- **ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION**

The structurally related substances 7-hydroxy-3,7-dimethyloctanal (*i.e.*, 7-hydroxycitronellal) and 4-isopropenyl-1-cyclohexenecarboxyaldehyde (*i.e.*, perilla aldehyde) participate in the same pathways of metabolism in rabbits, dogs, rats and humans. The predominant metabolic pathways involve either reduction of the aldehyde to yield the corresponding alcohol or oxidation of the aldehyde to yield the corresponding carboxylic acid. In both cases the metabolites are excreted as glucuronic acid conjugates predominantly in the urine.

Male rabbits (6) were administered an aqueous solution (20 ml) containing Tween 80 (0.02 g/100 ml) and 250-333 mg/kg bw of 7-hydroxycitronellal by stomach tube followed by 20 ml of water. Greater than 35% of the original dose is excreted in the urine as acidic and neutral metabolites within 72 hours [Ishida *et al.*, 1989]. The two principal urinary metabolites of 7-hydroxycitronellal are 7-hydroxycitronellic acid and 7-hydroxycitronellol isolated in a 5:2 ratio, respectively.

Another aldehyde structurally related to HMPCC, 4-isopropenyl-1-cyclohexenecarboxyaldehyde, commonly recognized as perilla aldehyde, was also investigated in the same study. Six male rabbits were given single oral doses of 666 to 800 mg/kg bw of 4-isopropenyl-1-cyclohexenecarboxyaldehyde by gavage followed by 20 ml of water. Approximately 50% of the original dose is isolated within 72 hours. The principal acidic urinary metabolites accounting for approximately 40% of the dose include corresponding carboxylic acid, 4-isopropenyl-1-cyclohexenecarboxylic acid and its aromatized derivative 4-isopropylbenzoic acid. The principal neutral metabolites accounting for approximately 10% of the dose include the corresponding alcohol, 4-isopropenyl-1-cyclohexenecarbinol and its dihydro isomer.

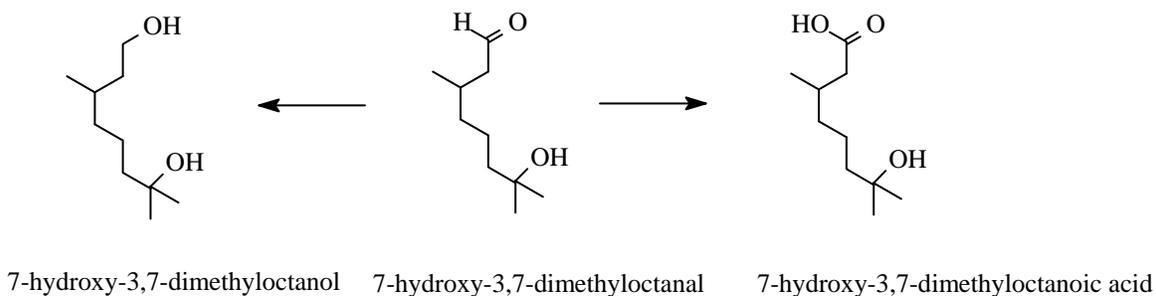
The group of substances, 4-isopropenyl-1-cyclohexenecarboxyaldehyde (perilla aldehyde), 4-isopropenyl-1-cyclohexenecarbinol (perillyl alcohol), and 4-isopropenyl-1-cyclohexenecarboxylic acid (perillic acid) have been the subject of extensive metabolic and toxicologic investigation in rats, dogs, and humans. The substances are currently being investigated as potential anti-carcinogenic agents for therapeutic use in humans. Patients with various advanced malignancies were treated with oral doses of 2,400 mg/m²/dose of 4-isopropenyl-1-cyclohexenecarbinol for four weeks. Peak plasma levels for the two main metabolites occur at 1.5 hours (4-isopropenyl-1-cyclohexenecarboxylic acid) and 3.5 hours (4-isopropenylcyclohexanecarboxylic acid) post-ingestion. The parent alcohol is not detected in the plasma. The major acid metabolites as well as a small amount of 4-isopropenyl-1-cyclohexenecarbinol (less than 1%) are detected in the urine [Ripple *et al.*, 1998].

