Human and Environmental Risk Assessment on ingredients of Household Cleaning Products

ISOEUGENOL

4-Hydroxy-3-methoxy-1-propen-1-yl benzene

CAS 97-54-1

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2 EXECUTIVE SUMMARY

Isoeugenol, a fragrance ingredient used to impart a spicy, carnation-like odour to numerous consumer products, has been chosen for a full risk assessment principally because of its known skin sensitising properties. While this risk assessment attempts to address all possible endpoints, the low volumes of use of isoeugenol and low levels of inclusion in these consumer products have led to this risk assessment giving a preponderant emphasis to dermal sensitisation.

Isoeugenol (phenol, 1-methoxy-4-prop-1-enyl) CAS 97-54-1, EINECS 202-590-7 is low molecular weight (164.2) substance that is generally a viscous liquid although it congeals at temperatures below 28°C. It has moderate water solubility (700 - 810 mg/l) and low lipophilicity (log Pow: c. 2.1). It has a low estimated vapour pressure of 0.21 Pa at 25°C and a low calculated Henry's constant (log H: -1.37).

Isoeugenol is used as an ingredient in fragrances and is found in a wide variety of consumer products. These include perfumes, skin-care products, deodorants, soaps, shampoos, detergents and other household cleaning and maintenance products. Maximum levels of isoeugenol in household cleaning products have been collected from manufacturers and are 60 ppm in laundry detergents, 70 ppm in fabric conditioners, 40 ppm in hard surface cleaners and less than 10 ppm in toilet cleaners and dish-wash products.

Isoeugenol used in Europe is produced primarily inside the European Union in quantities estimated to be 25,600 kg/year. It is estimated that 35% of this (9,000 kg/year) is used in household cleaning and maintenance products.

Environmental Assessment

Exposure: The current risk assessment is made according to the “HERA detergent scenario” and the EUSES local and regional methodology, tier 1 approach. Highest regional levels were calculated to be 2.73 x 10-5 mg/kg in sediments, 5.83 x 10-6 mg/l in surface water and 6.73 x 10-7 mg/kg in soil.

Hazards: Isoeugenol is readily biodegradable. The only acute toxicity study carried out on aquatic organisms shows that is toxic to daphnids (48h-EC50: 7.5 mg/l).

Possible no effect levels: In the absence of test data, assessment factors and QSARs have been used to give PNECS of 4.8 μg/l for aquatic organisms, 16.6 μg/kg bw for terrestrial organisms and 23.5 μg/kg bw for sediment-dwelling organisms.

Risk characterisation: *Risk characterisation:* Margins of exposure are well below 1 for all local and regional environmental compartments. Regional risk characterisation ratios are 1.22×10^{-3} for aquatic organisms, 4.06×10^{-5} for soil and 1.16×10^{-3} for sediments. Even if we use the global volume of isoeugenol, all regional ratios are below 10^{-3}.

Conclusion: Current use levels and volumes of isoeugenol in household cleaning products do not raise concern with regard to possible effects on the environment.

Human Health Assessment

Consumer exposure: This risk assessment has been restricted to direct or indirect exposure to consumers arising from the use of laundry detergents, fabric conditioners, hard surface cleaners, toilet cleaners, cleaning sprays and dish-washing products. In addition to considering exposure in terms of the quantities potentially entering the body, this assessment has focused on exposure in terms of the quantity likely to be deposited on the skin surface because this is the exposure factor that is critical to the induction of allergic contact dermatitis.

Highest exposures: Pretreatment cleaning of clothes with undiluted liquid detergent with no subsequent hand-rinsing and other kinds of accidental or unintentional exposure (0.7 μg/cm^2) represent the highest potential skin doses likely to induce or elicit allergic contact sensitization. Hand washing using laundry pre-treatment liquids is estimated to give the highest levels of direct or indirect exposure in terms of quantities penetrating the skin (0.00093 μg/kg bw/day). Total aggregate systemic exposure from all routes and all exposure scenarios is estimated to not exceed 0.0014 μg/kg bw/day.

Hazards: Studies on animals and humans demonstrate that isoeugenol is a skin sensitiser of moderate allergenic potency.. This is substantiated by clinical data that show widespread under-lying allergy to isoeugenol although very few cases of allergy are clearly attributable to the presence of isoeugenol in any specific consumer products.

Isoeugenol is rapidly metabolised and eliminated. Oral toxicokinetic studies show no signs of metabolic saturation. Skin penetration studies *in vitro* and *in vivo* show isoeugenol rapidly penetrates the skin. Isoeugenol has a moderate acute toxicity by dermal and oral routes (LD50 values > 1500 mg/kg). Inhalation is not considered a significant route of exposure. Systemic toxicity studies have shown that levels of 800 mg/kg/day are well tolerated by rats although these studies do not meet modern testing requirements. Evidence that no adverse systemic effects occur at levels of 70mg/kg bw/day is evident from multi-generation reproduction toxicity studies in rats. Developmental toxicity studies in single and multiple generations of rats have shown that the developmental NOAEL is 500mg/kg bw/day which is about twice the level of maternal toxicity. Isoeugenol is negative in bacterial and mammalian genotoxicity screens except in some studies where there is evidence that the results are the results of procedural artefacts. There are no data on the carcinogenic potential of isoeugenol.

Isoeugenol shows moderate skin and eye irritancy but shows no significant phototoxicity or photoallergenic potential.

Critical end-points and threshold levels: Skin sensitisation and systemic toxicity were considered to be the critical end-points. A No Expected Sensitization Level (NESL) of 250 μg/cm^2 has been determined using a “weight of evidence” approach from a large number of predictive tests carried out on animals and studies in human subjects. There is evidence to show that although the threshold for elicitation of allergic responses from non-occlusive exposure to prior-sensitised individuals may be as low as 80 μg/cm^2, these “thresholds”cannot be used in risk assessment as they are neither reliable nor unique determinants of elicitation.

In the absence of a NOAEL from conventional systemic toxicity studies, two measures were taken as a basis for risk assessment. One was a NOAEL of 70mg/kg bw/day from multiple generation developmental toxicity studies. The other was the Threshold of Toxicological Concern (TTC) of 30 μg/kg bw/day based on a large data set NOAELs of substances that have been similarly classified chemical structures.

Risk characterisation: Margins of exposure for the induction of skin sensitization from different exposure scenarios were found to vary between over a million and above 350. Aggregate margins of exposure for systemic effects from all products combined were over ten million based on the NOEL and above ten thousand based on the TTC (which already incorporates other safety factors).

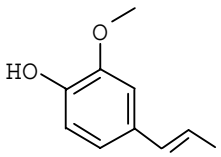
Conclusion: The use of isoeugenol at current levels in household cleaning products does not raise any safety concerns with regard to its potential to induce allergic contact dermatitis and adverse systemic effects.

3 SUBSTANCE CHARACTERISATION

3.1 CAS No and grouping information

Isoeugenol is an alkenyl phenol. The chemical structure, CAS number and various chemical names are in table 1:

Table 1. Identification

INCI name: Isoeugenol	CAS: 97-54-1 EINECS: 202-590-7
Chemical structure: $C_{10}H_{12}O_2$  Physical state: pale yellow, viscous, oily liquid at room temperature.	Other names: Phenol, 1-Methoxy-4-prop-1-enyl 4-Hydroxy-3-methoxy-1-propen-1-yl benzene 4-Propenylguaiacol 4-Hydroxy-3-methoxy-1-propenylbenzene, 3-Methoxy-4-hydroxy-1-propen-1-ylbenzene, 2-Methoxy-4-propenylphenol, 2-Methoxy-4-(1-propenyl)phenol, 1-Hydroxy-2-methoxy-4-propen-1-ylbenzene.

3.2 Chemical structure and composition

The environmental behaviour of a substance is determined by the physical chemical properties. These include the solubility in water, vapour pressure and the octanol/water partition coefficient. Some of these properties were estimated by so-called QSARs (EPIWIN). These estimates are based

on molecular fragments. The reliability of the data can be further improved by empirical data if required by uncertainty in the risk assessment.

The high water solubility and low partition coefficient of isoeugenol would suggest low potential for bioaccumulation and moderate concerns for the environmental compartment. Table 2 summarises the main physical properties of isoeugenol.

Table 2. General properties

Molecular weight	164.21				
Melting point	27.3°C	@ 981 mbar		Measured	Firmenich, 2003
Boiling point	266 °C			Measured	FMA
Flash point	132°C	@ 981 mbar		Measured	Firmenich, 2003
Vapour pressure	*0.003 mm Hg*	*@ 20 °C*		*Calculated*	*FMA*
	0.21 Pa	@ 25°C		Calculated	Biowin EPISuite
Log Pow	2.1	@ 25 °C		Measured	Givaudan,
Log Pow	*2.11*	*@ 40°C*		*Measured*	*Firmenich, 2003 (non GLP)*
Water solubility	810 mg/L	@ 25°C		Measured	Givaudan, 2003
Water solubility	*702 ± 70 mg/l*	*@ 20°C*		Measured	*Firmenich, 2003 (non GLP*)
Density	1.081 - 1.087	At 20°C		Measured	FMA
Density	*1.079 - 1.085*	*At 25°C*		*Measured*	*FMA*

Values in italics are only indicative and are not used in the risk assessment.

Henry's constant: Molecular Weight * Vapour Pressure/water solubility

= 0.0426 Pa m^3/mol

Log H = -1.37

3.3 Manufacturing & production/volume

The estimate volume of isoeugenol used in this risk assessment is based on a survey carried out by the International Fragrance Association (IFRA) over the year 2002. The respondents were composed of fragrance manufacturers who were members of the national member association of IFRA. The survey was restricted to the amount of isoeugenol that lost its identity as it was incorporated into fragrance formulations. This study was further restricted geographically to compounding intended for sale in the previous 15 member countries of the European Union as well as Norway and Switzerland.

Responding manufacturers were asked to declare the total quantity of isoeugenol used in all fragrance formulations. A further survey determined the proportion used in household laundry and cleaning

products including laundry detergents, laundry pre-treatment products, fabric softeners, hard-surface cleaners, hand dishwashing products and toilet cleaners.

Table 3. Use volumes in Europe (IFRA survey, 2002)

Year	IFRA global volume	Average percentage used in household & detergent products (IFRA)	Household & detergent volume
2002	26 tonnes/year	60%	15.4 tonnes/year

The majority of the total European isoeugenol tonnage, which includes uses outside the scope of HERA, is ultimately released down-the-drain, where depending on treatment it may reach the environment. Thus the environmental risk assessment also includes an overall assessment using the total European usage estimate of 26,000 kg/year.

3.4 Use applications summary

Although isoeugenol is not very substantive (i.e. does not adhere to rinsed fabrics and other lipophilic surfaces), it is quite commonly used as a minor ingredient [odour agent] in concentrated fragrance formulations that are incorporated into household products such as: detergents, fabric conditioners and other cleaning products. These concentrated fragrance formulations are not sold retail but are incorporated into consumer products. Maximum levels of isoeugenol in household cleaning products have been collected from major producers of these products and are 60 ppm (0.006%) in laundry detergents, 70 ppm (0.007%) in fabric conditioners, 40 ppm (0.004%) in surface cleaners and less than 10 ppm (0.001%) in dishwashing products and toilet cleaners (AISE and HERA, 2004).

The International Fragrance Association (IFRA) has applied a risk management quantitative limit of 200 ppm in the final consumer products (cosmetics, household cleaning and laundry products and other fragranced consumer products) (IFRA, 2004).

4 ENVIRONMENTAL ASSESSMENT

4.1 Environmental exposure assessment

Estimates of volume of use of isoeugenol

The estimates of exposure to the environment are primarily based on the estimated volume of use of isoeugenol in household laundry and cleaning products. This was determined in a survey conducted by the International Fragrance Association (IFRA). The survey was conducted by soliciting responses from fragrance manufacturers who are members of the national member associations of IFRA and was restricted to the amount of isoeugenol that lost its identity as it was incorporated into fragrance formulations during 12 months of calendar year 2002. This was further restricted geographically to formulations intended for sale in the current 15 member countries of the European Union as well as Norway and Switzerland. Responding manufacturers were asked to give a value for total isoeugenol use in all fragrance formulations and also for use in the following household laundry and cleaning products : laundry detergents, laundry pre-treatment products, fabric softeners, hard-surface cleaners, hand dishwashing products and toilet cleaners. After correcting for a conservatively estimated 60% response rate, the European volume of use was determined to be 15,400 kg/yr in these products (c. 60% of total isoeugenol usage of 26,000 kg/yr).

It is recognized that the majority of the total European tonnage is ultimately released in the same way as the HERA volume: down-the-drain to the environment. A more conservative assessment using the total European usage estimate (26’000 kg/year) is also presented in the addendum.

Exposure Pathways and Detergent Scenario

The "HERA detergent scenario" was used for the environmental exposure calculations. The entire tonnage was assumed to follow the domestic down-the-drain pathway to sewage treatment and to the environment. Releases from production and formulation activites fall outside of the scope of HERA and were not explicitly considered, at the local level, although both production and formulation losses are included in the regional risk assessment. For the calculation of the EUSES (European Union System for the Evaluation of Substances) regional tonnage, 7% of the EU tonnage was assigned to the region (replacing the default 10%), and the local emissions were not increased by the default factor 4, but by a factor of 1.5. Further explanation of and justification for these values can be found in Chapter 2.6 of the HERA methodology document. Available on the website - www.heraproject.com.

4.1.1 Environmental fate

The review of degradation data was based on proprietary test data submitted to the Research Institute for Fragrance Materials Inc. (RIFM). As the quality of the reports is variable, standard criteria were applied to determine the quality of data (Klimisch *et al.*, 1997).

Biodegradation Properties

Two tests are available:

1. In 1996, Givaudan conducted a Manometric Respirometry Test according to OECD Guideline 301 F. This study was performed in compliance with the principles of Good Laboratory Practice. In this study, isoeugenol reached 79% biodegradation after 28 days. The biodegradation started on day 2 and reached 79% at the end of the 10–day window period. Isoeugenol was tested at a concentration of 100 mg/L and the course of biodegradation was followed by measuring the Biological Oxygen Demand (BOD, mg O_2/l). Aniline, used as the reference substance, confirmed the validity of the study. On this basis, isoeugenol should be regarded as readily biodegradable under the conditions of this study.

In addition to that, isoeugenol is not inhibitory to micro-organisms at the tested concentration (100 mg/L). (Quest Int.Ltd., 1994).

This test followed OECD guideline 301F and was certified GLP. Thus, it can be classified as reliable without restriction [Code 1] (Klimisch *et al.*, 1997).

2. A second test was performed by Haarman & Reimer in 2000. The biodegradation of isoeugenol was tested at a concentration of 3 mg/l. A biodegradability rate of 14% after a period of 28 days was found by measuring the reduction of dissolved oxygen (BODTh). Isoeugenol was classified as “not readily biodegradable” (Haarmann & Reimer Gmbh, 2000).

Due to the fact that this test has not been performed according to internationally accepted test guidelines and did not follow the principles of GLP, it was attributed a score of 2 [reliable with restriction] (Klimisch *et al.*, 1997).

Biowin calculations give fast biodegradation results for linear and non-linear prediction models as well as for the linear and non-linear MITI models. These results support the result obtained in the first biodegradation test indicating that isoeugenol is readily biodegradable: a property that has been used in the risk assessment.

No further biodegradation studies (e.g. anaerobic biodegradation, degradation in soil) have been carried out. No information about abiotic degradability of isoeugenol (hydrolysis, photolysis) is available.

4.1.2 Removal

SimpleTreat™ calculation

Due to the absence of measured data on the removal of isoeugenol in sewage treatment plants, only the tier 1 estimate of removal could be used. This follows the default EUSES calculation that uses the SimpleTreat™ model.

A SimpleTreat™ calculation was used to determine removal of isoeugenol in waste-water treatment as well as its partitioning between air, water and sludge by taking relevant physico-chemical parameters detailed in section 3.2 into account. These calculations were based on the default rates assigned for readily biodegradable chemicals. Results are given in Table 4.

Table 4. Fate of chemicals in a wastewater treatment plant based on the Simple Treat Model

	Fraction of WWTP emission to			
	Air	Surface water	Sludge	Degraded
Isoeugenol	0%	12.5%	1.7%	85.8%

4.1.3 Monitoring studies

No data exist from the monitoring of concentrations of isoeugenol.

4.1.4 PEC Calculations

EUSES was applied to calculate the regional and local exposure to isoeugenol using the following parameters:

Industry category:	005 Personal / domestic use
Use category:	009 Cleaning/washing agents and additives
Fraction of tonnage for application:	100% to use as cleaning products
Fraction of chemical in formulation	0.2%
Production:	No
Formulation:	No
Processing:	No
Private use:	Yes
Recovery:	No

Use Pattern: Private Use - cleaning products

Fraction of tonnage released to air:	0
Fraction of tonnage released to waste water:	1
Fraction of tonnage released to surface water:	0
Fraction of tonnage released to industrial soil:	0
Fraction of main local source:	5×10^{-4}
Number of emission days:	365

Predicted Continental and Regional Environmental Concentrations (PECs):

As explained in the HERA methodology document, use of production tonnage for HERA means that the losses to the region during formulation are automatically included when 100% of the production tonnage is released to the environment. The regional and local PECs are as indicated in table 5.

Table 5: Local and Regional PECs

	PECLocal	**PECRegional**
Surface water (total) [mg/l]	1.90×10^{-5}	5.83×10^{-6}
Air [mg/m^3]	5.56×10^{-10}	4.49×10^{-10}
Agricultural soil (total) [mg/kg]	4.91×10^{-5}	6.73×10^{-7}
Sediment (total) [mg/kg]	9.28×10^{-5}	2.73×10^{-5}
Sewage (effluent) [mg/l]	1.31×10^{-4}	Not Applicable

Indirect Exposure to Humans:

For the calculation of indirect human exposure via drinking water, the EUSES calculations for indirect uptake via regional exposure can be used (taking into account that drinking water will not be sourced immediately downstream of wastewater emissions). These are shown in table 6, with the calculated uptake from a local source given for comparison. The total human uptake calculated by EUSES is also shown in this table, though known inadequacies with the current model for plant uptake mean that these calculated values mayconsiderably overestimate the uptake from food. Thus these total regional uptake values are conservative although not completely realistic for the HERA Human Health Assessment.

Table 6: Isoeugenol uptake by Humans – as calculated with EUSES

	Regional [mg/kg/day]		**Local [mg/kg/day]**	
	Drinking Water	Total Food + Water Uptake	Drinking Water	Total Food + Water Uptake
Isoeugenol	1.67×10^{-7}	2.87×10^{-7}	5.42×10^{-7}	9.99×10^{-7}

4.2 Environmental effects assessment

4.2.1 Toxicity

A review showed that available ecotoxicity data was based on unpublished reports submitted to the Research Instituite for Fragrance Materials Inc. (RIFM). EPIWIN™ calculations were used to complete the data. Here too, standard criteria were applied to determine the quality of data obtained from these study reports (Klimisch *et al.*, 1997).

4.2.1.1 Acute toxicity of isoeugenol to aquatic organisms

a) Algae EC_{50}: No data.

b) Daphnid EC_{50}:

The test describes in this section has been reported by RIFM and conducted by Haarman & Reimer in 2000. The test is conducted at different concentrations i.e.: 1.9, 3.8, 7.5, 15, 30, 60 mg/l. Ten daphnids are tested per dose level with the number of immobile daphnids being evaluated after 24 and 48 hours. At 24 h. and 48 h., no immobilization is recorded at 3.8 mg/L ($EC_{0)}$. The EC_{100} is determined to be 30 mg/l at 24 h. and 15 mg/l at 48 h. EC_{50} is estimated to be 10.7 mg/L at 24 h. and 7.5 mg/L at 48 hours (Haarmann & Reimer Gmbh, 2000).