An *in vivo* study conducted in male Wistar rats, confirmed that the oxidation of 4-isopropenyl-1-cyclohexenecarbinol to 4-isopropenyl-1-cyclohexenecarboxylic acid involved 4-isopropenyl-1-cyclohexenecarboxaldehyde as an intermediate. Groups of rats were intravenously administered the perillyl alcohol, perilla aldehyde, or perillic acid at a dose of 80 micromoles/kg bw (approximately 12.2, 12.0, or 13.3 mg/kg bw, respectively). Urine and bile were collected for two consecutive hours post administration. In all cases, the glucuronic acid conjugate of 4-isopropenyl-1-cyclohexenecarboxylic acid was the predominant metabolite detected in the urine and bile. Based on the results, the authors concluded that within two hours, approximately 56% of the original dose is oxidized to 4-isopropenyl-1-cyclohexenecarboxaldehyde, followed by the conversion to 4-isopropenyl-1-cyclohexenecarboxylic acid, and eventually excretion as a glucuronic acid conjugate [Boon *et al.*, 2000].

Female Wistar-Furth rats fed a diet of 2% 4-isopropenyl-1-cyclohexenecarbinol for a period of 3, 5, or 10 weeks, show 4-isopropenyl-1-cyclohexenecarboxylic acid and 4-isopropenylcyclohexanecarboxylic acid as major plasma metabolites. Unchanged 4-isopropenyl-1-cyclohexenecarbinol is not detected. The same plasma metabolites are identified four hours after female Wistar-Furth rats are administered a single dose of 1,000 mg/kg 4-isopropenyl-1-cyclohexenecarbinol *via* gavage. No trace of 4-

isopropenyl-1-cyclohexenecarbinol is found at any point, including 15 minutes post gavage. These results indicate that 4-isopropenyl-1-cyclohexenecarbinol is rapidly metabolized to 4-isopropenyl-1-cyclohexenecarboxaldehyde and then to 4-isopropenyl-1-cyclohexenecarboxylic acid. [Haag and Gould, 1994]. Two beagle dogs (male and female) administered 250 mg/kg bw of 4-isopropenyl-1-cyclohexenecarbinol by gavage exhibit peak plasma levels of oxidized metabolites of 4-isopropenyl-1-cyclohexenecarbinol (e.g. 4-isopropenyl-1-cyclohexenecarboxylic acid and 4-isopropenylcyclohexanecarboxylic acid) at one and five hours post administration, respectively. Analysis of blood specimens collected before dosing and at 19 points ranging from 10 minutes to 48 hours after dosing, indicate the presence of the oxidized metabolites 10 minutes post administration. The parent substance, 4-isopropenyl-1-cyclohexenecarbinol, is undetectable in the plasma. [Phillips *et al.*, 1995].

Figure 1 - Metabolism of (±)-7-Hydroxy-3,7-dimethyloctanal in Rabbits



- **SUMMARY FOR CHEMICAL ANALYSIS**

Based on pharmacokinetic and metabolic studies in rabbit, rats, dogs, and humans with 7-hydroxycitronellal and perilla aldehyde derivatives, it is anticipated that HMPCC will be rapidly absorbed *via* the oral route of exposure and primarily metabolized to the corresponding carboxylic acid and, to a lesser extent, the corresponding alcohol. Both metabolites are excreted primarily in the urine.

3 TEST PLAN

- **CHEMICAL AND PHYSICAL PROPERTIES**

3.1.1 Melting Point

Being a mixture of 3- and 4-(4-hydroxy-4-methylpentenyl)-3-cyclohexene carboxyaldehyde, HMPCC is a viscous liquid at ambient temperature. The calculated melting point for a single isomer of HMPCC according to the MPBPWIN program is between 65.64 and 89.01 °C depending on the method used with a mean of 77.32 °C [MPBPVP EPI Suite, 2000]. Given that the commercial product is a mixture of the 3- and 4-(4-hydroxy-4-methylpentenyl)-3-cyclohexenecarboxyaldehyde, the determination of a melting point for either the 3- or 4- isomer is not relevant. Based on these calculated values, the melting point of a single isomer of HMPCC is estimated to be 77.32 °C.

3.1.2 Boiling Point

The boiling point of HMPCC has been reported to be 280 °C [FMA, unpublished report] and 120-122 °C at 1.0 mm Hg [Bauer and Garbe, 1985]. The calculated boiling point for HMPCC according to the MPBPWIN program is 307 °C [MPBPVP EPI Suite, 2000]. This value is expected to be higher than that of 7-hydroxycitronellal, a structurally related substance also possessing the same two functional groups located at either end of the carbon skeleton, but containing three additional carbons. Therefore, the boiling point of HMPCC is anticipated to be higher than the 241 °C boiling point [FMA, unpublished report] (85-87 °C at 1.0 mm Hg [Bauer and Garbe, 1985]) reported for 7-hydroxycitronellal. Based on the consistency of the measured and calculated values, the boiling point of HMPCC is recognized to be 280 °C or 120-122 °C at a reduced pressure of 1.0 mm Hg.

3.1.3 Vapor Pressure

Although the vapor pressure of HMPCC determined from boiling point data is reported to be less than 0.001 mm Hg at 20 °C, it is recommended that the vapor pressure should be determined by a standardized methodology (Givaudan 1995). The calculated vapor pressure of HMPCC and 7-hydroxycitronellal has been reported to be 0.001 mm Hg at 20 °C [FMA, unpublished report]. The calculated vapor pressure for HMPCC according to the MPBPWIN program was 0.0000273 mm Hg at 25 °C [MPBPVP EPI Suite, 2000]..

3.1.4 n-Octanol/Water Partition Coefficients

The measured Log KOW for lylal is reported to be 2.1 at 25 °C (Givaudan-Roure, 1996). Log KOW was calculated resulting in values of 3.32 [KOWWIN EPI Suite, 2000] and 2.03 [Interactive Analysis LogP and LogW Predictor]. The log KOW of 7-hydroxycitronellal was calculated to be 2.11 [KOWWIN EPI Suite, 2000] while the experimentally determined value is 1.5 [Procter and Gamble, 1996].

3.1.5 Water Solubility

The calculated water solubility was estimated to be 1,045 mg/L [Interactive Analysis LogP and LogW Predictor] and 184.6 mg/L at 25 °C [WSKOWIN EPI Suite, 2000].

3.1.6 New Testing Required

None

- **ENVIRONMENTAL FATE AND PATHWAYS**

3.1.7 Photodegradation

The calculated half-life value for HMPCC has been reported to be 1.009 hours [AOPWIN EPI Suite, 2000]. The short half-life is consistent with the presence of a reactive hydroxyl OH and an aldehyde function.

3.1.8 Stability In Water

HMPCC is expected to be stable in aqueous solution given that it contains an unreactive tertiary alcohol group and aldehyde that is not readily oxidizable in water.

3.1.9 Biodegradation

Lyral was subjected to a biodegradability study using a OECD 301B guideline protocol. The % biodegradation (nominal) of lyral after 28 days (95% confidence limits) = 41.2 (11.2-71.2). Lyral was concluded to be not readily or ultimately biodegradable under test conditions (Quest, 1996). In a 20-day OECD closed bottle test, HMPCC showed measurable bio-oxidation (BOD/COD x100=10%) after 20 days incubation with activated sludge. Because HMPCC showed limited solubility in the test medium, it was directly injected into the reaction vessel. According to the authors, the test was intended to screen substances of potential biodegradation. [Waggy and Blessing, 1986]. The MITI linear and non-linear model predictions indicate that HMPCC should be readily biodegraded [BIOWIN EPI Suite, 2000]. In a OECD 301F study, lyral was reported to be 62% degraded after 28 days. Lyral was concluded to be not readily biodegradable (Givaudan, 1995).

The structurally related substance, 7-hydroxycitronellal at an initial dose of 52.5 mg DOC/l is completely biodegraded (99.8%) by day 19 in Method F biodegradability study in the Blue Book [Stickley, 1990]. In a more recent biodegradability test using a modified

Sturm procedure in an OECD 301B Guideline, 7-hydroxycitronellal was 93.7% biodegraded after 28 days. According to the authors, hydroxycitronellal is classified as readily and ultimately biodegradable [King, 1994].

3.1.10 Fugacity

Transport and distribution in the environment were modeled using Level III Fugacity-based Environmental Equilibrium Partitioning Model [Mackay, 1991, 1996a, 1996b] through the EPA EPI Suite 2000 program. The input parameters used were molecular weight, melting point (77.3°C), vapor pressure (0.001 m Hg), and boiling point (280°C).

The model predicts that HMPCC is distributed mainly to the soil (74.5%), but also is distributed to water (24.7%) and, to some extent, air (0.015%) and sediment (0.81%).