Due to the fact that this test has not been performed according to internationally accepted test guidelines and did not follow the principles of GLP, it was attributed a score of 2 [reliable with restriction] (Klimisch *et al.*, 1997).

c) Fish LC_{50}: No data.

d) Other data: Conservatively, an EC_{50} = 100 mg/l has been assumed for micro-organisms based on the study reported in section 4.1.1. (Givaudan SA, 1996).

As only one acute toxicity test to aquatic organisms is available, a comparison was made with EPIWIN calculation. The most sensitive species was derived from this comparision: A summary of the data is given in table 7:

Table 7: Ecotoxicological dataset – determination of the most sensitive species

		H&R result	**EPIWIN calculation**	**Most sensitive species**
Acute toxicity to Algae	96-h EC_{50}	-	21.7 mg/l	
Acute toxicity to Daphnid	48-h EC_{50}	7.5 mg/l	4.8 mg/l	**4.8 mg/l**
Acute toxicity to Fish	96-h LC_{50}	-	9.6 mg/l	

Based on the above comparison, the EPIWIN result for acute toxicity to Daphnid is taken as the most sensitive species. This was considered as acceptable with a conservative tier 1 approach.

4.2.1.2 Ecotoxicity – Aquatic: chronic test results

No chronic aquatic data were found/ available

4.2.1.3 Terrestrial – acute test results

No acute terrestrial data were found/ available

4.2.1.4 Terrestrial – chronic test results

No chronic terrestrial data were found/ available

4.2.1.5 Micro-organisms e.g. in Wastewater Treatment

No specific data on the toxicity of Isoeugenol on microorganisms were located. However, it can be concluded from the positive biodegradation result obtained in a ready biodegradability test (OECD 301F) that Isoeugenol is not significantly toxic to microorganisms in the aquatic environment at a concentration of 100 mg/L (cf. 4.1.1). Hence, conservatively an EC_{50} = 100 mg/L will be assumed for the PNEC microorganisms derivation.

4.2.1.6 Predicted No Effects Concentration calculations

Due to a general lack of data on terrestrial and sediment toxicity, equilibrium partitioning method was used to derived PNECs for these compartments from the existing data for the aquatic compartment. Assessment factors were used for deriving PNEC aquatic (from the most sensitive species in table 7) and PNEC STWmicroorganisms (from the result mentioned in 4.2.1.5). This approximation is considered acceptable as a worst-case scenario in a tier 1 risk assessment. The results are shown in table 8.

Table 8: PNECs

	EUSES methodology	**PNEC**
Aquatic organism [mg/l]	Assessment factor: 1000	4.79×10^{-3}
Terrestrial [mg/kg]	Equilibrium partitioning	0.0166
Sediment [mg/kg]	Equilibrium partitioning	0.0235
STW microorganisms (effluent) [mg/l]	Assessment factor: 100	1

4.3 Environmental risk characterisation

In the table below, the PEC/PNEC ratios (= Risk Characterization Ratios: RCR) (calculated with EUSES) are given below, based on the different exposure scenarios :

Table 9:Risk Characterization Ratios

	PEC/PNECLocal	**PEC/PNECRegional**
Aquatic organism [mg/l]	3.96×10^{-3}	1.22×10^{-3}
Terrestrial [mg/kg]	2.97×10^{-3}	4.06×10^{-5}

Sediment [mg/kg]	3.96×10^{-3}	1.16×10^{-3}
Sewage (effluent) [mg/l]	1.31×10^{-4}	Not defined

4.4 Discussion and conclusions

The absence of environmental concerns can be shown for current levels of use of isoeugenol in HERA products. The Risk Characterization ratios (PEC/PNEC) are well below 1 for all environmental compartments. These are largely driven by the low volume of use (i.e. tonnage distributed into the environment) as well as the high water solubility and low octanol/water partition coefficient of isoeugenol. In view of the conservative nature of these calculations, it can be assumed that isoeugenol presents a low risk to the environment and no immediate concerns.

The tier 1 used is a rough estimate of the overall risk for the environment. This approach was used due to the fact that only very few data are available and that some were of limited reliability. Hence, the missing data were derived from calculations. Even if the reliability of the estimated data could be questioned, the probability of under-estimation of the risk is low considering the assessment factor of 1000 used to derive the PNECs. Further reassurance is obtained from the extremely low risk characterization ratios (Table 9).

We can most likely consider that the tier 1 approach is relevant for isoeugenol as a conservative picture of the overall risk on the environment and say that at this stage there is no need to conduct a tier 2 assessment which would require further testing.

4.5 Addendum – "Total Tonnage" Scenario

4.5.1 Environmental risk characterization

The total tonnage used in Europe is 26 tonnes/year (IFRA survey). The exposure scenario presented in this section is a conservative alternative. It assumes that the entire tonnage is disposed of down-the-drain. The PEC/PNEC ratios for the HERA tonnage could be extrapolated to the overall tonnage by multiplying the PEC by the appropriate factor (1.7). This approach is valid from a mathematical point of view because of the linearity of the EUSES model. The results are shown in table 10.

Table 10: Risk Characterization Ratios

	PEC/PNECLocal	**PEC/PNECRegional**
Aquatic organism [mg/l]	6.73×10^{-2}	2.07×10^{-3}
Terrestrial [mg/kg]	5.05×10^{-6}	6.90×10^{-6}
Sediment [mg/kg]	6.73×10^{-1}	1.97×10^{-3}
Sewage (effluent) [mg/l]	2.23×10^{-6}	Not defined

All PEC/PNEC ratios are well below 1 strengthening the conclusion given in part 4.4 that isoeugenol does not present immediate concerns for the environment and that at this stage, there is no need to conduct a tier 2 risk assessment which would require further testing.

5 HUMAN HEALTH ASSESSMENT

5.1 Consumer Exposure

5.1.1 Product Types

This human health assessment focuses particularly on household cleaning products in keeping with the scope of the HERA initiative. Isoeugenol is only used as an ingredient of fragrances that are themselves relatively minor ingredients in these types of products (0.8 - 0.2% by weight). As a result of its relatively high water solubility, isoeugenol is not a major building block of the fragrances used in these types of products as it tends to be lost in the rinse water. None the less, it is used in all of the different categories. These include most notably laundry powders (maximum concentration: 60 ppm), laundry liquids (maximum concentration: 70 ppm), dish-washing liquids (maximum concentration: less than 10 ppm), hard surface cleaning products (maximum concentration: 40 ppm) and toilet cleaning products (maximum concentration: less than 10 ppm) (AISE and HERA, 2004).

5.1.2 Consumer Contact Scenarios

Based on the product types, the following consumer exposure routes were identified and assessed:

1. Direct skin contact with neat (laundry pre-treatment) or diluted consumer product (hand-washed laundry, hand dish-washing, hard surface cleaning);
2. Indirect skin contact via release from clothes fibres to skin;
3. Inhalation of detergent dust and of the fragrance emanating during product use and afterwards, from cleaned surfaces of fabrics, kitchen-ware and hard surfaces;
4. Oral ingestion of residues deposited on dishes;
5. Oral ingestion of residues in drinking water;
6. Accidental or intentional over-exposure.

In addition to systemic toxicity, this assessment looks particularly at exposure with regard to skin sensitization. With regard to skin sensitization there is now an extensive body of evidence to show that this is best expressed in terms of quantity per unit area (Boukhman and Maibach, 2001;Rees *et al.*, 1990;Friedmann *et al.*, 1990;White *et al.*, 1986;Upadhye and Maibach, 1992;Fowler and Finley, 1995) unless the area is less than a square centimeter (Rees *et al.*, 1990). For this reason, a separate section of the consumer exposure estimates given below, expresses exposure in terms of the quantity of isoeugenol per unit area deposited on the skin. There is no need to include factors for dermal penetration because the hazard end-points are all generated by placing isoeugenol on the outer surface of the skin. Where possible, these hazard end-point producing doses are expressed in terms of the

quantity of isoeugenol per unit area. Exposure leading to potential systemic toxicity is accounted for by estimates expressed as quantity of isoeugenol deposited on the outer surface of the skin per unit body weight per day. In vivo and in vitro skin absorption data suggest that about 50% of an applied dose of isoeugenol can be absorbed through the skin (section 5.2.3).

5.1.3 Consumer Exposure Estimates

These are based in part on exposure factors given in the Technical Guidance Document provided by the European Commission for the risk assessment of newly notified substances (TGD, 2003) and on a consolidated overview concerning habits and practices of use of detergents and surface cleaners in Western Europe that was issued by the European Soap and Detergent Industry Association, AISE (AISE/HERA, 2002). This table reflects consumers' use of detergents in g/cup, tasks/week, duration of task and other uses of products and is largely the basis for the exposure estimates in the following paragraphs. In some instances, e.g. habits & practices (H&P) of pre-treatment of clothes, additional H&P information for a targeted exposure assessment was directly provided by the member companies of AISE.

The quantity of product per unit area is also provided in this document and constitutes a critical element in the estimation of potentially skin sensitizing exposure to isoeugenol. The percentage weight fraction absorbed via the skin is taken as 50% based on the *in vitro* and *in vivo* studies (Liu and Hotchkiss, 1997b) reported in section **5.2.3**. This is taken into account in estimates of systemic exposure but is not a factor in estimating skin-sensitizing exposure because experimental tests and consumer exposure both entail placing the product on the exterior of the skin.

5.1.3.1 Direct skin contact from hand-washed laundry

Hand-washed laundry is a common consumer habit. During this procedure, the isoeugenol containing laundry solution with an estimated product concentration of 10 mg/ml comes in direct contact with the skin of hands and forearms. A hand-washing task typically takes 10 minutes (Table of Habits and Practices - (AISE/HERA, 2002;AISE/HERA, 2002)*). This table also reports a maximum frequency of 18 times per week (3 times/day) when using laundry powder, which seems highly exaggerated but nevertheless is used here as a worst case scenario. The table gives a lower frequency of hand washing with laundry liquid of 10 times per week (1.43 times/day), which still seems exaggerated.*

A. Estimation of potential <u>systemic exposure</u> to isoeugenol (Exp_{sys}):

For this exposure estimate, the terms are defined with the following values for the calculation of exposure in a worst-case scenario:

$\mathbf{F_1}$	weight fraction of isoeugenol in the product (AISE and HERA, 2004)	**0.00007** (70 ppm)

C	product concentration: (AISE/HERA, 2002)	**0.01** (10 mg/ml)
Kp	dermal penetration coefficient	**0.8 x 10^{-5} cm/h*** (Liu and Hotchkiss, 1997b)
t	duration of exposure or contact (AISE/HERA, 2002)	**10 min (0.167h)**
S_{der}	surface area of exposed skin	**1980 cm^2** (TGD, 2003).
n	product use frequency (tasks per day)	**3** (AISE/HERA, 2002)
BW	body weight	**60 kg**

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** The dermal penetration coefficient was calculated from the dermal flux (35,3 µg/cm2) that was determined in an in vitro dermal penetration* (Liu and Hotchkiss, 1997b) *according to the following algorithm:*

Kp = dermal flux/(exposure time x concentration of test solution);

Kp = (0.0353 mg/cm2)/(24h x 184 mg/cm^3) = ***0.8 x 10^{-5} cm/h***

The following algorithm is used to calculate exposure relevant to this end-point (assuming a specific gravity of 1.0 for both the product and the solution):

$$\mathbf{Exp_{sys} = F_1 \times C \times S_{der} \times Kp \times t \times n / BW}$$

$$\mathbf{Exp_{sys}} = [(7 \times 10^{-5}) \times (10\ mg/ml) \times (0.8 \times 10^{-5}\ cm/h) \times (0.167h) \times 3 \times (1980\ cm^2)]/\ 60$$

$$\mathbf{= 0.000093\ \mu g/kg\ bw/day}$$

B: Estimation of <u>potentially skin-sensitizing</u> exposure to isoeugenol (Exp_{sens}):

For this exposure estimate, the terms are defined with the following values for the calculation of exposure in a worst-case scenario:

F_1	weight fraction of isoeugenol in the product (AISE and HERA, 2004)	**0.00007** (70 ppm)
C	product concentration: (AISE/HERA, 2002)	**10 mg/ml**
T_{der}	film thickness on skin	**0.01cm** (TGD, 2003;Vermeire and et al., 1993)

The following algorithm is used to calculate exposure relevant to this end-point (assuming a specific gravity of 1.0 for both the product and the solution):

$Exp_{sens} = F_1 \times C \times T_{der}$

Exp_{sens} = [(0.00007) x (10 mg/ml) x (0.01cm)] **= 0.007 μg/cm^2**

5.1.3.1.1 Direct skin contact from laundry tablets and laundry powder

Placing tablets into the dispenser of the washing machine is unlikely to involve any significant transfer of isoeugenol from the tablet to the skin due to the encapsulated solid form of the product. Furthermore, contact time and contact with a very small area of the palm skin generally regarded as relatively impermeable (Wester and Maibach, 2002). As a result, dermal exposure to isoeugenol from this use is considered to be relatively insignificant.

5.1.3.1.2 Laundry pre-treatment of clothes

Consumers typically spot-treat clothing stains by hand using either a detergent paste (i.e. water/laundry powder = 1:1) or a laundry liquid, which is applied undiluted (i.e. concentration = 1000 mg/ml) directly on the garment. In this exposure scenario, only the skin surface of the hand (~ 840 cm2) is exposed.

The exposure to Isoeugenol is estimated according to the same algorithm from the HERA guidance document as is used in 5.1.3.1 above using the liquid detergent since this is the highest concentration of Isoeugenol.

A: Estimation of <u>systemic exposure</u> to isoeugenol (Exp_{sys}):

For this exposure estimate, the terms are defined with the following values for the calculation of exposure in a worst-case scenario:

F_1	weight fraction of isoeugenol in the product (AISE and HERA, 2004)	**0.00007** (70 ppm)
C	product concentration: (AISE/HERA, 2002)	**1000 mg/ml** (100%)
Kp	dermal penetration coefficient	**0.8×10^{-5} cm/h*** (Liu and Hotchkiss, 1997a)
t	duration of exposure or contact (AISE/HERA, 2002)	**10 min (0.167h)**
S_{der}	surface area of exposed skin	**840 cm^2** (TGD, 2003)
n	product use frequency (tasks per day)	**0.71 (= 5/7)**
BW	body weight	**60 kg**

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* *The dermal penetration coefficient was calculated from the dermal flux (35,3 µg/cm2) that was determined in an in vitro dermal penetration* (Liu and Hotchkiss, 1997a) *according to the following algorithm:*

Kp = dermal flux/(exposure time x concentration of test solution);

Kp = (0.0353 mg/cm2)/(24h x 184 mg/cm^3) = ***0.8×10^{-5} cm/h***

The following algorithm is used to calculate exposure relevant to this end-point:

$$\mathbf{Exp_{sys} = F_1 \times C \times S_{der} \times Kp \times t \times n / BW}$$

$$\mathbf{Exp_{sys}} = [7 \times 10^{-5} \times (1000 \text{ mg/ml}) \times (840 \text{ cm}^2) \times (0.8 \times 10^{-5} \text{ cm/h}) \times (0.167\text{h}) \times 0.71]/60$$

$$\mathbf{= 0.00093 \text{ µg/kg bw/day}}$$

This exposure estimate is very conservative in that it does not recognize use of water to dilute the detergent, a common practice and the fact that only a fraction of the two hands' surface skin will actually be exposed.

B: Estimation of potentially skin-sensitizing exposure to isoeugenol (Exp_{sens}):

For this exposure estimate, the terms are defined with the following values for the calculation of exposure in a worst-case scenario:

F_1	weight fraction of isoeugenol in the product	**0.00007** (70 ppm) (AISE and HERA, 2004)
C	product concentration:	**1000 mg/ml** (100%) (AISE/HERA, 2002)
T_{der}	film thickness on skin	**0.01 cm** (TGD, 2003;Vermeire and et al., 1993)

The following algorithm is used to calculate exposure relevant to this end-point:

$$Exp_{sens} = F_1 \times C \times T_{der}$$

$$Exp_{sens} = [7 \times 10^{-5} \times (1000 \text{ mg/ml}) \times (0.01 \text{ cm})] = 0.7 \ \mu g/cm^2$$

5.1.3.1.3 Direct skin contact from hand dishwashing

A: Estimation of systemic exposure to isoeugenol (Exp_{sys}):

The determination of Isoeugenol exposure from hand dishwashing also uses the algorithm discussed in chapter 5.1.3.1 is used to calculate the dermal exposure to Isoeugenol from hand dishwashing. The following assumptions have been made to address a reasonable worst-case scenario:

F_1	weight fraction of isoeugenol in the product (AISE and HERA, 2004)	**0.00001** (10 ppm)

C	product concentration: (AISE/HERA, 2002)	**(2.0 mg/ml)**
S_{der}	surface area of exposed skin	**1980 cm^2** (TGD, 2003)
Kp	dermal penetration coefficient	**0.8 x 10^{-5} cm/h*** (Liu and Hotchkiss, 1997b)
t	duration of exposure or contact (AISE/HERA, 2002)	**45 min (0.75 h)**
n	product use frequency (tasks per day)	**3** (AISE/HERA, 2002)
BW	body weight	**60 kg**

_____

** The dermal penetration coefficient was calculated from the dermal flux (35,3 µg/cm2) that was determined in an in vitro dermal penetration* (Liu and Hotchkiss, 1997b) *according to the following algorithm:*

Kp = dermal flux/(exposure time x concentration of test solution);

Kp = (0.0353 mg/cm2)/(24h x 184 mg/cm^3) = ***0.8 x 10^{-5} cm/h***

The following algorithm is used to calculate exposure relevant to this end-point:

$$\mathbf{Exp_{sys} = F_1 \times C \times S_{der} \times Kp \times t \times n / BW}$$

$$\mathbf{Exp_{sys}} = [1 \times 10^{-5} \times (2\ mg/ml) \times (1980\ cm^2) \times (0.8 \times 10^{-5}\ cm/h) \times (0.75h) \times 3]/60$$

$$\mathbf{= 0.000012\ \mu g/kg\ bw/day}$$

B: Estimation of potentially <u>skin-sensitizing exposure</u> to isoeugenol (Exp_{sens}):

For this exposure estimate, the terms are defined with the following values for the calculation of exposure in a worst-case scenario:

F_1	weight fraction of isoeugenol in the product (AISE and HERA, 2004)	**0.00001** (10 ppm)

C product concentration: **(1.0 mg/ml)**
(AISE/HERA, 2002)

T_{der} film thickness on skin **0.01cm** (TGD, 2003),
(Vermeire and et al., 1993)

_____

The following algorithm is used to calculate exposure relevant to this end-point (assuming a specific gravity of 1.0 for both the product and the solution):

$Exp_{sens} = F_1 \times C \times T_{der}$

Exp_{sens} = [0.00001 x (1.0 mg/ml) x (0.01 cm)] **= 0.0001 μg/cm^2**

5.1.3.1.4 Direct skin contact from hard surface cleaners

A: Estimation of <u>systemic exposure</u> to isoeugenol (Exp_{sys}):

During this procedure, the Isoeugenol -containing hard surface cleaning solution comes in direct contact with the skin of the hands. A hard surface-cleaning task takes at maximum 20 minutes (AISE/HERA, 2002). The exposure to Isoeugenol is estimated according to the following algorithm from the HERA guidance document:

$Exp_{sys} = F_1 \times C \times Kp \times t \times S_{der} \times n / BW$

For this exposure estimate, the terms are defined with following values for the calculation considering a worst-case scenario:

F_1 weight fraction of isoeugenol in the product **0.00004** (40 ppm)
(AISE and HERA, 2004)

C product concentration: **(12 mg/ml)**
(AISE/HERA, 2002)

Kp	dermal penetration coefficient	**0.8 x 10^{-5} cm/h*** (Liu and Hotchkiss, 1997a)
t	duration of exposure or contact (AISE/HERA, 2002)	**20 min (0.334 h)**
S_{der}	surface area of exposed skin	**840 cm^2** (TGD, 2003)
n	product use frequency (tasks per day)	**1** (AISE/HERA, 2002)
BW	body weight	**60 kg**

_____

** The dermal penetration coefficient was calculated from the dermal flux (35,3 µg/cm2) that was determined in an in vitro dermal penetration* (Liu and Hotchkiss, 1997b) *according to the following algorithm:*

Kp = dermal flux/(exposure time x concentration of test solution);

Kp = (0.0353 mg/cm2)/(24h x 184 mg/cm^3) = ***0.8 x 10^{-5} cm/h***

Exp_{sys} = [0.00004 x 0.012 x 840 x (0.8 x 10^{-5} cm/h) x (0.334 h) x 1] /60

= 0.000018 µg/kg bw/day

B: Estimation of potentially skin-sensitizing exposure to isoeugenol (Exp_{sens}):

For this exposure estimate, the terms are defined with the following values for the calculation of exposure in a worst-case scenario:

F_1	weight fraction of isoeugenol in the product (AISE and HERA, 2004)	**0.00004** (40 ppm)
C	product concentration:	**(12 mg/ml)** (AISE/HERA, 2002)
T_{der}	film thickness on skin (Vermeire and et al., 1993)	**0.01 cm** (TGD, 2003),

_____

The following algorithm is used to calculate exposure relevant to this end-point (assuming a specific gravity of 1.0 for both the product and the solution):

$$\mathbf{Exp_{sens} = F_1 \times C \times T_{der}}$$

$$\mathbf{Exp_{sens}} = [0.00004 \times (12 \text{ mg/ml}) \times (0.01 \text{ cm})] = \mathbf{0.0048\ \mu g/cm^2}$$

5.1.3.2 Indirect skin contact from wearing clothes

Residues of components of laundry detergents may remain on textiles after washing and can transfer from the textile to the skin. There are no data available showing how much isoeugenol is deposited on the fabric following a wash process. If 1 kg of clothes retains 600 ml rinse water (Henkel, 2002) and that rinse water contains 2.5 % (ZVEI and IKW, 1999) of the detergent (and thus isoeugenol) used then the concentration of isoeugenol in that rinse water can be calculated: 600 ml x 10 mg/ml x 2.5% x 0.007% = 0.01 mg.