3.1.11 New Testing Required

None

- **ECOTOXICITY**

3.1.12 Acute Toxicity to Fish

The 96 hour median lethal concentration LC50 for fathead minnows, *Pimephales promelas*, exposed to lyral is 11.8 mg/L (95% confidence interval = 10.2 to 13.6 mg/L). The 96 hour no observed effect concentration (NOEC) is 8.21 mg/L. In a semi-static test with guppies, the structural relative 3-cyclohexene-1-carboxaldehyde exhibited a 14-day LC50 of 10.2 micromoles/L or 21.4 mg/L [Deneer *et al.*, 1988]. The calculated LC50 values for HMPCC are on the same order of magnitude. The calculated 96-hour LC50 for HMPCC was reported to be 6.8 mg/L (aldehydes) and the 14-day LC50 was reported to be 20. mg/L [ECOSAR EPI Suite, 2000].

Given the current database of information, it will be necessary to perform an acute fish toxicity test for HMPCC.

3.1.13 Acute Toxicity to Aquatic Invertebrates

Measured and calculated aquatic invertebrate LC50 values are available for HMPCC. Based on a protocol in EPA Methods for Toxicity Tests with Aquatic Organisms (40GTW23), the 48-hour LC50 for HMPCC in *Daphnia magna* was determined to be 76 mg/L [Waggy and Blessing, 1986]. In *Daphnia magna*, a calculated 48-hour LC50 of 1.773 mg/L was determined [ECOSAR EPI Suite, 2000].

3.1.14 Acute Toxicity to Aquatic Plants

In an OECD 201 Guideline study, the acute toxicity of lyral measured as a 50% decrease in growth and reproduction of freshwater algae was estimated to be 72 hr EC50=25.4 mg/L based on average specific growth rate; 72-hr EC50=13.7mg/L calculated using the number of cells/mL; 72-hr EC50=13.8 mg/L using the area under the growth curve. The

72-hr NOEC=5.95 mg/L (Boeri, 2003). A calculated 96-hour EC50 of 7.091 mg/L was reported for green algae [ECOSAR EPI Suite, 2000].

3.1.15 New Testing Required

- NONE

HUMAN HEALTH TOXICITY

3.1.16 Acute Toxicity

The acute toxicity of HMPCC was reported to be low, with oral LC50s of 3,000 to greater than 5,000 mg/kg bw in rats and dermal LC50s of greater than 5000 mg/kg bw in rabbits [Opdyke, 1977; Mallory *et al.*, 1982; Myers *et al.*, 1987]. Similar results were reported with hydroxycitronellal [Opdyke, 1973].

Exposure to statistically generated saturated vapor of HMPCC for 6 hours, resulted in no deaths, no toxicity or no remarkable gross pathological lesions in exposed male or female rats [Myers *et al.*, 1987].

Groups of rats were exposed in a dynamic system to up to 558 ppm of 4,4-dimethyl-3-cyclohexenecarboxaldehyde vapor for 4 hours and observed for 14 days [Union Carbide, 1987]. Similarly groups of rats were exposed to up to 402 ppm 4,4-dimethyl-3-cyclohexenecarboxaldehyde vapor for 1 hour in a static system and observed for 14 days. Signs exhibited included lacrimation, peri-oral wetness and respiratory difficulties on day of exposure. No clinical signs or macroscopic lesions were reported post exposure. Some deaths occurred on days 1 or 2 post exposure at 558 ppm; however the authors considered the deaths to be related to exposure to acrolein, a reaction precursor and contaminant, since the 1-hour rat LC50 of acrolein is 26 ppm. Therefore, no mortalities were attributed 4,4-dimethyl-3-cyclohexenecarboxaldehyde exposure.

Given the results of oral, dermal, and inhalation studies, no additional acute toxicity tests in mammals are recommended.

3.1.17 *In vitro* and *In vivo* Genotoxicity

3.1.17.1 *In vitro*

HMPCC did not increase the number of revertants in *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 or in *Escherichia coli* strain WP2 *uvrA* when tested with or without metabolic activation at concentrations up to 5,000 micrograms/plate [Wagner and Klug, 1999]. In a two other Ames assays, lylal (HMPCC) was not mutagenic in the same strains with and without activation at concentrations up to 5,000 micrograms/plate (Cocchiara *et al.*, 2001; Takasago, 1999). Another structural relative of HMPCC, 2,4-dimethyl-3-cyclohexene-1-carboxaldehyde, also did not increase the number of revertants in *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and TA1538 when tested with or without metabolic activation [Vergnes and Morabit, 1995].