If 100% is transferred to the 1 kg of fabric, then the concentration in the fabric will be 0.01 mg/kg. Given the fabric density of 10 mg/cm2 (Procter and Gamble Company, 1996), it can be calculated that the isoeugenol is present at 1×10^{-7} mg/cm^2.

A: Estimation of <u>systemic exposure</u> to isoeugenol ($\mathbf{Exp_{sys}}$):

On this basis, the following algorithm recommended in the HERA guidance document can be used to estimate the dermal exposure to detergent residues in the fabric:

$$\mathbf{Exp_{sys} = F_1 \times C \times S_{der} \times n \times F_{2\,x}\, F_{3\,x}\, F_4/BW}$$

For the exposure estimate, the terms are defined with the following values for the calculation:

$\mathbf{F_1}$	proportion transferred	**100%**
C	fabric (Isoeugenol) load:	**1×10^{-7} mg/cm^2**
$\mathbf{S_{der}}$	Area of exposed skin:	**17'600 cm^2** (TGD, 2003).

F_2	fraction transfered to the skin	**1%** (Vermeire and et al., 1993)
F_3	percent weight fraction remaining on skin	**100%** (worst case)
F_4	percent weight fraction absorbed via skin	**50%** (0.052) for 24 hr (Liu and Hotchkiss, 1997a)*
BW	body weight	**60 kg**

* *the percentage weight fraction absorbed via the skin in 24 hours is taken as 50% based on in vitro* and *in vivo* studies (Liu and Hotchkiss, 1997b).

Exp_{sys} = [100% x (1 x 10^{-7} mg/cm^2) x (17,600 cm2) x 1% x 100% x 50%] / 60

= **1.5 x 10^{-7} µg /kg bw day**

B: Estimation of potentially skin-sensitizing exposure to isoeugenol (Exp_{sens}):

C	fabric (Isoeugenol) load:	**1 x 10^{-7}** mg/cm^2
S_{der}	Area of exposed skin: (TGD, 2003)	**17'600** cm^2
F_2	fraction transfered to the skin (Vermeire and et al., 1993)	**1%**

The following algorithm is used to calculate exposure relevant to this end-point (assuming a specific gravity of 1.0 for both the product and the solution):

Exp_{sens} = C x F_2/ S_{der}

Exp_{sens} = [(1 x 10^{-7} mg/cm^2) x 1%]/ (17'600 cm^2)

= 5.6 x 10^{-11} µg/cm^2

5.1.3.3 Inhalation of detergent dust during washing processes

According to studies on the release of dust per cup of laundry powder (van de Plassche et al., 1998) on average about 0.27 µg dust is released during consumer manipulation during machine laundering. Taking the worst case assumption that all released dust is inhaled and washing of laundry occurs 3 times daily, the exposure to isoeugenol of an adult with a body weight of 60 kg would be as follows:

$$\mathbf{Exp_{sys} = 7 \times 10^{-5} \times 270 \times 3 / 60 = 9.5 \times 10^{-7}\ \mu g/\ kg\ bw/day}$$

5.1.3.3.1 Inhalation of aerosols from cleaning sprays

Isoeugenol is present in surface cleaning sprays at concentrations below 10 ppm. The HERA guidance document specifies the algorithm to be used for calculation of consumers' worst-case exposure to isoeugenol –containing aerosols generated by the spray cleaner.

There is no significant dermal exposure from this type of exposure.

Estimation of systemically exposure to isoeugenol (Exp_{sys}):

F_1	weight fraction of isoeugenol in the product (AISE and HERA, 2004)	**0.00001** (10 ppm)
C'	product concentration in air:	**0.35 mg/m^3** * (Procter and Gamble Company, 1996)
Q_{inh}	ventilation rate	**0.8 m^3 /h**
T	duration of exposure	**0.17 h** (10 min) (AISE/HERA, 2002)
n	product use frequency (tasks per day)	**1.0** (AISE/HERA, 2002)
F_7	weight fraction of respirable particles	**1.0** (100% - worst case)
F_8	weight fraction absorbed or bioavailable	**75%**
BW	body weight	**60 kg**

** C' was determined by experimental measurements of the concentration of aerosol particles smaller than 6.4 microns in size which are generated upon spraying with typical surface cleaning spray products.*

For systemic exposure, the algorithm is as follows:

$$\mathbf{Exp_{sys} = F_1 \times C' \times Q_{inh} \times t \times n \times F_7 \times F_8 \ /BW}$$

$$\mathbf{Exp_{sys}} = [0.00001 \times (0.35\ mg/m^3) \times (0.8\ m^3/h) \times (0.17\ h) \times 1.0 \times 1.0 \times (75\ \%)] /60\ kg$$

$$\mathbf{= 6 \times 10^{-7}\ \mu g/kg\ bw/day}$$

5.1.3.4 Oral exposure

Oral exposure to isoeugenol can originate from residues on eating utensils and dishes washed in hand dish-washing detergents and from isoeugenol residues taken up via food and drinking water. On the basis of gavage toxicokinetic studies in rats (Badger *et al.*, 2002) where metabolites representing 85% of the administered dose were detected in the urine and another 10% were detected in the faeces, having possibly arisen from biliary excretion, it is assumed that 100% of exposure arising from the oral route is absorbed.

A. Oral exposure from drinking water

In addition to the described consumer exposure scenarios, oral exposures to FWA-1 can be assumed to originate also from drinking water. Modeling of the oral intake from drinking water using EUSES software (European Union System for Evaluation of Substances – see Table 6 in Section 4.1.4) has estimated the human total daily intake via food and drinking water for a male adult (70 kg):

$$\mathbf{Exp_{sys(oral\ via\ food\ \&\ drinking\ water)} = 2.87 \times 10^{-4}\ \mu g/kg\ bw/day}$$

In reality, this exposure estimate must be regarded as overly conservative. A considerable fraction of isoeugenol will be removed from surface water due to biodegradation and further purification during the drinking water treatment process.

B. Indirect exposure via dishwashing residues (hypothetical misuse)

Oral exposure to isoeugenol can originate from residues on eating utensils and dishes washed in hand dish-washing detergents and from isoeugenol residues taken up via drinking water.

The daily exposure isoeugenol from eating with utensils and dishware that have been washed in hand dish-washing detergents can be estimated according to the following factors:

F_1	weight fraction of isoeugenol in the product	**0.00001** (10 ppm) (AISE and HERA, 2004)
C	concentration of the product in dish wash solutions	**1.0 mg/cm^3** (AISE/HERA, 2002)
T_a	amount of water left on dishes after rinsing (Schmitz, 1973)	**5.5 x 10^{-5} ml/cm^2**
S_a	area of dishes in daily contact with food (FRANCE, 1990)	**5400 cm^2**
BW	body weight	**60 kg**

Using these factors the following algorithm gives the exposure:

$$\mathbf{Exp_{sys(oral\ dish\ deposition)} = F_1 \times C' \times T_{a'} \times S_a / BW}$$

$$= [0.00001 \times (1.0\ mg/cm^3) \times (5.5 \times 10^{-5}\ ml/cm^2) \times (5400\ cm^2)]/60\ kg$$

$$\mathbf{= 0.5 \times 10^{-4}\ \mu g/kg\ bw/day}$$

5.1.3.5 Accidental or intentional over-exposure

Accidental or intentional over-exposure can occur to all of the product types containing isoeugenol but would not be a factor for long-term systemic exposure. Accidental exposure to the skin may occur due to accidental splashes or spills of undiluted formulated products and this could have significance to skin sensitization even though such contact would not be expected to occur in a repeated manner.

Estimation of potentially <u>skin-sensitizing</u> exposure to isoeugenol ($Exp_{sens(accid./\ miss\text{-}use)}$):

For this exposure estimate, the terms are defined with the following values for the calculation of exposure in a worst-case scenario:

F_1	weight fraction of isoeugenol in the product (AISE and HERA, 2004)	**0.00007** (70 ppm)
C	product concentration:	**1.0** (undiluted product)
T_{der}	film thickness on skin (Vermeire and et al., 1993).	**0.01 cm** (TGD, 2003),

The following algorithm is used to calculate exposure relevant to this end-point (assuming a specific gravity of 1.0 for both the product and the solution):

$$\mathbf{Exp_{sens(accid./\ miss\text{-}use)} = F_1 \times C \times T_{der}}$$

$$\mathbf{Exp_{sens(accid./\ miss\text{-}use)}} = 0.00007 \times 1.0 \times 0.01 \text{ cm} = \mathbf{0.7\ \mu g/cm^2}$$

5.1.3.6 Aggregate Systemic Exposure

The overall body burden of consumers to isoeugenol by skin contact through the use of isoeugenol-containing house-hold laundry and cleaning products and by all exposure routes* is calculated to be:

$$\mathbf{Exp_{sys} = 0.0014\ \mu g/kg\ bw/day}$$

*Considering the contribution of the different routes of exposure, the exposure via the skin represents the major route of exposure (ca. 75.7 % of the total systemic exposure) with the oral route being more prominent (ca. 24.2 % of the total systemic exposure) than the inhalation route (ca. 0.11%).

The aggregate exposure is an unrealistic, worst-case of the body burden of isoeugenol. It combines several scenarios; each using highly conservative or worst case assumptions and it is virtually impossible that each of these conservative input parameters will apply concurrently in all cases for this overall exposure estimate. It further assumes, again very conservatively, that these unlikely circumstances will be repeated regularly over a substantial period of time.

5.1.3.7 Highest skin exposure for allergic contact sensitisation

Pretreatment of clothes with a liquid detergent and accidental exposure from splashes and spills represent the highest potential skin exposure doses likely to induce or elicit allergic contact sensitization. This exposure level (**0.7 μg/cm^2**) exceeds the exposure doses from all other products by at least two orders of magnitude. In essence, both of these exposure scenarios are extremely similar and equally improbable. The scenario for pretreatment (hand application with undiluted detergent without any post-application rinsing) is in fact a form of intentional misuse and hence resembles the scenario where accidental exposure to splashed dish washing detergent is also not followed up with any attempt to rinse the product from the skin. Both scenarios are therefore unlikely to occur concurrently. This level (**0.7 μg/cm^2**) is therefore taken as the "highest exposure scenario of relevance to allergic contact dermatitis".

5.2 Hazard Assessment

5.2.1 Acute Toxicity

5.2.1.1 Acute Oral Toxicity

The acute oral toxicity of isoeugenol has been evaluated in a single study in which groups of five male and five female Osborne-Mendel or Sherman strain rats were administered by intubation different quantities of undiluted test material (procedures which would no longer be used). The LD_{50} was calculated to be 1560 mg/kg with 95% confidence limits of 1290-1880 mg/kg. Deaths occurred between 1 hour and 7 days and clinical signs were reported to be scrawny appearance and coma (Taylor *et al.*, 1964).

Conclusion

Isoeugenol shows a moderate degree of oral toxicity consistent with that of other similar phenolic substances. A significant degree of gastro-intestinal stress may have been caused by the direct intubation of undiluted isoeugenol although the report does not indicate signs of this.

5.2.1.2 Acute Inhalation Toxicity

There are no test data available to evaluate the acute inhalation toxicity of isoeugenol.

Conclusion

It is not possible to assess the acute inhalation toxicity of isoeugenol

5.2.1.3 Acute Dermal Toxicity

Only one study is reported. In this, 5 doses were applied undiluted to intact skin of 3 male and 3 female rabbits per dose group under occlusion for 24 hours. At the lowest dose of 0.8 ml/kg no effects were seen. At 1.25 ml/kg, 1/6 animals died. Erythema and skin haemorrhaging were noted but this healed in surviving animals during the observation period. At a dose of 1.57 ml/kg 2/6 deaths were reported. Intradermal haemorrhage and eschar formation were noted but this too healed in surviving animals. At a dose of 1.98 mg/kg, 4/6 deaths occurred. Necropsy showed that the principle changes were eschar formation and bruising of the skin and congestion of the lungs. At 3.15 ml/kg and 5 ml/kg all animals died. Necropsy revealed the same effects plus pulmonary congestion of the lungs and haemorrhaging of the treated skin and visceral organs. The LD_{50} was calculated as 1.77 ml/kg (RIFM, 1979a).

Conclusion

On the basis of a single test performed over 20 years ago, it can be considered that isoeugenol is of moderate dermal toxicity when applied to the skin during a 24 hour period of occlusion. No systemic or dermal effects were observed under these conditions when the administered dose was 0.8 ml/kg body weight (c. 860 mg/kg).

5.2.1.4 Acute Toxicity by intraperitoneal injection

The intraperitoneal LD_{50} in Sprague-Dawley albino rats was 261 mg /kg in males and 309 mg/kg in females and was determined by injecting five groups of 2 male and 2 female animals with aqueous isoeugenol (RIFM, 1984).

Conclusion

Administration by intraperitoneal injection indicated moderate toxicity. Various pharmacological effects such as anaesthetic and anticonvulsant effects are observed at doses that are close to the median lethal dose.

Acute toxicity studies:

Conclusion

Isoeugenol is harmful by ingestion and by skin contact. Dermal effects are not manifested at doeses below 860 mg/kg.

5.2.2 Irritation

5.2.2.1 Skin irritation

a) Skin irritation in animals

In comparative studies on the irritancy of different materials to the skin of different species, undiluted isoeugenol was applied under occlusion to the dorsal skin of albino angora rabbits and guinea-pigs for 24 hours, then the patches were removed, and a second application of undiluted isoeugenol was made 30 minutes later. Readings were made visually and by examination of excised skin following intravenous injection of saline Evans blue. Under these conditions, it was concluded that undiluted isoeugenol was severely irritating to the skin of rabbit and guinea-pig (Motoyoshi *et al.*, 1979;RIFM, 1985c). Occlusive patch testing of miniature swine with undiluted isoeugenol however, was reported to give no signs of irritant effects (Motoyoshi *et al.*, 1979).

In another test, 3 albino rabbits were patched under occlusion with 0.5 ml of a 1% solution of isoeugenol in alcohol for 24 hours on intact and abraded skin and gave a primary irritation index of zero (IFF Inc., 1972).

The other irritancy data reported here were from screening tests carried out preliminary to, or during sensitization studies. Irritation was observed during the challenge of control guinea pigs in a Buehler sensitization study when isoeugenol at 2% in petrolatum was applied under occlusion. Under the same conditions, 1% isoeugenol in petrolatum was not irritating (Kaminsky and Szivos, 1986). In another Buehler test, irritant effects were observed during the induction phase following 6 hours occlusion of 75% isoeugenol in diethyl phthalate. Similar patch testing conditions however, produced no primary irritation at concentrations of 50% or less in the same solvent (RIFM, 1986). When the solvent was a mixture of 80% ethanol and 20% distilled water, no irritant effects were seen at isoeugenol levels of 2.5% or less but they were seen at 5% and 10%. At levels of 25% and higher, necrosis was also observed (RIFM, 1986;RIFM, 1986). In another series of Buehler tests, induction doses of isoeugenol at 5 and 10% in white petrolatum gave irritant reactions, but not at concentrations below 10% (Itoh, 1982).

In a Closed Epicutaneous Test on guinea pigs, occlusive patches of isoeugenol in petrolatum showed no primary irritation at 3%. At concentrations of 20 and 30%, irritant effects in the form of faint erythema were observed (RIFM, 1985a). In an Open Epicutaneous Test, the irritating concentration was determined to be 100% while at 30% in ethanol irritant effects were only seen on day 7, and at 10% they were seen at day 14 (RIFM, 1985c). In the same test, concentrations of 10% and higher in an unspecified vehicle were found to be irritant (Klecak *et al.*, 1977). During an ear swelling test on Balb/cBy mice, where irritancy was measured by increases ear thickness, it was found that concentrations of isoeugenol in an unspecified vehicle of 5, 15% and 20% all showed irritant effects (Thorne *et al.*, 1991). In another ear swelling test on the same strain of mice, 10% isoeugenol in acetone:olive oil (4:1) produced no irritation (Garrigue *et al.*, 1994).

Conclusion

Undiluted isoeugenol is a severe irritant to animal skin, but does not meet the criteria of corrosivity. Although results of different tests vary considerably for biological and methodological reasons, a clear dose/response relationship is apparent with concentrations producing no irritant effects under occlusion only at the 1% level while under non-occluded conditions, concentrations at least ten times higher being tolerated.

b) Skin irritation in human subjects

A limited number of irritancy studies have been carried out on human volunteers. In one a solution of 32% isoeugenol in acetone was found to be moderately irritating when applied to the skin of 50 adult males in occlusive patches over 48 hours (Motoyoshi *et al.*, 1979). In closed patch tests applied for 72 hours, ethanol solutions of 2% isoeugenol gave irritant effects in one of 30 volunteers while similar studies on dermatitic patients produced irritant reactions in three (of 35 patients) at 5% in ethanol and an irritant reaction in one (of 30 patients) at 0.1% in ethanol (Fujii *et al.*, 1972).

In another occluded patch test was on dermatitic patients, one of 54 patients exhibited erythema after occlusive patch testing of an unspecified concentration of isoeugenol in a cosmetic cream base (Takenaka *et al.*, 1986).