In Chinese hamster ovary cells, HMPCC (at concentrations tested up to 900 micrograms/ml) did not induce an increase in the number of chromosome aberrations in the presence or absence of S9 at any test concentration compared to control solutions [Gudi and Schadly, 2000]. Lylal was considered to induce structural chromosomal aberrations in the presence of S9, but not in the absence of S9. Lylal did not induce numerical chromosome aberrations in the presence or absence of S9. Since the statistically significant increase in the percentage of structurally aberrant cells at 400 ug/ml in the non-activated 4-hour group (7%) was only 1% outside of the historical solvent control range (0-6%) and in the non-activated 20-hour group (3.5%) was within the historical control range, these increases were not considered by the study authors to be biologically significant (Gudi R. and Schadly, E.H., 2000).

3.1.17.2 *In vivo*

Groups of mice were intraperitoneally injected with up to 900 mg/kg bw of HMPCC in corn oil [Gudi and Krsmanovic, 2000]. At 24 or 48 hours, mice were killed, femurs were exposed, and bone marrow was removed. The number of micronucleated

normochromatic erythrocytes was counted and the proportion of polychromatic erythrocytes to total erythrocytes was determined. HMPCC does not induce micronucleated polychromatic erythrocytes in the mouse bone marrow assay. In a second micronucleus assay, dose levels of 225, 450 or 900 mg/kg of lylal produced no significant increases in micronucleated polychromatic erythrocytes. A slight to moderate reduction in the ratio of PCE to total erythrocytes, relative to vehicle control (Cocchiari et al., 2001).

When administered to mice by intraperitoneal injection, 7-hydroxycitronellol (up to 1,204 mg/kg bw) and 7-hydroxycitronellal (up to 861 mg/kg bw) did not induce an increase in the incidence of micronuclei in mouse bone marrow [Wild *et al.*, 1983].

In *Drosophila*, 7-hydroxycitronellal (37 mM) or 7-hydroxycitronellol (10 mM) did not increase the number of sex-linked recessive lethal mutations as compared to controls [Wild *et al.*, 1983].

3.1.17.3 Conclusions

The genotoxicity database on HMPCC and 7-hydroxycitronellal shows no mutagenic potential in the Ames assay. In a mammalian assay, there was no evidence of an increase in the incidence of chromosomal aberrations in the presence or absence of S9. In whole animals, the genotoxicity results for HMPCC, 7-hydroxycitronellol, and 7-hydroxycitronellal showed no evidence of an induction of bone marrow micronuclei in mice. In *Drosophila*, 7-hydroxycitronellol or 7-hydroxycitronellal did not induce an increase in the number of sex-linked recessive lethal mutations. Based on these results no additional genotoxicity tests are recommended.

3.1.18 Repeat Dose Toxicity

Repeat-dose inhalation and oral studies have been conducted for HMPCC and structural relatives of HMPCC, including 7-hydroxycitronellal, dimethyl-3-

cyclohexenecarboxaldehyde, and perilla aldehyde (4-isopropenyl-1-cyclohexenecarboxaldehyde) derivatives.

3.4.3.1 Subacute Studies

The oral administration of lylal to male and female rats for 28 consecutive days at dose levels of 15, 150 and 1000 mg/kg/day in Arachis oil BP resulted in treatment related effects in animals of either sex treated with 1000 mg/kg/day and in certain end points in females and males treated with 150 mg/kg/day. The No Observed Effect Level (NOEL) was, therefore considered to be 15 mg/kg/day for female and male rats. The histopathologic change detected at 150 mg/kg/day was confined to adaptive liver changes in three males. In isolation this was considered not to represent "serious damage" to health as defined by the criteria given in the EC labeling guide of Commission Directive 2001/59/EC. Therefore, 150 mg/kg/day may be regarded as a "No Observed Adverse Effect Level" (NOAEL) for female and male rats (Dunster et al., 2006).

Rats were exposed to 0, 50, 125 or 250 ppm of 4,4-dimethyl-3-cyclohexenecarboxaldehyde vapor, 6 hours/day for 9 exposures [Norris and Kintigh, 1994]. Additional rats were assigned to the control and high concentration groups for inclusion in a 4-week recovery period. No overt signs of toxicity were reported. There was an increased incidence of higher values for bilirubin, urobilinogen and amorphous phosphates in all treated males on day 11 and an increased incidence of bilirubin and urobilinogen in females at the highest concentration on day 12. Other reported effects included initial decreased body weight gain, increased water consumption, increased serum urea nitrogen values, exposure-related increase in renal tubular immunohistochemical staining for *alpha*-2micro-globulin in males, increased relative liver (mid- and high doses) and kidney weights (high dose) in males, swollen periocular tissue (mid- and high doses), periocular encrustation (high dose), alopecia (high dose) corneal lesions (high-dose), and increased urine osmolalities in males on day 11 (mid- and high doses). After the recovery period, females exposed to the highest concentration had decreased total erythrocytes, hemoglobin, hematocrit, and mean corpuscular

hemoglobin concentration values, showed increased segmented neutrophils and decreased monocytes, and showed slightly increased protein in the urine. The authors considered that dimethyl-3-cyclohexenecarboxaldehyde appears to be an ocular and respiratory irritant at vapor concentrations of 125 ppm and higher. There were no observable adverse effects reported at 50 ppm.