Additional information on the irritancy of isoeugenol to human skin has been obtained from irritancy screens carried out preliminary to sensitization testing. During this type of screen for 11 separate human maximization tests, occluded isoeugenol was found to be non-irritant at 8% in petrolatum on a total of 323 volunteers (RIFM, 1979e;RIFM, 1980d;RIFM, 1980e) but when 8% isoeugenol was mixed with an equal amount of eugenol in petrolatum, the same procedures gave irritant effects in 22 subjects (RIFM, 1980d). In another irritancy screen carried out prior to a human maximization study, isoeugenol at 16% in petrolatum gave irritant effects in 25 subjects (RIFM, 1980e). Other studies (all at lower levels) produced no irritant reactions in screening tests for human repeated insult patch tests (RIFM, 1964;RIFM, 1973;RIFM, 1980a;RIFM, 1980b;RIFM, 1980c).

Conclusion

Results in human volunteers mirror those seen in animal studies although all studies have been carried out under occlusion. One case of an irritant reaction has been claimed when one dermatitic patient was occlusively patch tested with an ethanolic solution of 0.1% isoeugenol. More consistent data is available from screening studies associated with sensitization tests. An occluded dose of 8% isoeugenol (c. 4 mg/cm^2) in petrolatum has been consistently tolerated in these studies.

5.2.2.2 Eye irritation

Two eye irritation tests (each on 3 albino rabbits) indicated that isoeugenol at 1% and 1.25% in denaturated alcohol (0.1 ml applied without rinsing) produced irritant effects (mild conjunctival irritation at 1%, intense conjunctival irritation involving chemosis and sicharge at 1.25%) but after 4 and 7 days respectively following dosing, the eyes were normal (RIFM, 1963;RIFM, 1972).

Conclusion
Only limited tests have been performed. These show low-level reversible effects even at concentrations of 1%.

5.2.3 Skin sensitization

5.2.3.1 Predictive tests using animals

Tests that use Freund's Complete Adjuvant to potentiate potentiate the induction of skin sensitization are useful for determining whether a substance is a significant allergen or not. The skin sensitization potential of isoeugenol has been evaluated in different tests systems. In the guinea pig maximization test according to the Magnusson-Kligman protocol (OECD, 1992), positive results were obtained (Table 11) showing that isoeugenol has a clear potential to induce cell-mediated contact allergy.

Table 11 : Guinea Pig Maximization Tests on isoeugenol

Induction		**Challenge**	**Results**	**Reference**
Intra-dermal	**Topical**			
5% in saline	30% in Petrolatum	1% in Petrolatum 3% in Petrolatum 10% in Petrolatum	1/20 2/20 10/20	(RIFM, 1985b)
5%	25% in Petrolatum	"subirritant" concentratum	some sensitization	(Klecak *et al.*, 1977)
0.15% in saline	25% in Acetone PEG 400	5% in Acetone PEG 400	100% sensitization	(Basketter and Scholes, 1992;Barratt and Basketter, 1992)
0.15% in saline	25% in Acetone PEG 400	5% in Acetone PEG 400	100% sensitization	(Hilton *et al.*, 1996) (may be same study as reported above)
0.15% in DOBS saline	25% in Acetone PEG 400	5% in Acetone PEG 400	10/10	(Kimber *et al.*, 1991) (may be same study as reported above)
1.0% in Ethanol	100%	100%	10/10	(Tsuchiya *et al.*, 1982;Tsuchiya *et al.*, 1985)
Modified test no intra-dermal administration of Isoeugenol	3% in Petrolatum	0.5% in Petrolatum	10/10	(Maurer and Hess, 1989)

Other aduvant tests (Freund's Complete Adjuvant Test and Optimization Test) also revealed the sensitization potential of isoeugenol (Table 12) while the Cumulative Contact Enhancement Test (Table 13) showed a dose-response relationship as well as vehicle effects.

Table 12: Freund's Complete Adjuvant Tests (FCAT, Optimization Test) on isoeugenol

Induction concentration	**Challenge concentration**	**Results**	**Comments**	**References**
1% in Ethanol 3% in Ethanol 10% in Ethanol	1% in Ethanol 3% in Ethanol 10% in Ethanol	5/10 (FCAT) 9/10 10/10	Intra-dermal induction. Topical challenge (FCAT)	(RIFM, 1985b)
50% in Adjuvant	"subirritant concentration"	(FCAT) Sensitisation observed	FCAT. Results only reported in summary form	(Klecak *et al.*, 1977)
5% in Ethanol	5% in Ethanol	(FCAT) 8/8	FCAT. Results only reported in summary form	(Tsuchiya *et al.*, 1982;Tsuchiya *et al.*, 1985)
3% in Acetone	0.3% in Acetone 1% in Acetone 3% in Acetone	(FCAT) Moderate sensitisation at all concentrations	Modified FCAT. Results only reported in summary form	(Hausen *et al.*, 1995)
0.1% in 30% Ethanol	Intra-dermal challenge : 0.1% in 30 % Ethanol Topical challenge : 0.5% in Petrolatum	Optimization test 17/20 20/20	Optimization test. Like FCAT except intra-dermal and topical challenges	(Maurer *et al.*, 1979)

Table 13: Cumulative contact enhancement tests (CCET) on isoeugenol

Induction conditions	Challenge conditions	Results	Comments	References
100%	100%	5/10	Standard CCET	(Tsuchiya *et al.*, 1982;Tsuchiya *et al.*, 1985)
100%	100% 30% in Ethanol 10% in Ethanol	6/6 6/6 2/6	Multi-dose CCET	(Tsuchiya *et al.*, 1982;Tsuchiya *et al.*, 1985)
10% in Ethanol	10% in Ethanol	0/9	Standard CCET	(Tsuchiya *et al.*, 1985)
10% in Ethanol	10% in liquid paraffin (low viscosity)	2/9	Standard CCET	(Tsuchiya *et al.*, 1985)
10% in Ethanol	10% in liquid paraffin (high viscosity)	0/9	Standard CCET	(Tsuchiya *et al.*, 1985)
10% in liquid paraffin (low viscosity)	10% in liquid paraffin (low viscosity)	8/10	Standard CCET	(Tsuchiya *et al.*, 1985)
10% in liquid paraffin (low viscosity)	10% in Ethanol	8/10	Standard CCET	(Tsuchiya *et al.*, 1985)
10% in liquid paraffin (low viscosity)	10% in liquid paraffin (high viscosity)	7/10	Standard CCET	(Tsuchiya *et al.*, 1985)
10% in liquid paraffin (high viscosity)	10% in liquid paraffin (high viscosity)	1/10	Standard CCET	(Tsuchiya *et al.*, 1985)
10% in liquid paraffin (high viscosity)	10% in Ethanol	1/10	Standard CCET	(Tsuchiya *et al.*, 1985)
10% in liquid paraffin (high viscosity)	10% in liquid paraffin (low viscosity)	6/10	Standard CCET	(Tsuchiya *et al.*, 1985)

The allergenic potential of isoeugenol is also evident from non-adjuvant tests. Early studies using the Modified Draize Test on Guinea Pigs had already indicated this (Table 14).

Table 14: Modified Draize Tests (Guinea Pigs) on isoeugenol

Induction conditions (intra-dermal)	**Challenge conditions (intra-dermal)**	**Results**	**Comments**	**References**
1% in peanut oil	1% in peanut oil	2/2	Old study	(Griepentrog, 1961)
0.1% in saline	0.1% in saline	Sensitization reported	No details were reported	(Klecak *et al.*, 1977)

Tests that do not use Freund's Complete Adjuvant offer a better opportunity of determining non-sensitizing conditions than those that do. The allergenic potential of isoeugenol is also evident from these non-adjuvant tests. In the Buehler Test (Table 15), a clear dose/response relationship was observed but because of the dose levels chosen, no test displayed a non-inducing dose although this would seem to be close to 1% when the skin at the site of induction was intact (Kaminsky and Szivos, 1986;Kaminsky and Szivos, 1990).

Table 15: Buehler Tests on isoeugenol

Induction conditions (topical)	Challenge conditions (topical)	Results	Comments	References
10% in diethylphthalate	3% in diethylphthalate 10% in diethylphthalate 30% in diethylphthalate	2/20 1/20 5/20	Standard test	(RIFM, 1987a)
5% in ethanol/water 80/20)	3% in diethylphthalate 9% in diethylphthalate 30% in diethylphthalate	0/20 0/20 1/20	Standard test	(RIFM, 1986)
4% in petrolatum for first 5 inductions, the 1% in petrolatum for 6th induction	2% in petrolatum re-challenge at 1%	5/10 reactions at 24 hours, 1/10 at 48 hours 5/10 reactions at 24 hours, 1/10 at 48 hours	Standard test with intact skin	(Kaminsky and Szivos, 1986;Kaminsky and Szivos, 1990)
As above	As above 2% then re-challenge at 1% in petrolatum	2/10 reactions at 24 hours, 1/10 at 48 hours 7/10 reactions at 24 hours, 2/10 at 48 hours	Use of abraded skin in induction phase	(Kaminsky and Szivos, 1986;Kaminsky and Szivos, 1990)
30% in petrolatum for first 5 inductions, then 20% for the 6th induction	Challenge at 2% in petrolatum re-challenge at 1%	8/10 reactions at 24 hours, 4/10 at 48 hours 9/10 reactions at 24 hours, 2/10 at 48 hours	Standard test with intact skin	(Kaminsky and Szivos, 1986;Kaminsky and Szivos, 1990)
3% in petrolatum	1% in petrolatum	5/8 reactions at 24 hours, 4/8 reactions at 48 hours	Standard test with intact skin	(Kaminsky and Szivos, 1986;Kaminsky and Szivos, 1990)

Induction conditions (topical)	Challenge conditions (topical)	Results	Comments	References
3% in petrolatum	1% in petrolatum	9/10 reactions at 24 hours, 5/10 reactions at 48 hours	Abraded skin at sites of induction	(Kaminsky and Szivos, 1986;Kaminsky and Szivos, 1990)
30% in petrolatum	1% in petrolatum	7/10 reactions at 24 hours, 6/10 reactions at 48 hours	Abraded skin at sites of induction	(Kaminsky and Szivos, 1986;Kaminsky and Szivos, 1990)
1% in petrolatum	challenge : 1% in petrolatum re-challenge : 1% in petrolatum	1/9 reactions at 24 hours, 0/9 reactions at 48 hours, 1/9 reactions at 24 hours, 0/9 at 48 hours	Standard test with intact skin	(Kaminsky and Szivos, 1986;Kaminsky and Szivos, 1990)
1% in petrolatum	challenge : 1% in petrolatum re-challenge : 1% in petrolatum	3/9 reactions at 24 hours, 2/9 at 48 hours, 3/9 reactions at 24 hours, 1/9 at 48 hours	Abraded skin at sites of induction	(Kaminsky and Szivos, 1986;Kaminsky and Szivos, 1990)
30% in petrolatum	challenge : 1% in petrolatum re-challenge : 1% in petrolatum	7/9 reactions at 24 hours, 3/9 at 48 hours, 8/9 reactions at 24 hours, 2/9 at 48 hours	Standard test with intact skin	(Kaminsky and Szivos, 1986;Kaminsky and Szivos, 1990)

Induction conditions (topical)	Challenge conditions (topical)	Results	Comments	References
10% in petrolatum	0.1% in petrolatum 1% in petrolatum	8/20 16/20	Standard test with extra challenges with chemical analogues	(Goh and Yuen, 1994)
	0.1% acetyl isoeugenol 0.1% eugenol	2/6 1/6	Cross-challenges only on animals that had been sensitived to isoeugenol at 0.1%	
	1% acetyl isoeugenol 1% eugenol	3/6 1/6	Cross-challenges only on animals that had been sensitived to isoeugenol at 1%	

Epicutaneous Tests, involving open application and closed patch testing (Table 16), showed no clear dose/response relationship except in the challenge doses that were able to elicit reactions.

Table 16: Epicutaneous tests (open: OET & closed: CET) in Guinea Pigs on isoeugenol

Induction conditions (topical)	Challenge conditions (topical)	Results	Comments	References
10% (vehicle not specified)	1% (vehicle not specified)	Sensitization observed	Standard OET but only summary of results reported	(Klecak *et al.*, 1977)
100%, 30%, 10% and 3% in ethanol	30% in ethanol	No reactions	Standard OET	(RIFM, 1986)
100%,30%,10% and 3% in ethanol	100% in ethanol 30% in ethanol 10% in ethanol 3% in ethanol	6/6 6/6 5/6 2/6	Standard multi-dose OET	(Tsuchiya *et al.*, 1982;Tsuchiya *et al.*, 1985)
8% (vehicle not specified)	8% vehicle not specified)	No reactions	Standard OET but only summary of results reported	(Klecak, 1979)
10% in petrolatum	1% in petrolatum 3% in petrolatum 10% in petrolatum	7/20 14/20 15/20	Standard CET (48 hours occlusion at induction and challenge)	(RIFM, 1985b)
10% (vehicle not reported)	1% (vehicle not reported)	16/20	CET with (48 hours occlusion)	(Ishihara *et al.*, 1986)

In the murine tests (Table 17), the Mouse Ear Swelling Test (MEST) confirmed the allergenicity of isoeugenol. The Local Lymph Node Assay (LLNA) also gave positive reactions in numerous tests. From LLNA dose response data, the concentration estimated to induce a threshold positive response (stimulation index = 3) can be calculated by linear interpolation. This measure termed the EC3 value, (Basketter *et al.*, 1999b)**,** provides a quantitative estimate of the relative skin sensitising potency that has been shown to correlate well with NOELs established from human studies (Gerberick *et al.*, 2001b;Griem *et al.*, 2003;Schneider and Akkan, 2004). EC3 values have been obtained from over forty different Local Lymph Node Assays carried out on isoeugenol. Although intra-laboratory differences and vehicle effects have been observed, a weighted mean EC3 value based on the number of dose levels from all of tests giving finite EC3 values has been calculated to be 2% (500 µg/cm^2) (RIFM/COLIPA, 2004).

Some insight into the mechanism has been provided by local lymph node assays conducted with and without an inhibitor of epidermal cytochrome P4501A which showed that the inhibition of this enzyme increased degree of allergenic reaction (Scholes *et al.*, 1994).

Table 17 : Murine tests (Mouse ear swelling test : MEST, Local Lymph Node Assay : LLNA)on isoeugenol

Induction conditions **(AOO = acetone:olive oil [4:1])**	**Challenge conditions**	**Results**	**Comments**	**References**
5% (vehicle not specified)	5% (vehicle not specified)	Significant ear-swelling after 24 hours	MEST	(Yamazaki *et al.*, 1998)
10%, 25%, 50% and 75% in AOO	as for induction	Sensitization at all dose level	MEST	(Garrigue *et al.*, 1994)
3% and 10% (vehicle not specified)	as for induction	100% mice were sensitized at both levels	MEST	(Thorne *et al.*, 1991)
	-			
	-			

Induction conditions **(AOO = acetone :olive oil [4:1])**	**Challenge conditions**	**Results**	**Comments**	**References**
				()
5%, 10% and 25% in AOO	-	Sensitization at all levels	LLNA	(Hilton *et al.*, 1996)
1.3, and 5% in AOO	-	Stimulation Index was 4.16 at 1.3%	LLNA : Only two doses	(Dearman *et al.*, 1999)
2.5%, 5%, and 10% in AOO	-	Sensitization effects at all doses	LLNA	(Basketter and Scholes, 1992)

0.25%, 0.5%, 1%, 2.5% and 5% in AOO	-	Number of Labs with positive effects 0.25% (1/5) 0.5% (0/5) 1% (1/5) 2.5 (3/5) 5% (5/5)	LLNA : Interlaboratory comparison (5 labs) sensitization effects (Stimulation Index > 3) recorded	(Loveless *et al.*, 1996)
2.5%, 5% and 10% in AOO	-	Stimulation Indexes (SI) : 3.2 at 2.5%, 4.8 at 5% and 9.5 at 10%	LLNA : Stimulation Indexes (SI) were recorded but EC3 not calculated	(Gardner *et al.*, 1996)
2.5%, 5% and 10% in AOO	-	Stimulation Indexes (SI) : 8.5 at 2.5%, 12.1 at 5% and 16.5 at 10%	LLNA : Stimulation Indexes (SI) were recorded but EC3 not calculated	(Bertrand *et al.*, 1997)
2.5%, 5% and 10% in AOO	-	EC3: 3.3%, 3.5% or 3.8% depending on method of calculation	LLNA : Comparison of different methods of calculations EC3	(Basketter *et al.*, 1999a)

Induction conditions **(AOO= acetone:olive oil [4:1])**	**Challenge conditions**	**Results**	**Comments**	**References**

5 concentrations 0.5%, 1%, 2.5%, 5% and 10% in following solvents acetone/olive oil (AOO) dimethyl sulphoxide (dmso) methyl ethyl ketone (mek) dimethyl formamide (dmf) propylene glycol (pg) ethanol/water [50/50] (e/w) ethanol/water [90/10] (E/W)	-	EC3 values as indicated 1% (AOO) (250 µg/cm^2) 0.9% (dmso) (225 µg/cm^2) 1% (mek) (250 µg/cm^2) 1.4% (dmf) (350 µg/cm^2) 2.5% (pg) (625 µg/cm^2) 4.9% (e/w) (1225 µg/cm^2) 1.8% (E/w) (450 µg/cm^2)	LLNA To determine effect of using 7 different vehicles	(Wright *et al.*, 2001b;Wright *et al.*, 2001a)
0.25%, 0.5%, 1%, 2.5% and 5% in AOO	-	EC3 : 1.54% (390 µg/cm^2)	LLNA	(RIFM, 2001)
0.25%, 0.5%, 1.%, 2.5% and 5% in AOO	-	EC3 : 0.64% (160 µg/cm^2)	LLNA	(RIFM, 2001)
Not given	-	EC3 : 1.3% (325 µg/cm^2)	LLNA : Report of unpublished study	(Basketter *et al.*, 2002;Basketter *et al.*, 2003;Dearman *et al.*, 1999)

Induction conditions (AOO = acetone:olive oil [4:1])	Challenge conditions	Results	Comments	References
0. 5%, 1% and 5% in AOO	-	EC3 values between 0.5% and 2.6% (125-653 µg/cm^2). Mean of 300 µg/cm^{2}with SD of 0.6%	29 separate LLNA studies where isoeugenol was used as a positive control	(Basketter and Cadby, 2004)

5.2.3.2 In vitro tests for the sensitisation potential of isoeugenol

A number of experimental in vitro techniques provided indications of the positive allergenicity of iseugenol (Dearman *et al.*, 1994;Dearman *et al.*, 1999;Guironnet *et al.*, 2000;Sieben *et al.*, 2001;Verrier *et al.*, 1999a;Verrier *et al.*, 1999b;Verrier *et al.*, 2001). The methods used in these studies have not been validated or related in any quantitative way to studies in animals or humans.

5.2.3.3 Tests in human volunteers

In human volunteers, the Human Maximization Test (Kligman, 1966) has been extensively used (Table 18) with 25 separate tests having been undertaken on a total of 660 volunteers. As most studies were performed at the concentration of 8% (sensitising over 100 of 484 test participants), this series of studies provides little information on possible no effect levels. Under these conditions (2cm x 2cm induction patches and a dose volume of 0.3 mL) a concentration of 8% results in an applied dose of 6000 µg/cm^2.

Table 18: Human maximization tests (HMTs) on isoeugenol

Induction conditions (pet. : petrolatum)	**Challenge conditions**	**Results**	**References**
10% in Petrolatum	10% in Petrolatum	19/25	(RIFM, 1979c)
8% in Petrolatum	8% in Petrolatum	0/25	(RIFM, 1971)
8% in Petrolatum	8% in Petrolatum	20/24 (in Japanese-Americans)	(RIFM, 1979c)
8% in Petrolatum	8% in Petrolatum	8/29	(RIFM, 1979e)
8% in Petrolatum	8% in Petrolatum	5/29	(RIFM, 1980d)
8% in Petrolatum	8% in Petrolatum	10/32	(RIFM, 1980d)
8% in Petrolatum	8% in Petrolatum	0/25	(RIFM, 1980d)
8% in Petrolatum	8% in Petrolatum	21/33	(RIFM, 1980d)
8% in Petrolatum	8% in Petrolatum	7/25	(RIFM, 1980d)
8% in Petrolatum	8% in Petrolatum	5/29	(RIFM, 1980d)
8% in Petrolatum	8% in Petrolatum	4/28	(RIFM, 1980d)
8% in Petrolatum	8% in Petrolatum	Only irritant Reactions in 25	(RIFM, 1980d)
8% in Petrolatum	8% in Petrolatum	4/27	(RIFM, 1980d)
8% in Petrolatum	8% in Petrolatum	21/3	(RIFM, 1980d)
8% in Petrolatum (with 8% Eugenol)	8% in Petrolatum (with 8% Eugenol)	10/22	(RIFM, 1980d)
8% in Petrolatum with 8% Dipropylene glycol	8% in Petrolatum with 8% Dipropylene glycol	8/35	(RIFM, 1980d)
8% in Petrolatum with 8% Limonene	8% in Petrolatum with 8% Limonene	9/25	(RIFM, 1980d)
1% in Petrolatum with 20% fragrance compound	1% in Petrolatum with 20% fragrance compound	0/25	(RIFM, 1980d)

Induction conditions (pet. : petrolatum)	Challenge conditions	Results	References
0.6% in Petrolatum with 20% fragrance compound	0.6% in Petrolatum with 20% fragrance compound	Only irritant reactions in 30	(RIFM, 1980d)
1.8% in Petrolatum with 20% fragrance compound	1.8% in Petrolatum with 20% fragrance compound	1/29	(RIFM, 1980d)
0.6% in Petrolatum with 20% fragrance compound	8% in Petrolatum with 20% fragrance compound	4/35	(RIFM, 1980d)
1.8% in Petrolatum (contains 20% fragrance compound)	8% in Petrolatum	4/34	(RIFM, 1980d)
1% in Petrolatum	1% in Petrolatum	6/7	(Kligman and Gollhausen, 1986)
8% in petrolatum (90% cis-isoeugenol)	8% in petrolatum (90% cis-isoeugenol)	21/31	(RIFM, 1980d)
5% in hydrophilic ointment	1% in hydrophilic ointment	5/25	(RIFM, 1979e)

The Human Repeat Patch Test (HRIP Test) has been extensively used to study the potency and possible induction thresholds of isoeugenol (under 24 hour occlusion). The results of 10 HRIP Tests are shown in Table 19. Negative reactions were obtained when induction and challenge concentrations were 0.5% (260 µg/cm^2) while positive results were obtained in tests where induction and challenge concentrations were 1% (800 µg/cm^2) or higher. One test was negative when the concentration was 1.25% (970 µg/cm^2) but two other tests carried out at this concentration gave marginally positive scores.

Table 19: Human Repeated Insult Patch Tests (HRIPT) on isoeugenol

Induction conditions	Challenge conditions	Results	Comments	References
1.25% in 95% Ethanol (970 µg/cm^2) Nine 24 hour semi-occluded patches	1.25% in 95% Ethanol	2/40	11 male & 29 female volunteers re-challenge at 5 months gave 1/40	(RIFM, 1964)
1.25% in 95% Ethanol (970 µg/cm^2) Nine 24 hour semi-occluded patches	1.25% in 95% Ethanol	0/41	7 male and 34 female volunteers	(RIFM, 1964)
1% in SDA Ethanol (800 µg/cm^2) Nine 24 hour semi-occluded patches	1% in SDA Ethanol	2/38	10 male and 28 female volunteers	(RIFM, 1973)
0.5% in SDA Ethanol (260 µg/cm^2) Nine 24 hour semi-occluded patches	0.5% in SDA Ethanol	2/53	Re-challenge after 2 weeks gave no reactions	(RIFM, 1980b)
10% in Petrolatum (11,800 µg/cm^2) Nine 48 hour occluded patches	10% in Petrolatum	16/25	7 male and 18 female volunteers	(RIFM, 1979d)
5% in SDA Ethanol (5,900 µg/cm^2) for first 3 weeks. Thereafter at 2.5% (semi-occlusive) (2,950 µg/cm^2) Nine 24 hour occlusive patches	2.5% in SDA Ethanol	3/49	Irritation with 5% isoeugenol under occlusion gave irritant reactions. Induction changed to 2.5% semi-occlusion	(RIFM, 1987b)
1.25% in unknown vehicle	1.25 in unknown vehicle	1/81	Details not provided	(Thompson *et al.*, 1983)
1% in unknown vehicle	1% in unknown vehicle	1/38	Details not provided	(Thompson *et al.*, 1983)
0.5% in unknown vehicle	0.5% in unknown vehicle	0/56	Details not provided	(Thompson *et al.*, 1983)
8% in Ethanol (2,500 µg/cm^2) Ten 48-72 hour occluded patches	8% in Ethanol	9/73	Severe induction conditions	(Marzulli and Maibach, 1980)

5.2.3.4 Clinical patch testing on patients

There are many published reports of studies in which isoeugenol produces positive reactions in patients in routine diagnostic patch testing. Although there have been numerous reports of patients giving frank allergic responses to isoeugenol in clinical patch testing on dermatological patients, many of these studies do not establish a clear causal relationship according to currently accepted criteria (Lachapelle, 1997;Lachapelle and Maibach HI, 2003;Maibach and Hostynek, 2003).

A recent publication (Hostynek and Maibach, 2004) has pointed out that reactions seen in dermatological clinics, while genuinely allergic in nature, may only occur under the severe conditions use in clinical diagnosis and may not relate to adverse effects from the use of consumer products. In a separate publication, the same authors (Hostynek and Maibach, 2003c) have also defined criteria by which possible causality can be assessed. These criteria have been applied by these authors to a number of other proposed allergens (Hostynek and Maibach, 2003b;Hostynek and Maibach, 2003a).

The same criteria have been used here to assess the strength of a causal link between the observed clinical reaction and everyday exposure to an isoeugenol-containing product. These relatively rare cases are shown in Table 20.

Table 20: Clinical patch testing with isoeugenol establishing a possible causative link to presence in a consumer product

Reference	Patch test conditions	Cases	Products
(Novak, 1974)	0.25% in vegetable oil	Ten patients two reacted to isoeugenol	Patients sensitized to a fragrance in an antiphlogistic ointment were tested to components of the fragrance
(Cordoba *et al.*, 2000)	2% in Petrolatum	Patient sensitive to body milk	Isoeugenol only component of product producing positive reaction

Authors reporting on one of the biggest multi-centre studies stated that "we observe what we seek" (Eiermann *et al.*, 1982). Isoeugenol is one of the eight components of the "Fragrance Mix" used by dermatologists to detect possible sensitivity to fragrances. This mix was first proposed (Larsen, 1975;Calnan *et al.*, 1980), on the basis of the components of a fragrance used in a popular Triadcortyl cream (Mycolog®, Squibb Corp.) (Larsen, 1979) and it was concluded that the use of this ointment in treating eczematous and ulcerous skin may have contributed significantly to the cases of clinical dermatitis that had been ascribed to this substance (Larsen, 1979).

Clinical patch testing of patients who have already shown positive reactions to the "Fragrance Mix" frequently gives positive reactions to isoeugenol although in such cases, it is rare that isoeugenol is the only component of this "Fragrance Mix" to produce positive reactions. In the cases reported in Table 21**,** no clear causal link could be established with the use of consumer products using the criteria of Hostynek and Maibach (Hostynek and Maibach, 2003c). In a large multi-centre study covering nearly 60,000 patients tested in German clinics from 1996 to 2002 (Schnuch *et al.*, 2004), the frequency of reactions to isoeugenol and in patients reacting to the fragrance mix has been about 13%. These patients have frequently reacted to other constituents of the fragrance mix (for instance 47.6% and 56.7% of patients reacting to chemically-dissimilar geraniol and amylcinnamic aldehyde respectively, also reacted to isoeugenol).

It has been reported that while the proportion of patients reacting to the "Fragrance Mix" has been relatively constant over 17 years, there is a 5% yearly increase in the proportion of patients reacting to isoeugenol (Buckley *et al.*, 2000a) having reached an average 16.7% and 15.4% of "Fragrance Mix-sensitive" males and females respectively. However, the full significance of these findings has been questioned (Wesley NO and Maibach, 2003).

Table 21: Clinical patch testing of isoeugenol in "Fragrance Mix-sensitive" patients.

References	Patch test conditions	Number tested	Number reacting	Scores	Comments (see below)
(Temesvari *et al.*, 2002)	No dose reported 24 hours occlusion Finn Chambers®	160	24	Not given	A,B
(Sieben *et al.*, 2001)	No dose reported	32	9	Not given	A,B
(Brites *et al.*, 2000)	1% in Petrolatum 48 hours occlusion	226	45	Not given	A,B
(Buckley *et al.*, 2000b)	1% in Petrolatum 48 hours occlusion over 15 years	1112	231	Not given	A,B
(Katsarma and Gawkrodger, 1999)	1% in Petrolatum Finn Chambers® or Scanpore®, 48 hours occlusion	40	8	Not given	A,B
(Katsarou *et al.*, 1999)	1% in Petrolatum Finn Chambers® or Scanpore®, 48 hours occlusion	38	9	Not given	A,B
(Johansen *et al.*, 1996d)	Different concentrations (serial dilution study on isoeugenol -sensitive patients who had previously reacted to Fragrance-Mix)	19	18	Different scores recorded for different patients	B
(Johansen and Menne, 1995)	1% or 2% in Petrolatum 48 hours occlusion in Finn Chambers® or Scanpore®, tape	367	68	+ to +++ reactions	A,B
(Becker *et al.*, 1994)	No conditions given	50	3	Not given	A,B
(de Groot *et al.*, 1993)	1%, 3% and 5% in Petrolatum (serial dilutions)	6	1	Not given	B

References	Patch test conditions	Number tested	Number reacting	Scores	Comments (see below)
(Safford *et al.*, 1990)	2% in Petrolatum 48 hours occlusion in Finn Chambers®	20	4	Not given	B
(Enders *et al.*, 1989)	1% in Petrolatum	162	27	Not given	A,B
(Santucci *et al.*, 1987)	1% in Petrolatum 48 hours occlusion in Finn Chambers® or Scanpore®	54	12	Not given	A,B
(Rudzki and Grzywa, 1986)	Not given	42	19	Not given	A,B
(Angelini *et al.*, 1985)	1% in Petrolatum	144	6	Not given	A,B
(Romaguera *et al.*, 1983)	Not reported	80	7	Not given	A,B
(Calnan *et al.*, 1980)	2% in Petrolatum	172	48	Not given	A,B
(Bordalo *et al.*, 2000)	Not given	50	8	Not given	A,B
(Schnuch *et al.*, 2002)	1% in Petrolatum 48 hour patch tests	4900 consecutive patients	173	51 gave + reactions to 1% isoeugenol and to 8% Fragrance-Mix. 60 gave + reactions to 1% isoeugenol but ++ or +++ reactions to 8% Fragrance -Mix. 56 gave ++ or +++ reactions to both the Fragrance-Mix and Isoeugenol 6 gave ++ or +++ reactions to isoeugenol but only + reactions to Fragrance-Mix	A,B
(Ohela and Saramies, 1983)	5% isoeugenol in Petrolatum	520	15	Not given	A,B

Comments : *A : Not a primary study. Review of several studies or multicentre study.*

B : Patients probably reacted to other test materials in the same study.

C : Abstract only in English.

In patients already classified as "perfume sensitive" (Table 22) or only “cosmetic-sensitive” (Table 23), similar frequencies of positive reactions to isoeugenol have been observed. Here too no clear causal link between specific exposure to isoeugenol could be established using the criteria of Hostynek and Maibach (Hostynek and Maibach, 2003c).

Table 22: Clinical patch testing of isoeugenol in “perfume-sensitive” patients as well as patients reacting to other fragrance ingredients.

References	Patch test conditions	Number tested	Number reacting	Scores	Comments (see below)
(Wohrl *et al.*, 2001)	1% in Petrolatum 48 hours occlusion	747 “Perfume-sensitive” patients	40	Not given	A,B
(Larsen *et al.*, 1996)	4% in Petrolatum 48 hours occlusion using Finn Chambers® or Scanpore®	167 “Perfume-sensitive” patients	23	Irritant reactions in 6 allergic reactions in 23	A,B
(Safford *et al.*, 1990)	2% in Petrolatum	8 “Perfume-sensitive” patients	0	-	-
(Meynadier *et al.*, 1986)	2.5% in Petrolatum	21 “Perfume-sensitive ” patients	7	Not given	A,B
(Larsen, 1977)	2% and 5% in Petrolatum	21 “Perfume-sensitive ” patients	5	Not given	A,B
(Frosch *et al.*, 1995a)	1% in Petrolatum 48 hours occlusion in Finn Chambers®	1072 “Perfume-sensitive ” patients	30	20 ++ to +++ 10 + or ?	A,B
(Gutman and Somov, 1968)	Not reported	97 “Perfumery plant workers with occupational eczema”	0	-	-

References	Patch test conditions	Number tested	Number reacting	Scores	Comments (see below)
(Ruhnek *et al.*, 1989)	1% in Petrolatum	367 "Perfume-sensitive "	15	9 ++ to +++ 4 + 2 doubtful	A,B
(Hausen, 2001)	2% in Petrolatum 24 hours occlusion using Finn Chambers® or Scanpore®	102 "Peru balsam-sensitive" patients	28	7 +, 11 ++ & 10 +++ reactions	A,B
(Bruynzeel *et al.*, 1984)	5% in Petrolatum	1 "Peru balsam-sensitive" patients	1	Not given	B
(Hjorth, 1961c)	5% in Petrolatum 48 hours occlusion in Lysaplast patches	74 "Peru balsam-sensitive" patients	45	Not given	A,B
(Hjorth, 1961c)	2% in Petrolatum 48 hours occlusion in Lysaplast patches	55 "Peru balsam-sensitive" patients	33	Not given	A,B
(Hjorth, 1961c)	0.5% in Petrolatum 48 hours occlusion in Lysaplast patches	22 "Peru balsam-sensitive" patients	20	Not given	A,B
(Hjorth, 1961b)	2% in Petrolatum 48 hours occlusion in Lysaplast patches	17 "Peru balsam-sensitive" patients	6	Not given	A,B
(Hjorth, 1961a)	5% in Petrolatum 48 hours occlusion in Lysaplast patches	28 "Peru-balsam and vanillin-sensitive" patients	25	Not given	A,B
(Hjorth, 1961a)	5% in Petrolatum 48 hours occlusion in Lysaplast patches	32 "Peru-balsam and vanillin-sensitive" patients	15	Not given	A,B
(Van Joost *et al.*, 1984)	8% in Petrolatum	242 patients sensitive to Peru-balsam, wood tar, eugenol and coumarin	36	Not given	A,B
(Goncalo *et al.*, 1988)	Not reported	31 "Oak moss-sensitive" patients	9	Not given	A,B

References	Patch test conditions	Number tested	Number reacting	Scores	Comments (see below)
(Stinchi *et al.*, 1997)	Not reported	6 "Lichen-sensitive" patients	2	Not given	B
(Wojnarowska and Calnan, 1986)	Not reported	16 "Musk Ambrette photo-sensitive" patients	3	Not given	B
(Ducombs *et al.*, 1986)	Not reported	3 Musk Ambrette photo-sensitive" patients	1	Not given	B
(Van Joost *et al.*, 1984)	2% in Petrolatum	5 "wood tar-sensitive" patients in 667 patients	5	Not given	B
(Tanaka *et al.*, 2004)	1% in Petrolatum 48 hours occlusion in Finn Chambers® or Scanpore®	2261 consecutive dermatitis patients concomittent reactions in 40 patients sensitive to trans-isoeugenol 19 patients sensitive to isoeugenyl acetate 4 patients sensitive to isoeugenyl benzoate 16 patients sensitive to isoeugenyl phenyl acetate 4 patients sensitive to isoeugenyl methyl ether 2 patients sensitive to isoeugenyl benzyl ether	40 36 13 3 15 0 0	Not given	

Comments : *A : Not a primary study. Review of several studies or multicentre study.*

B : Patients probably reacted to other test materials in the same study.

C : Abstract only in English.

Patients who are allergic to another "marker" of "fragrance allergy: Peru balsam, have been shown to react also to isoeugenol even though it is not a known constituent of this natural exudate. Significant correlations have also been found to sensitivity to isoeugenol and other materials that contain no isoeugenol or any of its obvious analogues such as wood tars and Oakmoss and other lichen products. Isoeugenol and showed cross-sensitivity to coumarin while patients who had Musk Ambrette-induced photoallergy were also found to react to isoeugenol.

Cross-reactions have been observed with structurally-similar substances. In some cases isoeugenol and eugenol have been reported to cross-react (Van Joost *et al.*, 1985;Buckley *et al.*, 2000a;LeCoz, 2002;Beswick *et al.*, 1999;Johansen *et al.*, 1996a) while in others, this is claimed to be more rare than structural similarity would predict (Buckley *et al.*, 2001). Mechanistic explanations have been published (Barratt and Basketter, 1992) showing that isoeugenol and eugenol are metabolized to quite different haptens.

Patients who were sensitive to vanillin (Hjorth, 1961c), isoeugenyl acetate, isoeugenyl benzoate, isoeugenyl phenylacetate, isoeugenyl methyl ether and isoeugenyl benzyl ether also reacted to iseugenol in some cases (Tanaka *et al.*, 2002) and (Tanaka *et al.*, 2004).