3.1.18.1 Subchronic Studies

Groups of female rats or Syrian golden hamsters were exposed to 211 micrograms of 7-hydroxycitronellal/cubic meters as part of a complex fragrance mixture (50 mg/cubic meters for 4 hours/day, 5 days/week for 13 weeks [Fukayama *et al.*, 1999]. Twelve animals per test group were exposed in a whole body inhalation experiment. Aerodynamic mean diameter of particle size was 0.5 um in rats and 1.4 um in hamsters. Animals were sacrificed 1 to 2 days following exposure. Hematological examination at week 13, involved measurement of white blood cell count, mean corpuscular volume, hemoglobin concentration, and hematocrit. Clinical chemistry examinations were performed at weeks 6 or 7 and week 13. At necropsy, gross pathological examination was performed on 24 organs and tissues including the uterus, testes and ovaries. Histopathological examination was performed on the trachea, lungs, adrenals, brain, esophagus, heart, kidneys, liver pancreas, spleen, sternum, testes, uterus and bone marrow taken from the femur. No toxicologically significant effects on animal survival, behavior, body weights or weight gains, organ weights, or in hematology, clinical chemistry, or urinalysis parameters were reported and no gross pathological or histopathological findings were observed in either species.

Numerous animal studies have shown high dietary levels of perilla aldehyde (4-isopropenyl-1-cyclohexenecarboxyaldehyde), its corresponding alcohol and acid may be protective against known animal carcinogens. The perilla aldehyde metabolite, 4-isopropenyl-1-cyclohexenecarbinol (discussed above in Section 2.5), has been shown to inhibit the growth of pancreatic, mammary, and liver tumors in animals. It has been studied in animals as a chemotherapeutic agent for neuroblastoma, prostate and colon

cancer, and has possible chemotherapeutic applications for skin and lung cancer (Belanger, 1998; Crowell, 1997; Stark *et al.*, 1995; Burke *et al.*, 1997; Mills *et al.*, 1995; Ren and Gould, 1998; Haag and Gould, 1994; Reddy *et al.*, 1997: no robust summaries included).

In a 90-day study, dose levels 40, 120, or 400 mg/kg bw per day of 4-isopropenyl-1-cyclohexenecarbinol was given by gavage to groups of rats and dogs of unspecified number and strain [National Cancer Institute, 1996]. Clinical signs observed with increasing dose included hyper-excitement and a clear mouth discharge. The animals were provided food and drinking water *ad libitum* and monitored for survival, behavior and changes in hematology. In rats, no agent related deaths, abnormal hematology, clinical chemistry or gross lesions were reported at dose levels up to and including 400 mg/kg bw per day. Histopathological examination of major organs and tissues including the ovaries and gonads failed to reveal any alterations that could be associated with administration of the test substance [National Cancer Institute, 1996].

3.1.18.2 Chronic Studies

Two groups of male and female rats were fed 7-hydroxycitronellal at dietary concentrations of 0.1% (10 rats/sex) or 0.5% (20 rats/sex), respectively, for 2 years. These levels correspond to calculated average daily intakes of 50 or 250 mg 7-hydroxycitronellal/kg bw. Control animals were fed a basal diet. Animals were observed for appearance and behavior and body weights were determined regularly during the study. At the end of the study, rats were necropsied and microscopic examinations were performed on the liver, heart, pancreas, adrenals, spleen, brain, and gross lesions. The number of animals that survived the 2-year duration of the study was 5 of 10 and 31 of 40, respectively. Low survival was attributed to the occurrence of spontaneous diseases that occurred at the same rate in both test and control animals. Administration of 7-hydroxycitronellal at dose levels up to 250 mg/kg bw/d resulted in no evidence of systemic toxicity [Bar and Griepentrog, 1967].

3.1.19 Reproductive/Developmental Toxicity

A one-generation reproduction study was conducted with HMPCC in rats (OECD 415). HMPCC in the vehicle, Arachis oil BP, was administered orally (via gavage) at dosages of 0, 25, 100 and 500 mg/kg body weight/day to 24 Sprague-Dawley CrI:CD[®] (SD) IGS BR rats per sex per group (Hoberman, 2006). After 10 weeks of treatment for males and two weeks of treatment for females, animals within each dose group were paired for mating. Pregnant females were allowed to give birth and maintain their offspring until Day 21 post partum at which time all surviving females and offspring were sacrificed. Females continued to be dosed during the gestation and lactation phases. The No Observable Adverse Effect Level (NOAEL) for developmental and reproductive toxicity was identified as 25 mg/kg body weight/day. The NOAEL for general maternal toxicity was 100 mg/kg body weight/day.

Skin sloughing was observed in the pups at the two highest dose levels, which far exceed current exposure from consumer products. The observed skin effects occurred several days after birth, and after shedding the pups appeared normal. It was not clear from the study that was conducted whether these effects were due to pre- or post-natal exposure. The OECD 415 study protocol is not designed to elucidate individual effects in the pups. As such, the results needed further clarification.

High doses of HMPCC may produce a functional zinc deficiency in the dams resulting in the observed effects in the offspring. This phenomenon has been observed with other materials. Skin changes, particularly those observed in the HMPCC study (acanthosis and hyperkeratosis), are among the most sensitive manifestations of zinc deficiency. The high level of perinatal death in the high dose group may also be relevant to prenatal zinc deficiency. The importance of this mechanism is that it is maternally-mediated (not direct developmental toxicity) and is only produced at high dose levels when the threshold is exceeded. A second study with a developmental component was designed to study this phenomena.

To determine if the observed effect is a result of pre- or post-natal exposures, a second reproductive toxicity study with postnatal observations has been conducted in Crl:CD[®](SD) rats (Lewis and Hoberman, 2007).

Groups of presumed pregnant female rats were dosed once daily via gavage with HMPCC in Arachis oil at dosages of 0 or 500 mg/kg/day in a final volume of 4 ml/kg. The 500 mg/kg/day dosage was selected because it was the highest dose tested in the first study and is the dosage at which effects were observed in both the dams and the offspring. Groups I-III (n = 10) comprised the main part of the study. Groups Ia and II were dosed from gestation day 0 through parturition, Groups Ib and III were dosed from postnatal days 1 through 21. Groups Ia and Ib were vehicle controls. Body weights, food intake, and clinical observations were recorded. Pups were individually identified, monitored, and weighed daily. On postnatal day 2, blood was taken from the dams for clinical chemistry and zinc level analyses. On postnatal day 21, all dams and pups were euthanized.

Groups IV-V (n = 5) comprised the satellite part of the study. These pregnant female rats were dosed from gestation day 0 through 14. On gestation day 15, blood was taken for clinical chemistry and zinc level analyses, the dams and fetuses were euthanized, and liver samples from the dams were snap-frozen for subsequent measurement of metallothionein levels.

Dosage Group	N	HMPCC (mg/kg/day)	Dosage Administration
Ia	10	0	Day 0 of presumed gestation through parturition
Ib	10	0	Days 1 through 21 <i>post partum</i>
II	10	500	Day 0 of presumed gestation through parturition
III	10	500	Days 1 through 21 <i>post partum</i>
IV	5	0	Days 0 through 14 of presumed gestation
V	5	500	Days 0 through 14 of presumed gestation

Treated Throughout Gestation

In the dams treated with 500 mg/kg/day HMPCC during gestation (Group II), mortality and adverse clinical signs were observed. Non-significant reductions in body weight gains were observed during gestation in treated rats in comparison to vehicle-treated rats (Group Ia). However, maternal body weights were increased during the lactation period (post-dosage), with terminal body weights statistically significantly increased compared to vehicle control rats. The increased maternal body weights during the lactation period are reflective of reduced live litter size and reduced physiological needs of the dams. The absolute and relative liver weights did not significantly differ. On lactation day 2, glucose, blood urea nitrogen, and alkaline phosphatase levels, and the albumin/globulin ratio were significantly increased in HMPCC-treated rats.

Increased incidences of stillbirths, reduced liveborn pup numbers, and increased incidences of pup deaths near parturition all contributed to a statistically significant reduction in the viability index resulting from gestational HPMCC treatment compared to vehicle controls. Live litter sizes were significantly reduced throughout the entire lactation period in the HMPCC-treated group. Pup weights were reduced at birth and on lactation days 2 through 5 in the HMPCC-treated group. However, by lactation day 14, pup body weights in the HMPCC-treated group were greater than controls.