Table 23: Clinical patch testing of isoeugenol in "cosmetic-sensitive" and other dermatitic patients

References	Patch test conditions	Number tested	Number reacting	Scores	Comments (see below)
(Hendriks and van Ginkel, 1999)	2% in Petrolatum with + 1% sorbitan sesquioleate	757 "Cosmetic-sensitive" patients	16	Not given	A,B
(Haba *et al.*, 1993)	5% in Petrolatum	64 "Cosmetic-sensitive" patients	4	Not given	A,B,C
(Dooms-Goossens *et al.*, 1992)	No dose reported 48 hours occlusion	462 "Cosmetic-sensitive" patients	33	Not given	A,B
(Remaut, 1992)	2% in Petrolatum 48 hours occlusion in Finn Chambers® or Scanpore®	115 "Cosmetic-sensitive" patients	5	Not given	A,B
(Itoh *et al.*, 1986;Itoh *et al.*, 1988)	5% (vehicle and patches not reported)	310 "Cosmetic-sensitive" patients	13	Not given	A,B,C
(Asoh and Sugai, 1986;Asoh and Sugai, 1987)	Not reported	258 "Cosmetic-sensitive" patients	22	Not given	A,B,C

References	Patch test conditions	Number tested	Number reacting	Scores	Comments (see below)
(Broeckx *et al.*, 1987)	Not reported	156 "Cosmetic-sensitive" patients	16	Not given	A,B
(Hayakawa and Japan Patch Test Research Group, 1986)	1% in Petrolatum 48 hours occlusion in closed patch tests	117 "Cosmetic-sensitive" patients	7	Not given	A,B,C
(de Groot *et al.*, 1988)	3% in Petrolatum 48 hours occlusion in Van der Bend® Chambers	119 "Cosmetic-sensitive" patients	2	Not given	A,B
(Asoh and Sugai, 1985)	Dose not reported Finn Chambers® or Scanpore®	122 "Cosmetic-sensitive" patients	4	Not given	A,B,C
(Adams and Maibach, 1985)	Dose not reported Finn Chambers® or A1- test patches 48 hours occlusion	399 "Cosmetic-sensitive" patients	10	Not given	A,B
(Emmons and Marks, Jr., 1985)	4% in Petrolatum 48 hours occlusion in Finn Chambers® or Scanpore®	16 "Cosmetic-sensitive" patients	0	-	A
(de Groot *et al.*, 1985)	8% in Petrolatum 48 hours occlusion under Silver patches	179 "Cosmetic-sensitive" patients	36	Not given	A,B
(Ishihara *et al.*, 1981)	5% (vehicle and conditions not reported)	155 "Cosmetic-sensitive" patients	8	Not given	A,B,C
(Ishihara *et al.*, 1979)	1-5% in Petrolatum	133 "Cosmetic-sensitive" patients	3	Not given	A,B,C
(Schorr, 1974)	Dose not reported 48 hours occlusion	70 "Cosmetic-sensitive" patients	2	Not given	A,B
(Nishimura *et al.*, 1984)	5% (vehicle and conditions not reported)	212 "Cosmetic-sensitive" patients	9	Not given	A,B,C

References	Patch test conditions	Number tested	Number reacting	Scores	Comments (see below)
(Eiermann *et al.*, 1982)	Dose vehicle not reported A-1 test strips or Finn Chambers® for 48 hours	149 Dermatitis patients	10	Not given	A,B
(Ishihara *et al.*, 1981)	5%	159 Dermatitis patients	11	Not given	A,B,C
(Itoh, 1982)	5% in Petrolatum 48 hours occlusion	155 Dermatitis patients	8	Not given	A,B,C
(Nagareda *et al.*, 1992)	1% in Petrolatum in Finn Chambers® or Scanpore®	22 Dermatitis patients	3	Not given	A,B,C
(Hayakawa and Japan Patch Test Research Group, 1986)	1% in Petrolatum 48 hours occlusion	117 Dermatitis patients	7	Not given	A,B,C
(White *et al.*, 1999)	1% in Petrolatum	155 Consecutive dermatitis patients	8	3 questionable reactions also observed	
(Angelini *et al.*, 1997)	Not reported	19546 Consecutive dermatitis patients	39	Not given	A,B
(Shah *et al.*, 1997)	Dose not reported 48 hours occlusion	83 Children	Some reactions	Not given	A,B
(Stables *et al.*, 1996)	Dose not reported 48 hours occlusion	95 Children	2	Not given	A,B
(Shah *et al.*, 1996)	Dose not reported 48 hours occlusion	63 Consecutive dermatitis patients	1	Not given	A,B
(Frosch *et al.*, 1995b)	1% in Petrolatum 48 hours occlusion in Finn Chambers® or Scanpore®	702 Consecutive dermatitis patients	17	6 irritant reactions also observed 6 additional reactions observed when 1% sorbitan sesquioleate added to patch test vehicle	A,B

References	Patch test conditions	Number tested	Number reacting	Scores	Comments (see below)
(de Groot *et al.*, 1993)	5% in Petrolatum	677 Consecutive dermatitis patients	15	Not given	A,B
(Hashimoto *et al.*, 1990)	1% in Petrolatum 48 hours occlusion using Finn Chambers® or Scanpore®	106 Consecutive dermatitis patients	2	Not given	A,B,C
(Miranda *et al.*, 1990)	Not reported	50 Consecutive dermatitis patients	15	Not given	A,B
(Malanin and Ohela, 1989)	5% in Petrolatum 24 or 48 hours occlusion in Finn Chambers®	1967 Consecutive dermatitis patients	90	Not given	A,B
(Storrs *et al.*, 1989)	4% in Petrolatum 48 hours or 72 hours occlusion in Finn Chamber® ® or Scanpore®	1012 Consecutive dermatitis patients	24	5 additional questionable reactions	A,B
(Macfarlane *et al.*, 1989)	Not reported ICDRG recommendations followed	403 Consecutive dermatitis patients	1	Not given	A,B
(Rademaker and Forsyth, 1989)	Not reported ICDRG recommendations followed	125 Children with dermatitis	4	Not given	A,B
(Nethercott *et al.*, 1989)	5% in Petrolatum 48- hours or 72-hours occlusion A1- test strips or Finn Chambers® or Scanpore®	89 Consecutive dermatitis patients including 19 with eyelid dermatitis	4	Not given	A,B

References	Patch test conditions	Number tested	Number reacting	Scores	Comments (see below)
(Ohela and Saramies, 1983)	5% (vehicle and conditions not reported) in Finn Chambers®	520 Dermatitis patients	15	Not given	A,B
(Johansen *et al.*, 1997)	1% in Petrolatum 48 hours occlusion in Finn Chambers® or Scanpore®	884 Dermatitis patients	78	+ to +++ reactions	A,B
(Johansen *et al.*, 1996b)	1% in Petrolatum 48 hours occlusion in Finn Chambers® or Scanpore®	335 Dermatitis patients	27	+ to +++ reactions	A,B
(Frosch *et al.*, 1995a)	1% in Petrolatum 48 hours occlusion in Finn Chambers® or Scanpore®	1072 Dermatitis patients	20	+ to +++ reactions with an additional 10 questionable reactions	A,B
(Rudzki and Grzywa, 1986)	Conditions not specified	5315 Dermatitis patients	299	Not given	A,B
(Ishihara, 1977;Ishihara, 1978)	5% in Petrolatum 24 hours occlusion	82 Dermatitis patients	2	Not given	A,B,C
(Rudner, 1977;Rudner, 1978)	2% (vehicle not reported) A1- test and Dermicel® 48 hours occlusion	273 Consecutive dermatitis patients	14	Not given	A,B
(Cronin, 1985)	2% in Petrolatum	1836 2461	31 48	Not given Not given	A,B A,B
(Ferguson and Sharma, 1984)	2% in Paraffin in Finn Chambers®	241 Consecutive dermatitis patients	13	Not given	A,B
(Asoh *et al.*, 1985)	2% (vehicle not reported) 48 hours occlusion	25 Dermatitis patients	2	Not given	A,B,C
References	**Patch test conditions**	**Number tested**	**Number reacting**	**Scores**	**Comments (see below)**

(Mid-Japan Contact Dermatitis Research Group, 1984)	5% in Petrolatum 2% in Petrolatum 1% in Petrolatum 48 hours occlusion in A1- patches or Torii-ban patches or Finn Chambers®	357 357 357 Patients with facial dermatitis	13 11 11	Not given Not given Not given	A,B,C
(Nishimura *et al.*, 1984)	5% vehicle and conditions not reported	275 Non-cosmetic dermatitis patients	17	Not given	A,B,C
(T.Sugai *et al.*, 1983)	Dose not reported Finn Chambers® or Scanpore®	152 Dermatitis patients	9	Not given	A,B,C
(Emmons and Marks, Jr., 1985)	4% in Petrolatum 48 hours occlusion in Finn Chambers® or Scanpore®	15 Dermatitis patients	0	-	B
(Emmons and Marks, Jr., 1985)	4% in Petrolatum open application under Scanpore® tape	15 Dermatitis patients	0	-	B
(Itoh *et al.*, 1986;Itoh *et al.*, 1988)	Dose not reported Finn Chambers® or Scanpore®	408 Consecutive dermatitis patients	24	Not given	A,B,C
(Goodfield and Saihan, 1988)	Not reported	120 Consecutive dermatitis patients	4	Not given	A,B
(Santucci *et al.*, 1987)	5% in Petrolatum 48 hours occlusion in Finn Chambers® or Scanpore®	1200 Consecutive dermatitis patients	14	Not given	A,B
(Takenaka *et al.*, 1986)	0.05-0.5% in a base cream or in 99% Ethanol	54 Dermatitis patients	1	Not given	A,B,C

References	Patch test conditions	Number tested	Number reacting	Scores	Comments (see below)
(Addo *et al.*, 1982)	2% in Paraffin 48 hours occlusion in A1- Test patches or Scanpore®	457 Consecutive dermatitis patients	8	Not given	A,B
(Ishihara *et al.*, 1981)	5% (vehicle and patch test conditions not reported)	159 Consecutive dermatitis patients	11	Not given	A,B,C
(Ishihara *et al.*, 1979)	1-5% in Petrolatum	86 Dermatitis patients	4	Not given	A,B,C

Comments : *A : Not a primary study. Review of several studies or multicentre study.*

B : Patients probably reacted to other test materials in the same study.

C : Abstract only in English.

In patients judged as sensitive to cosmetics, isoeugenol was judged to be one of the most common eliciting allergens (Dooms-Goossens *et al.*, 1992;Schnuch *et al.*, 2002).

Patients suffering from special dermatological conditions also reacted to isoeugenol (Table 24).

Table 24: Clinical patch testing of isoeugenol in patients with special conditions (see comments)

References	Patch test conditions	Number tested	Number reacting	Scores	Comments (see below)
(Meding *et al.*, 2003)	2% (vehicle not reported) 48 hours occlusion in Finn Chambers®	45	1	Not given	B
(Kiec and B.Krecisz, 2002)	Not reported 1% in Petrolatum 48 hours occlusion	46 dental nurses 79 dentists 223 nurses	1 0 5	Not given - Not given	A,B,E A,B,F
(Francalanci *et al.*, 2000)	5% in Petrolatum 48 hours occlusion in Finn Chambers®	54	1	Not given	A,B,G
(S.Freeman and R.Stephens, 1999)	No dose reported 48 hours occlusion	75	1	Not given	A,B,G
(Lucke *et al.*, 1998)	1% in Petrolatum	55	3	Not given	A,B,H
(Heule *et al.*, 1998)	1% in Petrolatum	47	2	Not given	A,B.I
(Armstrong *et al.*, 1997)	1% in Petrolatum 48 hours occlusion	48	1	Not given	A,B,J
(Virgili *et al.*, 1997)	1% in Petrolatum	44	1	Not given	A,B,H
(Haba *et al.*, 1993)	5% in Petrolatum	7	0	-	A,B,C,K
(Abifadel *et al.*, 1992)	1% in Petrolatum 48 hours occlusion	20	0	-	A,B.L
(Goh and Kwok, 1986)	5% (vehicle and patches not reported)	38	5	Not given	A,B,K
(Addo and Frain-Bell, 1987)	2% in Paraffin 48 hours occlusion	4 (with previous sensitivity to isoeugenol)	4	Not given	B,M

References	Patch test conditions	Number tested	Number reacting	Scores	Comments (see below)
(Addo *et al.*, 1982)	2% in Paraffin 48 hours occlusion in A1-Test patches or Scanpore®	50	6	Not given	A,B,M
		32	1	Not given	A,B,N
(Ishihara *et al.*, 1979)	1-5% in Petrolatum	55	1	Not given	A,B,C,K
(Ishihara, 1978)	5% (vehicle not reported)	34	4	Not given	A,B,C,K
(Nishimura *et al.*, 1984)	5% (vehicle and conditions not given)	35	5	Not given	A,B,C,K
(Schauder and Ippen, 1997)	1% in Petrolatum	41	7	Not given	A,B.M

Comments :
- *A : Not a primary study. Review of several studies or multicentre study.*
- *B : Patients probably reacted to other test materials in the same study.*
- *C : Abstract only in English.*
- *D : Suspected occupational eczema in bakeries*
- *E : Suspected occupational eczema from dental work*
- *F : Suspected occupational eczema in hospital nurses*
- *G : Patients with Cheilitis*
- *H : Patients with Vulval Dermatoses*
- *I : Patients with Psoriasis*
- *J : Patients with Orofacial Granulomatosis*
- *K : Patients with Facial Melanosis*
- *L : Patients with Atopic Dermatitis*
- *M : Patients with Photosensitivity dermatitis/Actinic Reticuloid Syndrome.*
- *N : Patients with Polymorphic Light Eruptions*

Conclusion

Isoeugenol shows a definite skin sensitization potential in a wide variety of predictive test systems and is classified as a moderate skin sensitizer accoding to ECETOC standards. Non-adjuvant tests in animals and maximized tests carried out on human subjects offer a sound basis for a “weight of evidence” judgment on what exposure levels are unlikely to induce allergy in naïve individuals

during use of household products. The local Lymph Node Assay places this level at around 500 µg/cm^2 (with a some degree of variability) while the Human Repeated Insult Patch Test places this at around 260 µg/cm^2 on the basis of two tests carried out on a total of 97 subjects..

Numerous patch tests carried out on dermatitic patients have indicated that acquired allergy to isoeugenol is wide-spread even though most of these clinical studies were not carried out under conditions that enable establishment of an unambiguous causal role of isoeugenol in the patients' dermatitis.

5.2.4 Phototoxicity and photo-allergenicity

5.2.4.1 In vitro Phototoxicity

No phototoxic effects were seen from 10% methanolic isoeugenol in screening studies using yeast (*Saccharomyces cerevisiae)* exposed to UV doses up to 97.2 J/cm^2 UV light for 18 hours (Weinberg and Springer, 1981;Tenenbaum *et al.*, 1984). On the other hand, while concentrations of 5% (in paraffin) produced no phototoxic effects in studies where another yeast (Candida utilis) was exposed 1.2 mW/cm^2 UV-A, when the light source was changed to a normally non-toxic 15 minute flux of approximately 1350 mJ/cm^2 of UV-B, minimal phototoxic effects were seen (Addo *et al.*, 1982).

Conclusion
There is some evidence to show that isoeugenol is potentially (but minimally) phototoxic when irradiated with UV-B but not UV-A.

5.2.4.2 Phototoxicity in humans

No effects were seen with 5 ul/cm^2 (5.4 mg/cm^2) isoeugenol applied under occlusion for 24 hours to the lower untanned backs of 10 subjects and these sites were then irradiated with UV-A and visible light (25 mW/cm^2 UV-A) (RIFM, 1979b). Similarly, no effects were seen in 10 subjects in another study when a 6 hour closed patch application of 5% isoeugenol in a hydrophilic ointment was followed by irradiation with UVA and visible light from a xenon-arc solar simulator (20 J/cm^2 of UVA) (Kaidbey and Kligman, 1980b).

Conclusion
No phototoxicity was seen in studies using visible and UV-A light.

5.2.4.3 Photoallergy in humans

Two predictive tests involved successive 24 hour occlusive exposure to isoeugenol followed by 3-MED irradiation with UV-A. Induction was performed at 5% and challenge at 1% concentrations in 25 subjects in each test but failed to give any evidence of photo-allergy or photo-irritation (RIFM, 1979b;Kaidbey and Kligman, 1980b). Another 24 hour closed patch test carried out on 25 subjects using 5% isoeugenol in hydrophilic ointment followed by UV-A and UV-B irradiation repeated 6 times and then followed by challenge testing at 1% isoeugenol and the same UV-A and UV-B exposures, also gave no reactions (Kaidbey and Kligman, 1980a).

In clinical studies on 745 suspected photoallergic patients, photopatch testing of 1% isoeugenol gave 2 reactions (Wennersten G *et al.*, 1984). However, in other studies, photopatch testing of 1%

isoeugenol in petrolatum gave no effects (Hashimoto *et al.*, 1990;Nagareda *et al.*, 1992;Schauder and Ippen, 1997).

Conclusion
Predictive tests failed to demonstrate a potential for isoeugenol to cause photoallergies. Such tests have however failed to demonstrate this potential in other photoallergens such as musk ambrette and 6-methylcoumarin. Clinical photopatch tests produced a low rate of response of uncertain linkage to the causality of isoeugenol.

5.2.5 Repeated Dose Toxicity

5.2.5.1 Oral route

Repeated dose studies were conducted in the 1950s and 1960s. They demonstrate a low degree of toxicity upon repeated exposure to isoeugenol. In one of these studies isoeugenol was administered to ten rats at a dietary concentration of 1% over 16 weeks and to another ten rats at 0.1% over 28 weeks. All animals were examined for pathological macroscopic and microscopic changes but none were seen (Food and Drug Administration, 1954). In another study, isoeugenol was administered at a dose of 1% in the diet of an unspecified number of rats and gave no effects after 16 weeks (Bar and Griepentrog, 1967). A similar study on 5 male and 5 female Osborne-Mendel rats also given 0.1% isoeugenol in their diet showed no effects on growth or haematology or any macroscopic and microscopic tissue changes (Hagan *et al.*, 1967). A 13 week gavage study in Fisher 344 rats and B6C3F1 mice was carried out at doses of 37.5, 75, 150, 300 and 600 mg/kg but the results of this study are not yet available (National Toxicology Program, 2003).

More recent developmental toxicity studies provide some insight into the effects of repeated dosing of isoeugenol (see section 5.2.8.). In developmental studies in which isoeugenol was administered by gavage to rats on gestational days 6 through 19. Maternal toxicity was expressed mainly as reduced body weight and gestational weight gain. The lowest observed effect level (LOAEL) was at (250 mg/kg/day); a NOAEL was not established in this study (George *et al.*, 2001). In a second developmental study using the same dosing regimen (National Toxicology Program, 1999), animals dosed at 500 mg/kg but not at 250 mg/kg/day showed an increase in the incidence of piloerection and lethargy. Maternal body weight and gravid uterine weight exhibited significant decreases compared with concurrent control weights at 500 mg/kg/day but not at 250 mg/kg/day (see section 5.2.8.1). A third study was carried out over three continuously breeding generations of male and female Sprague-Dawley rats. In this, isoeugenol was administered by gavage at doses of 70, 230 and 700 mg/kg/day. Decreases in mean bodyweight were seen only in the mid- and high-dose males and high-dose females in the F_0 and F_1 generations. In the 230 mg/kg-dosed and 700 mg/kg-dosed males and 700 mg/kg-dosed females, there were signs of general toxicity as noted by hyperkaeratosis and hyperplasia in non-glandular stomachs and decreased body weight (Layton *et al.*, 2001).

Conclusion

Data are from old feeding studies lack the rigor, diversity and numbers of animals, multiplicity of dose levels and width of observation of modern studies. None-the-less, they show that dietary levels of 1% (approximately 800 mg/kg/day) are well tolerated in rats for periods exceeding 28 weeks. Evidence from 3-generation reproduction studies indicates some possibly adverse effects in pregnant animals given gavage doses of 250 and 230 mg/kg/day. The appearance of hyperkaeratosis and hyperplasia in non-glandular stomachs are probably due to the administration by intubation which delivers a bolus of an irritant substance. Body weight depression may be due to general toxicity and to these irritant gastric effects.

5.2.5.2 Other routes

No data are available.

5.2.6 Genetic Toxicity

5.2.6.1 Bacterial tests

Ten studies of the mutagenicity of isoeugenol in various *Salmonella typhimurium* strains (TA 98, TA 97, TA 100, TA 102, TA 1535, TA 1537 and TA 1538) gave no effects either with or without metabolic activation (Hsia *et al.*, 1979;Nestmann *et al.*, 1980;Florin *et al.*, 1980;Douglas *et al.*, 1980;Sekizawa and Shibamoto, 1982;RIFM, 1983;Huang *et al.*, 1985;Mortelmans *et al.*, 1986;Fujita and Sasaki, 1987;Heck *et al.*, 1989). In most of these studies, doses up to the limits of toxicity (around 0.6 mg/plate) was carried out. A negative result was also reported (no details available) in a *Salmonella typhimurium* –reversion assay carried out under the US National Toxicology Program (National Toxicology Program, 2003).

Negative results were also obtained in *Escherichia coli* strain WP2 uvrA trp- with and without metabolic activation (Sekizawa and Shibamoto, 1982) and in the SOS Chromotest using *Escherichia coli* strain PQ37 (Ohshima *et al.*, 1989).

No effects were also seen in a yeast gene conversion assay with an without metabolic activation (Nestmann and Lee, 1983).

The Rec-assay/DNA-repair test in *Bacillus subtilis* strains H 17 (Rec+) and M 45 (Rec-) was negative in one study (Oda *et al.*, 1979) but gave positive effects in another (Sekizawa and Shibamoto, 1982). In the latter study the zone of inhibition at a dose of 0.8 mg isoeugenol/disk was 23.4 mm for M 45 (Rec-) and 18.2 for H 17 (Rec+) giving a difference of only 5.2 mm. In this study, isoeugenol was administered neat. The authors explain that the oily nature of the test material did not permit ready diffusion in an aqueous agar layer. Furthermore, the Rec+ cells grew faster (doubling in 48 minutes) than the Rec- cells (doubling at 75 minutes). The Rec+ cells may therefore have grown too fast, giving a visible lawn of bacteria before the sample diffused effectively thereby giving rise to a smaller inhibition zone than the Rec cells (Sekizawa and Shibamoto, 1982). In the study that was negative (Oda *et al.*, 1979), isoeugenol was administered in dimethylsuphoxide and hence would be expected to have diffused more efficiency that the neat test material that was administered in the positive study (Sekizawa and Shibamoto, 1982). The significance of this positive response in the Rec assay is therefore doubtful and may be attributed to an artifact.

5.2.6.2 In vitro studies in mammalian cells

Key studies, where there were no confounding factors, were negative. No effects were seen in unscheduled DNA synthesis assays with hepatocytes isolated from male Fischer 344 rats (Burkey *et al.*, 1998) and from male Fischer 344 rats and female B6C3F1 mice exposed to isoeugenol by 18-hour incubation at concentrations up to 1.0 mM of this substance (Burkey *et al.*, 2000). A negative result was also reported (no details available) in a chromosome aberration assay carried out under the US National Toxicology Program (National Toxicology Program, 2003).

A Sister Chromatid Exchange (SCE) assay was conducted using human lymphocytes gave positive effects at isoeugenol concentrations of 0.5 mM (82 mg/L) (Jansson *et al.*, 1986). The number of SCE per treated cells were 10.3 at 0.25 mM (44 mg/l) and 14.0 at 0.5 mM (88 mg/l). These effects were significantly less than for other substances that were also tested in the same study such as vanillin. In another SCE study, isoeugenol had no effect on the frequency of SCEs induced by mitomycin C in cultured Chinese hamster ovary cells at concentrations of 10, 33.3, 100 uM (1.8 - 17.6 mg/l) with cytotoxicity being observed at 333 μM (58.6 mg/l) (Sasaki *et al.*, 1989).

Positive results in a SCE assay are generally not regarded as evidence of a mutagenic response, especially in cases of high toxicity where lysosome breakdown due to cytotoxicity, and not from the direct action of the test substance on DNA. These effects are observed at concentrations of test substance that produce high levels of cytotoxicity, involving lysosomal breakdown and release of DNAase which induces increased exchanges, chromosome aberrations and DNA double-strand breaks (Bradley *et al.*, 1987;Zajac-Kay and Ts'o, 1984). Indeed, isoeugenol gave no evidence of an increase in the frequency of chromatid breaks and sister chromatid exchanges (SCE) in Chinese hamster ovary (CHO) cells (Sasaki *et al.*, 1989) at lower concentrations, but showed an increase in sister chromatid exchanges in human lymphocytes at higher concentrations of 0.25 mM (44 mg/l) and 0.5 mM (88 mg/l) 0.5 mM (82 mg/ml) (Jansson *et al.*, 1986).

Conclusion

Based on the overall weight of the evidence, Isoeugenol is not considered to be genotoxic. It is consistently negative in bacterial screens except for one of two DNA-repair tests in Bacillus subtilis: a procedure for which there are no standard protocols and which is not a preferred assay in current testing strategies. Studies in key mammalian cell systems (chromosomal aberration assay and negative UDS assay) were negative. Sister chromatid exchanges were observed in human lymphocytes but these would seem to be due to cytotoxicity at the chosen dose levels.

5.2.7 Carcinogenicity

No data are available. The planned National Toxicology Program bioassay has not been completed (National Toxicology Program, 2003).

5.2.8 Developmental Toxicity/Teratogenicity

5.2.8.1 Oral route

A developmental toxicity study was conducted in which pregnant Sprague-Dawley outbred albino rats were given doses of 250, 500 or 1000 mg isoeugenol/kg/day by gavage on gestational days 6 through 19. There were no treatment-related maternal deaths and at termination of day 20, the clinical signs associated with isoeugenol exposure included dose-related evidence of sedation and aversion to treatment (rooting behaviour) in all dosed groups, as well as in increased incidence of

piloerection in the 1000 and 500 mg/kg/day groups. Maternal body weight, weight gain, and gestational weight gain were reduced in all doses in a dose-related manner. Gravid uterine weight was significantly decreased at the mid and high doses whereas maternal relative liver weight was increased at all 3 dose levels. During treatment (gestational days 6 to 19), maternal relative food consumption was significantly decreased at the high dose but prenatal mortality (resorption or late foetal death) was unaffected. At 1000 mg/kg/day, average foetal body weight/litter was decreased by 7% in males and 9% in females. The incidences of foetal morphological anomalies were statistically equivalent among groups, except for an increase in unossified sternebrae noted at the highest dose. Pharmacological activity (sedation), maternal toxicity (reduced body weight and corrected weight gain), and aversion to dosing were noted at doses of 250 mg/kg/day and higher of gestational days 6 - 19. The lowest-observed-adverse effect level (LOAEL) for maternal toxicity was therefore 250 mg/kg/day. The lowest-observed-adverse effect level (LOAEL) for developmental toxicity was 1000 mg/kg/day based on intra-uterine growth retardation and mildly delayed skeletal ossification. The no-observed-adverse effect level (NOAEL) for developmental toxicity was 500 mg/kg/day (George *et al.*, 2001).

In another study, timed-mated CD rats were orally administered isoeugenol (250, 500, or 1000 mg/kg body weight/day) on gestation days 6 through 19. At the 250 mg/kg dose the only maternal change was an increase in liver weight. At the 500 mg/kg dose the incidence of piloerection and lethargy was increased and maternal body weight and gravid uterine weight exhibited significant decreases compared with concurrent control weights. These trends were amplified at the 1000 mg/kg dose level. No morphological abnormalities were observed in foetal skeletons at the two lower dose levels. However at the 1000 mg/kg dose level, average weight for male or female foetuses was slightly reduced (91-93% of that of control animals). At this dose level there was also a significant increase in the incidence of skeletal variations with 14/179 foetuses exhibiting unossified sternebrae (National Toxicology Program, 1999).

A three-generation continuous breeding test (reported only as an abstract) was performed in which isoeugenol was administered by gavage at four doses (0, 70, 230 and 700 mg/kg) to 20 adult male and female Sprague-Dawley rats (F_0 generation) and dosing was continued after post-natal day 21 to male and females of the subsequent F_1 generation. There was a dose-related decrease was seen in mean bodyweight of the mid- and high-dose males and high-dose females in the F_0 and F_1 generations. Feed consumption was decreased by 12-26% in high-dose males of the F_0 and F_1 generations. The aggregate mean number of live male pups born to F_0 parents across was decreased by 21% in the high-dose group. There were also decreases in overall male, female and combined F_2 pup weights. In the 230 mg/kg-dosed and 700 mg/kg-dosed males and 700 mg/kg-dosed females, there were signs of general toxicity as noted by hyperkaeratosis and hyperplasia in non-glandular stomachs and decreased body weight. Mild reproductive toxicity at 700 mg/kg was indicated by decreased male and female pup weights and by decreased number of male pups per litter during F_0 cohabitation (Layton *et al.*, 2001).

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indicated by decreased male and female pup weights and by decreased number of male pups per litter during F_0 cohabitation (Layton *et al.*, 2001).

Conclusion:

Studies in single or multiple generations of rats, have shown that the NOAEL for developmental toxicity was 500 mg/kg/day. Higher doses produced delayed skeletal ossification and *in utero* growth retardation. However these effects were seen at intake levels superior to those producing maternal toxicity. This maternal toxicity was observed at 700 mg/kg/day. In males, similar effects were seen at a lower dose of 230 mg/kg/day but not at the next lowest dose of 70 mg/kg/day. The appearance of hyperkaeratosis and hyperplasia in non-glandular stomachs and decreased body weight may be due to the route of administration whereby the test material is administered by intubation thereby delivering a bolus of a substance that is shown to have clearly irritant properties at high concentrations.

5.2.8.2 Other routes

No data are available.

5.2.9 Toxicokinetics

5.2.9.1 Biotransformation

Studies on the biotransformation of isoeugenol following intravenous and oral administration to rats (Badger *et al.*, 2002) have shown that isoeugenol is metabolized by direct sulphation and glucuronidation of the phenolic hydroxyl group. The distal carbon of the propenyl group is also hydroxylated and this metabolite is sulphated and may be subsequently methylated. These pathways are shown in Figure 1.

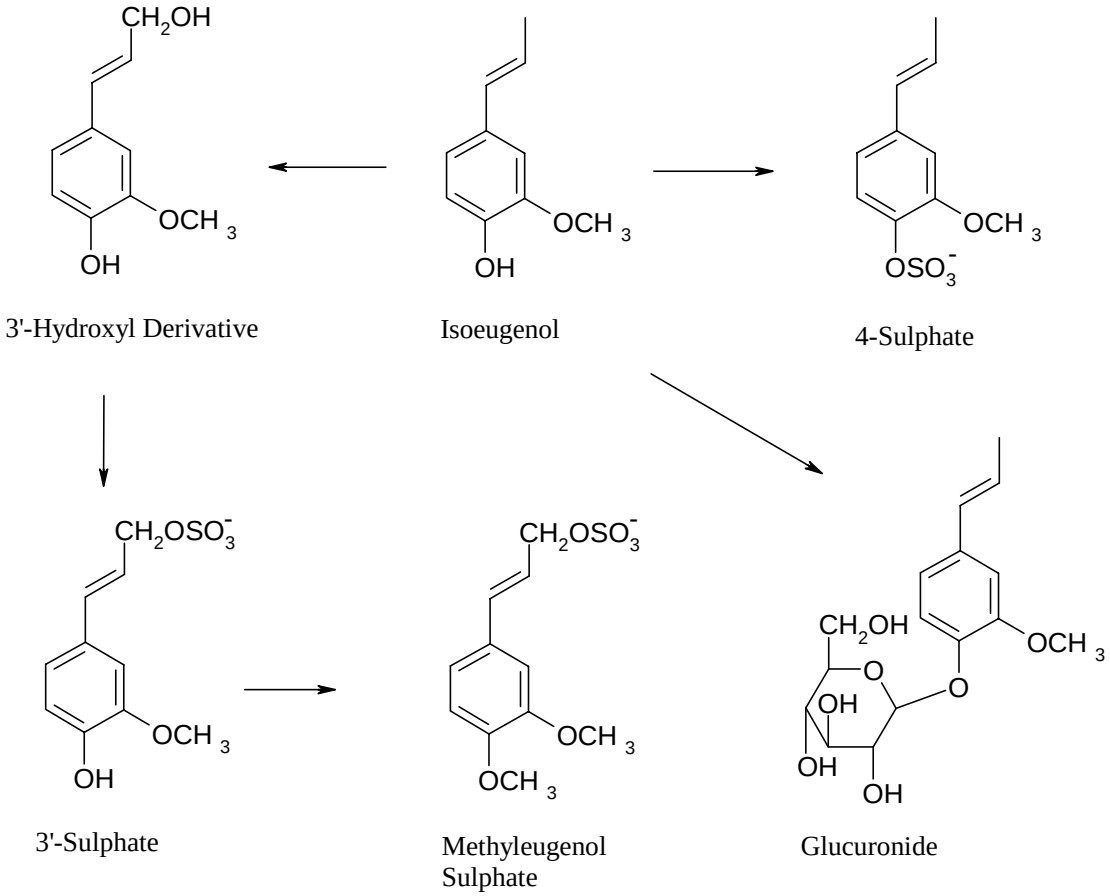

Figure 1. Putative metabolic scheme for isoeugenol following oral administration after Badger et al.

After oral administration, the major urinary metabolites were found to be the 4-sulphate (33.8%) followed by the glucuronide (20%) and the 3'-sulphate (19.1%) (Badger *et al.*, 2002).

5.2.9.2 In vitro studies – Skin penetration

In a study on freshly excised human skin, a dose of 92.2 mg/cm2 radio-labelled isoeugenol (184 mg/cm3) was applied to the surface of the skin and radioactivity was measured in the skin, on the

surface of the skin and in receptor fluid. The recovery of radioactivity in the receptor fluid after 72 hours was 30% and an additional 8.4% was detected in the skin. Total uptake was 38.4% and total recovery was 60% (Liu and Hotchkiss, 1997b).

5.2.9.3 Studies in animals

a) Inhalation route:

No data are available.

b) Dermal route in rats:

Skin penetration studies

A single application of radio-labelled isoeugenol to the skin of rats showed absorption after 24 hours of between 36.6% and 48.7% of the applied dose. A total of 25% of applied dose was recovered as radioactive urinary metabolites (Liu and Hotchkiss, 1997b).

Toxicokinetics

The relative extent of sulfation and glucuronidation of the major urinary metabolites of isoeugenol was evaluated in the *in vivo* skin absorption study reported above. At a dose of 2.6 mg/cm^2, sulphate conjugates accounted for 88.9% of the urinary metabolites while 11.1% were glucuronide conjugates. The sulfate-conjugated metabolites were 42.1% of conjugated isoeugenol, 4.6% of conjugated 3,4-dihydroxypropylbenzene, 3.5% of conjugated 3,4-diyhdroxyallylbenzene and about 50% unknowns. Unchanged isoeugenol accounted for under 1% (Liu and Hotchkiss, 1997a).

c) Oral route in rats:

Toxicokinetics

An oral toxicokintetic study carried out under the US National Toxicology Program has been reported as a summary (Fuciarelli *et al.*, 2001). After administration of a single 150 mg/kg dose of test material in corn oil to female Fischer 344/N animals, there was no evidence for saturation of isoeugenol metabolism in female rats by the gavage route of administration. Cmax was 2.64 (µg/mL). The area under the curve (AUC) was 5.93 (µg-hr/mL at 8 hours). Plasma concentration-versus time profiles for isoeugenol revealed that isoeugenol is absorbed rapidly (the absorption half-life was approximately 15-20 minutes post-dose) from the gastrointestinal tract. Although saturation of metabolism was not determined by comparing the pharmacokinetics at different dose levels, the short half-life provided evidence that lack of saturation occurred at 150 mg/kg . Administration of 37 mg/kg isoeugenol to another ten female rats gave a maximum plasma concentration (Cmax) of 1.07 (µg/mL) and the AUC was 1.57 (µg-hr/mL at 8 hours). Bioavailability was approximately 20% (Fuciarelli *et al.*, 2000).

In another study, also carried out under the US National Toxicology Program and reported only as a summary, twenty-one male and female Fischer 344 rats were given three different doses of

isoeugenol as a single gavage bolus in corn oil. At doses of 140, 70, 35 and 17 mg/kg, the time variation of the concentration in plasma of isoeugenol was characterized by an early absorption phase occurring within 5-20 minutes post dosing followed by at least one secondary peak which prevented estimation of toxicokinetic parameters. The authors did not indicate whether this may have been due to entero-hepatic recycling. Maximum plasma levels (Cmax) values increased with dose. The areas under the curves (AUCs) were significantly higher for females than males but increased supraproportionately with dose for both sexes. At doses of 140, 70 and 35 mg/kg, the clearance [Cl(app)] values were significantly greater for males as compared to females and also increased portionately with dose for both sexes. Bioavailability at all three doses was significantly different between the sexes [10.5%/16.5% (Males/Females)]. At the lowest dose of 17 mg/kg bioavailability was also significantly greater in female rats (17%) as compared to male rats (11%). Overall, low bioavailability was evident at all doses and only a small amount of the administered dose reached systemic circulation. Isoeugenol was rapidly cleared from circulation suggestive of extensive metabolism and/or excretion (Fuciarelli *et al.*, 2001).

In other more completely reported studies, gavage administration of 156 mg/kg [^{14}C]-Isoeugenol (μCi/kg) in corn oil to male Fischer 344-rats, led to approximately 10% of the administered dose being recovered in the faeces (possibly following absorption and then biliary excretion), less than 0.1% was recovered as CO_2 or expired organics and less than 0.2% was detected in selected tissues. About 85% was detected in urine; a level that was reached after 24 hours. No parent Isoeugenol was detected in the blood at any to the time-points analysed. Incubation of urine samples with B-glucuronidase caused dramatic peak shifts in the HPLC profile (Badger *et al.*, 1999). Results of this study reported in greater detail in a subsequent publication show that isoeugenol is rapidly metabolized and is excreted predominantly in the urine as phase II conjugates of the parent compound (Badger *et al.*, 2002).

d) Oral route in mice:

In another study, also carried out under the US National Toxicology Program and reported only as a summary, administration of a single 150 mg/kg dose of test material in corn oil to female B6C3F1 animals, produced no evidence for saturation of isoeugenol metabolism in female rats by the gavage route of administration. Maximum plasma level (Cmax) was 5.43 μg/mL and the area under the curve (AUC) was 9.48 μg-hr/mL at 8 hours. Plasma concentration-versus time profiles for isoeugenol revealed that isoeugenol is absorbed rapidly (the absorption half-life was approximately 15-20 minutes post-dose) from the gastrointestinal tract. Administration of 37 mg/kg isoeugenol to another group of female mice gave a maximum plasma concentration (Cmax) of 2.18 μg/mL and an AUC of 1.86 μg-hr/mL at 8 hours. Bioavailability was approximately 20% (Fuciarelli *et al.*, 2000).

In another study, also carried out under the US National Toxicology Program and reported only as a summary, forty-two male and female B6C3F1 mice were given three different doses of isoeugenol as a single gavage bolus in corn oil. At doses of 140, 70 and 35 mg/kg, the time variation of the concentration in plasma of isoeugenol was characterized by an early absorption phase occurring within 5-20 minutes post dosing followed by at least one secondary peak which prevented estimation of toxicokinetic parameters. AUCs were significantly higher for females than males but increased proportionately with dose for both sexes. The clearance [Cl(app)] values were significantly greater for males as compared to females. Overall, low bioavailability revealed that only a small amount of the administered dose reached systemic circulation, and the compound was rapidly cleared from circulation suggestive of extensive metabolism and/or excretion.

Bioavailability at a dose of 35 mg/kg was not significantly different between the sexes [33.9%/36.2% (M/F)] (Fuciarelli *et al.*, 2001).

e) Intravenous route in rats:

An intravenous toxicokintetic study carried out under the US National Toxicology Program has been reported as a summary (Fuciarelli *et al.*, 2001). Intravenous dosing of 37mg/kg isoeugenol in Fischer 344/N rats revealed that elimination of isoeugenol was bi-phasic exhibiting a rapid initial distribution and slower terminal elimination phase. Terminal half-life was estimated as 2.4 hours. Total clearance (Cltot) was estimated as 4900 mL/hr-kg and the area under the time/plasma concentration curve (AUC) was 7.6 μg-hr/mL for elimination of isoeugenol from rat plasma. Vss for isoeugenol in rat plasma was 3910 mL/kg (Fuciarelli *et al.*, 2001).