All pups except one had flaking skin first observed on lactation day 6, 7 or 8 in the HMPCC-treated group. In one litter, 5 of 12 pups had skin peeling, a more severe observation, on lactation day 7 only; this observation was reduced to flaking skin on lactation day 8. All flaking and peeling observations were transient and all pups appeared normal by lactation day 16.

For Groups IV and V, no clinical chemistry differences were observed on gestation day 15. Zinc levels on gestation day 15 were slightly higher in HMPCC-treated rats than vehicle control rats.

Treated Throughout Lactation

No deaths or clinical signs were observed in dams treated with HMPCC during lactation only (Group III). Terminal body weights were significantly increased in rats treated with HMPCC during lactation compared to vehicle-treated controls (Group Ib). The absolute and relative liver weights were significantly increased in HMPCC-treated rats.

On lactation day 2, cholesterol levels were significantly decreased in HMPCC-treated rats. Creatinine, aspartate aminotransferase, and inorganic phosphorous levels, and the albumin/globulin ratio were significantly increased in HMPCC-treated rats. Zinc levels on lactation day 2 were slightly higher in HMPCC-treated rats than vehicle control rats.

Viability and lactation indexes were significantly reduced in the HMPCC-treated rats. Pup body weights were reduced, achieving statistical significance on lactation days 10 through 21, in the HMPCC-treated group compared to the vehicle control.

Essentially all pups born to dams in Group III (postnatal HMPCC exposure) had peeling skin, the more severe observation; this observation was significantly increased compared to vehicle-treated controls. Two litters had pups with flaking skin observed on lactation day 7 which progressed to peeling skin on lactation day 8. The peeling skin was first observed on lactation day 7, 8, 9 or 10 and persisted to necropsy in 112 of the 123 surviving pups.

This study was designed to answer two specific questions: 1) whether the skin peeling observed in pups in the first study resulted from prenatal or postnatal exposure; and 2) whether the skin peeling was a consequence of maternally-mediated zinc deficiency.

1) Postnatal. The data strongly suggest that the skin peeling and flaking observed in pups from dams treated with 500 mg/kg HMPCC resulted from postnatal

exposure. This effect occurs at extremely high doses of HMPCC that are maternally toxic, even lethal. Treatment of dams during the lactation period (postnatal) with 500 mg/kg/day HMPCC resulted in statistically significant increases in skin peeling in offspring. While skin flaking was observed (and a few instances of peeling) in nearly all pups from the group treated with HMPCC during gestation only, these effects were transient and all pups appeared normal by lactation day 16. The transient effect is most likely the result of residual HMPCC in the body of the dams treated only during gestation.

2) Not zinc related. No zinc deficiency was observed in the blood taken on gestation day 15 or lactation day 2. Slightly elevated levels of zinc were observed in HMPCC-treated rats (in both gestation and lactation groups), though this effect is of questionable biological importance. The metallothionein data are not yet available.

• TEST PLAN TABLE

Chemical	Physical-Chemical Properties					
	Melting Point	Boiling Point	Vapor Pressure	Partition Coefficient	Water Solubility	
CAS No. 31906-04-4 3 and 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde	Calc	A	A, Calc	A, Calc	A, Calc	
Chemical	Environmental Fate and Pathways					
	Photodegradation	Stability in Water	Biodegradation	Fugacity		
CAS No. 31906-04-4 3 and 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde	Calc	NA	A, R, Calc	Calc		
Chemical	Ecotoxicity					
	Acute Toxicity to Fish	Acute Toxicity to Aquatic Invertebrates	Acute Toxicity to Aquatic Plants			
CAS No. 31906-04-4 3 and 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde	A, Calc	A, Calc	A, Calc			
Chemical	Human Health Data					
	Acute Toxicity	Genetic Toxicity <i>In Vitro</i>	Genetic Toxicity <i>In Vivo</i>	Repeat Dose Toxicity	Reproductive Toxicity	Developmental Toxicity
CAS No. 31906-04-4 3 and 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde	A	A	A	A	A	A

Legend	
Symbol	Description
R	Endpoint requirement fulfilled using category approach, SAR
Test	Endpoint requirements to be fulfilled with testing
Calc	Endpoint requirement fulfilled based on calculated data
A	Endpoint requirement fulfilled with adequate existing data
NR	Not required per the OECD SIDS guidance
NA	Not applicable due to physical/chemical properties
O	Other

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