In another study, male and female Fischer 344 rats were dosed intravenously with 17 mg isoeugenol/kg, the time variation of the concentration in plasma of isoeugenol was biphasic as in the above study. The estimates of toxicokinetic parameters following intravenous administration were CO of 10.8/10.9 (males/females) μg/mL; Vapp of 11.0/12.0 (males/females) L/kg; tl/2-alpha of 7.98/7.54 (males/females) min; tl/2-beta of 69.1/79.5 (males/females) min; systemic clearance Cl of 110/105 (males/females) mL/min-kg and AUC of 155/162 (males/females) ug-min/mL. No significant differences between the sexes in rats were observed. Values are indicative of distribution to extravascular tissues, high tissue uptake and high tissue binding; and/or extensive first-pass metabolism (Fuciarelli *et al.*, 2001).

Following administration of 15.6 mg/kg [^{14}C]-Isoeugenol to male Fischer-344 rats, (100 μCi/kg) to male Fischer-344 rats, the parent substance disappeared rapidly from the blood. By 24 hours, it was found that 10% of administered radiocarbon was recovered in the faeces. A total of 82% of the administered radioactivity was excreted into the urine by this time. After 72 hours, less than 0.1% was recovered as CO2 or expired organics, and less than 0.2% was detected in selected tissues. At the first measurement (about 2 minutes after administration), 10.5% of the administered radiocarbon dose but only 7% of parent isoeugenol was detected in the blood. Both of these diminished rapidly so that levels of parent isoeugenol in the blood had diminished to below 1% after 60 minutes. The half-life in blood ($t_{1/2)}$ was 12 minutes. Systemic clearance was 1.9 L/min/kg and the mean residence time of 11.6 minutes indicate that isoeugenol is rapidly eliminated from the blood of rats. Excretion characteristics were similar to those of oral administration. The total amount of radioactivity remaining in selected tissues by 72 h was less than 0.25% of the dose following either oral or intravenous administration. Results of this study show that isoeugenol is rapidly metabolized and is excreted predominantly in the urine as phase II conjugates of the parent compound (Badger *et al.*, 1999).

f) Intravenous route in mice:

In another study reported only as an abstract, plasma concentration-versus-time profiles after intravenous dosing ten female B6C3F1 animals with 37mg/kg isoeugenol revealed that elimination of isoeugenol was bi-phasic exhibiting a rapid initial distribution and slower terminal elimination phase. Estimates of the terminal half-life, (Total clearance Cltot) and the area under the time/plasma concentration curve (AUC) were 3.3 hours, 4000 mL/hr-kg and 9.2 μg-hr/mL for elimination of isoeugenol from mouse plasma. Vss for isoeugenol in mouse plasma was 2000 mL/kg (Fuciarelli *et al.*, 2000).

In another study, male and female B6C3F1 mice were dosed intravenously with 35 mg isoeugenol/kg, the time variation of the concentration in plasma of isoeugenol was biphasic as in the above study. The estimates of toxicokinetic parameters following IV administration were: CO of 17.2/18.1 (males/females) µg/mL; Vapp of 25.2/16.0 (males/females) L/kg; tl/2-alpha of 7.95/10.4 (males/females) min; tl/2-beta of 118/102 (males/females) min; Cl of 148/108 (males/females) mL/min-kg and AUC of 237/323 (males/females) ug-min/mL. The differences between the sexes in mice observed for Cl and AUC were statistically significant. These values are indicative of distribution to extravascular tissues, high tissue uptake and high tissue binding; and/or extensive first-pass metabolism (Fuciarelli *et al.*, 2001).

5.2.9.4 Other studies

None were found.

Conclusion

Studies on the metabolism of isoeugenol show that sulphate conjugation and glucuronidation followed by excretion into the urine, constitute the major route of elimination. By all routes studied, elimination is rapid and extensive. In oral toxicokinetic studies, there is no evidence of saturation of metabolism which was rapid and led to a low bioavailability of parent isoeugenol with only a small proportion (down to 10% in male rats) reaching the systemic circulation. Slightly higher bioavailability was seen in mice.

5.2.10 Neurotoxicity

Studies were carried out on spontaneous motor activity and catatonia in mice. Isoeugenol in corn oil had no effects at the tested doses of 100 mg/kg and 200 mg/kg (deMello and Carlini, 1973). Administration of isoeugenol in corn oil by injection had no effects on rope climbing performance in male Wistar rats at 10, 40 and 80 mg/kg but at 160 mg/kg non-specific effects including severe depression and paralysis of the hindquarters were observed (deMello and Carlini, 1973).

Conclusion

Intravenous injection of isoeugenol produced effects at 160 mg/kg that may be ascribed to general toxicity or neurotoxicity.

5.2.11 Endocrine assays

Isoeugenol gave no 17-β-estradiol mimetic effects in an *in vitro* assay that uses a strain of Saccharomyces cerevisiae whose genome has been modified by the incorporation of the DNA sequence of the human oestrogen receptor and expression plasmids (Miller *et al.*, 2001).

Isoeugenol (at a concentration of 16.4 mg/L) failed to give any indication of potential endocrine-receptor binding capacity in another study on genetically modified Saccharomyces cerevisiae into which two expression plasmid had been incorporated (Nishihara *et al.*, 2000).

Isoeugenol was considered to be a "non-binder" and did not displace estradiol when tested at a concentration of 164 µg/L in an *in vitro* system consisting of the uterine cytosol of Sprague-Dawley rats to which radio-labelled 17-beta-estradiol had been added for competitive binding studies (Blair.R.M. *et al.*, 2000). In a competitive binding assay with methyltrienolone for a recombinant rat androgen receptor, the IC_{50} values for isoeugenol (for inhibition of tritiated methyltrienolone) was found to be 0.0002 and the relative binding activity was 0.0015 % placing

isoeugenol among the weak binders (estradiol had an RBA over 500 times greater) (Fang *et al.*, 2003).

Conclusion

Isoeugenol gave negative results in several assays but showed very weak competitive binding properties to an androgen receptor. These tests systems are still experimental and their significance to true endocrinal effects is not known. None the less, other studies (section **5.2.9**) showed that isoeugenol did not adversely interfere with reproduction and development in a multi-generation reproduction study.

5.2.12 Cytotoxicity

A number of *in vitro* studies point to the intrinsic cytotoxicity of isoeugenol. Four *in vitro* measures of cytotoxicity have been rated for isoeugenol (effects on oxidative mechanism in fat cells, on ciliary activity of tracheal cells, on cell growth of Ascites sarcoma cells and membrane damage to lung fibroblasts) to show that this substance is significantly cytotoxic (Curvall *et al.*, 1984).

A 71% inhibition of noradrenaline-induced respiration in isolated hamster brown fat cells infused with a 1 millimolar solution (164 mg/l) of isoeugenol was observed (Pettersson *et al.*, 1982).

A five millimolar concentration of isoeugenol (820 mg/l) produced ciliostasis in chicken embryo tracheal organ cultures within 6 minutes (Pettersson *et al.*, 1982).

Inhibition of mouse cell growth by isoeugenol was shown in Ascites sarcoma BP 8 cells. Although 1 millimolar (164 mg/l) solutions of isoeugenol showed 96% inhibition, 0.1 millimolar solutions (16.4 mg/l) showed only 13% inhibition which was not statistically significant (at $p>0.001$) (Pilotti *et al.*, 1975).

In another study, plasma membrane damage caused by 25 millimolar (4.1 g/l) isoeugenol, assessed as leakage of a cytoplasmic nucleotide marker from pre-labelled human diploid embryonic lung fibroblasts was above 90% of maximal possible release (Thelestam *et al.*, 1980).

The cytotoxicity of isoeugenol to hepatocytes isolated from male Fischer 344 rats and female B6C3F1 mice was evaluated by measuring release of lactate dehydrogenase. The LC50 values calculated as micromole/L were on the region of 200 and 300 (35.2 - 52.8 mg/l) This has been ascribed to the formation of a quinone methide derivative (Burkey *et al.*, 2000;Bertrand *et al.*, 1997). If phase II conjugation reactions were to become overwhelmed following administration of isoeugenol, levels of the reactive quinone methide metabolite of isoeugenol would increase, and might explain the presence of acute systemic toxicity at highly exaggerated doses (Badger *et al.*, 2002).

Conclusion

The cytotoxicity of isoeugenol is consistent with observations reported on similar phenolic substances. The levels used in these *in vitro* studies are difficult to link to doses administered in *vivo*.

5.3 Risk Characterisation

5.3.1 Hazard Summary

Isoeugenol shows moderate acute toxicity by the oral and dermal routes and is classified as harmful by these routes according to the criteria outlined in the European Dangerous Substances Directive. Isoeugenol does however, display moderate cytotoxicity and it is possible that if phase II conjugation reactions were to become overwhelmed following administration of isoeugenol, levels of reactive metabolites such as quinone methides would increase, and might explain the presence of acute systemic toxicity at highly exaggerated doses.

Undiluted isoeugenol is irritating to the skin and eyes of animals and is classified as an irritant to skin and eyes according to the official criteria. However, there is sound evidence to show that when diluted to concentrations of 8%, no dermal irritation is observed.

Studies *in vitro* show that about 50% of the administered dose of isoeugenol is absorbed into or passed through the skin in 24 hours. A conservative estimate of the dermal penetration constant is 0.8×10^{-5} cm/h.

The key hazard shown by isoeugenol is its skin sensitization potential. This is manifested in a wide variety of predictive test systems. Patch tests carried out on dermatitic patients have indicated that acquired allergy to isoeugenol is widespread. However, these clinical studies were carried out under conditions that are predisposed to detecting allergies that may not manifest themselves in the normal use of consumer products (Hostynek and Maibach, 2004). With a few exceptions, these clinical studies did not establish an unambiguous role of isoeugenol as the cause of the patients' dermatitis (Hostynek and Maibach, 2003c).

Although isoeugenol shows some marginal photo-toxic effects in un-validated *in vitro* systems using UV-B, isoeugenol is not photo-toxic to human skin in the presence of UV-A. Predictive photo-allergenicity screens in humans were negative. One clinical study has given positive photo-patch results in poly-reactive patients but no causal relationship was confirmed.

Studies on the metabolism of isoeugenol show that in the dermal route, sulphate conjugation is the major route of biotransformation. In oral toxico-kinetic studies in mice and rats, metabolism was rapid with no evidence of saturation of metabolism at doses of over 100 mg/kg. Metabolism by both routes was characterised by a low bioavailability with only a small proportion (down to 10% in male rats) reaching the systemic circulation. Slightly higher bioavailability was seen in mice.

Isoeugenol is not considered to be genotoxic. It is consistently negative in standard bacterial screens. In a DNA-repair test in Bacillus subtilis, isoeugenol was negative in one study but positive in another under circumstances where artifacts may have arisen Studies in standard mammalian cell systems were negative. One study showed a moderate increase in sister chromatid exchanges in human lymphocytes although here too, this may be explained as an artifact of the methodology and cytotoxic doses used.

Insufficient data are available from orthodox systemic toxicity studies to allow the determination of a clear no observed adverse effect level for isoeugenol. Two 16-week feeding studies carried

out over 30 years ago show that levels of 800 mg/kg/day in the diet are well tolerated. In view of the limitations of this study and bearing in mind recent considerations of structure-based thresholds of toxicological concern (Kroes *et al.*, 2004), to assume with confidence that the true NOEL for systemic exposure to isoeugenol by the oral route falls above a certain conservative value. Isoeugenol has a chemical structure corresponding to Class I in the "Decision Tree" procedure of (Cramer *et al.*, 1978). The 5th percentile of NOELs of a large number of similarly classified chemicals gives such a threshold, which with a 300 fold safety factor in relation to limitations of these animal tests in relation to human exposure, gives a threshold of toxicological concern (TTC) for isoeugenol of 1800 μg/capita/day (30 μg/kg bw/day). An indication of the NOAEL for systemic toxicity has been obtained from a multi-dose reproduction toxicity study where histological changes in the non-glandular stomachs and decreased body weight were seen in rats at a dose of 230 mg/kg but not at 70 mg/kg. While these effects may be due to the test procedure involving intubation of a bolus of an irritant directly into the forestomach, it may be conservatively be considered that the No Observed Adverse Effect Level (NOAEL) of 70 mg/kg/day

Single-generation and multiple-generation studies have shown that the NOAEL for developmental toxicity was 500 mg/kg/day. Higher doses produced delayed skeletal ossification and *in utero* growth retardation. However these effects were seen at intake levels superior to those producing maternal toxicity.

5.3.2 Exposure summary

Based on information from the Habits and Practices tables, it can be concluded that skin exposure for topical effects and for systemic toxicity resulting from the use of isoeugenol in household laundry and detergent products is the major route of exposure to isoeugenol. Using the algorithms recommended in the HERA methodology document it has been estimated that *ca*. 75% of systemic body burden from the use of these products (1 x 10^{-3} μg/kg bw/day) results from dermal absorption, resulting almost entirely from direct skin contact of concentrated or diluted detergent products. Highly conservative estimates of oral intake of isoeugenol in food and drinking water or from residues present on eating utensils and crockery give a value of nearly 3 x 10^{-4} μg isoeugenol/kg bw/day. Inhalation of isoeugenol from detergent powder dusts or to aerosol sprays will give rise to only 1.5 x 10^{-6} μg isoeugenol/kg bw/day. This represents an extremely minor fraction of overall systemic exposure. A highly conservative estimate of aggregate systemic exposure has been calculated as 0.0014 μg/kg bw/day (1.4 x 10^{-3} μg/kg bw/day).

For topical effects, the highest anticipated exposures will be 0.7 μg/cm^2 arising from the use of liquid detergents in laundry pre-treatment or from accidental or unintentional exposure.

5.3.3 Rational for identification of critical endpoints

Dermal exposure is the main exposure route for consumers and consequently it is necessary for human risk assessment to consider direct dermal effects such as skin irritation and sensitization as well as systemic toxicity due to dermally absorbed isoeugenol. There is a substantial amount of data available for assessing the skin irritation and skin sensitization potential of isoeugenol and for assessing the risks associated with these effects due to the use of consumer product formulations containing isoeugenol. Exposure levels are too low in household cleaning products for isoeugenol to contribute significantly to irritant effects. However, the possibility that allergic contact sensitization might be produced by low-level exposures to isoeugenol coupled with a background of numerous reports of clinical allergy to this substance justify attribution of this effect as a critical endpoint.

Dermal penetration studies with excised skin have shown that isoeugenol have shown that isoeugenol has the potential to penetrate the skin and become systemically available. There are no long-term, systemic toxicity studies using the dermal route. Adequate repeat dose studies by the oral route are also lacking at this time. However, systemic effects after dermal exposure can also be assessed using some conservative assumptions. On the basis of effects seen in a multi-dose reproduction toxicity study rats, a no effect level can be obtained. A lower limit for systemic toxicological concern can also be obtained from recent data-based theoretical approaches.

No other critical endpoints were identified. Isoeugenol was not considered to be mutagenic or genotoxic on the weight of evidence. Studies on the teratogenicity, embryotoxicity and toxicity to reproduction caused by isoeugenol show that maternal toxicity is not observed at doses above those which produce adverse effects in their male partners. Delayed development was observed in rodents, but only at doses above those already giving rise to maternal toxicity.

5.3.4 Quantitative evaluation of data – No effect levels

Skin sensitisation:

Using a "weight-of-evidence" approach, the No Expected Sensitisation Level (NESL) for isoeugenol derived from a large number of studies carried out in animals and human volunteers has been determined to be **250 μg/cm^2** (see **Appendix 1**). Attempts have been made to define elicitation thresholds in subjects who have already been sensitized to isoeugenol (see **Appendix 2**). However, there is convincing evidence that these levels are themselves subject to a number of variable factors that are more artefacts of their measurement than true no-effect-levels that can be used in risk assessment (see **Appendix 3**).

Systemic effects: An old chronic feeding study in rats shows that levels around 400 mg/kg/day in the diet are well tolerated. However, this study is inadequate. In a multi-dose reproduction toxicity study, hyperkaeratosis and hyperplasia in non-glandular stomachs and decreased body weight were seen at a dose of 230 mg/kg but not at 70 mg/kg. While these effects may be due to the test procedure involving intubation of a bolus of an irritant directly into the forestomach, it may be conservatively be considered that the No Observed Adverse Effect Level (NOAEL) of **70 mg/kg/day (70'000 μg/kg/day)**.

5.4 Risk Assessment

5.4.1 Margin of Exposure calculations

5.4.1.1 Margin of exposure: for contact allergy (skin sensitization)

Taking the No Expected SensitisationLevel (NESL) for isoeugenol as **250 μg/cm^2** (Section 5.3.4.), it is possible to determine Margins of Exposure (MOE_{sens}) using the dermal exposure estimates from Section 5.1.3. These exposure estimates are all based on exceptional "worst-case" scenarios. Direct exposure from product use assumes that the consumer does not take normal precautions to rinse or wipe hands after use. Isoeugenol is assumed to remain on the skin after use and hands have dried. Indirect and accidental exposures are also assumed to be the result of highly unlikely scenarios. For this reason, it is reasonable to neglect the additional effects of multiple uses of the same or different products over this period.

The Margins of Exposure are as follows:

5.4.1.1.1 Exposure scenario: Direct skin contact

A. Hand-washed laundry. The $MOE_{sens}$was calculated by dividing No Expected Sensitizing Level (NESL) of **250 μg/cm^2** by the estimated exposure from hand washing detergents of **0.007 μg/cm^2**

MOE_{sens} = **250 μg/cm^2 /0.007 μg/cm^2. = > 35,000**

B. Pre-treatment of clothes (liquid detergent). The $MOE_{sens}$was calculated by dividing the No Expected Sensitizing Level (NESL) of **250 μg/cm^2** by the estimated exposure from pre-treatment of clothes using a liquid detergent of **0.7 μg/cm^2**.

MOE_{sens} = **250 μg/cm^2 /0.7 μg/cm^2.. = > 350**

C. Hand dish-washing. The $MOE_{sens}$was calculated by dividing the No Expected Sensitizing Level (NESL) of **250 μg/cm^2** by the estimated exposure from hand dish-washing of **1 x 10^{-4} μg/cm^2**.

MOE_{sens} = **250 μg/cm^2 /1 x 10^{-4} μg/cm^2 = 2,500,000**

D. Hard surface cleaning. The $MOE_{sens}$was calculated by dividing the No Expected Sensitizing Level (NESL) of **250 μg/cm^2** by the estimated exposure from hard surface cleaning of **4.8 x 10^{-3} μg/cm^2**.

MOE_{sens} = **250 μg/cm^2 /4.8 x 10^{-3} μg/cm^2 = > 50,000**

5.4.1.1.2 Exposure scenario: Indirect skin contact

From wearing clothes. The $MOE_{sens}$was calculated by dividing the No Expected Sensitizing Level (NESL) of **250 μg/cm^2** by the estimated exposure from wearing clothes of 5.6 x 10^{-11} μg/cm^2.

MOE_{sens} = **250 μg/cm^2 / 5.6 x 10^{-11} μg/cm^2 = > 4 x 10^{12}**

5.4.1.2 Accidental or intentional over-exposure

The MOE_{sens} was calculated by dividing the No Expected Sensitisation Level (NESL) of **250 μg/cm^2** by the estimated exposure from accidental over-exposure of **0.7 μg/cm^2**.

MOE_{sens} = **250 μg/cm^2 /0.7 μg/cm^2 = > 350**

5.4.1.3 Margin of exposure: Systemic effects

For systemic effects from exposure to isoeugenol, two measures are used in this risk assessment:

(a) the No Observed Effect Level from a reproductive toxicity study of **70,000 μg/kg bw/day;**

(b) the Threshold of Toxicological Concern (TTC) of **30 μg/kg bw/day***

***** Threshold of Toxicological Concern already incorporates a 300-fold safety factor.

The Margins of Exposure (MOE) are as follows:

5.4.1.3.1 Exposure scenario: Direct skin contact from hand-washed laundry

The MOE was calculated by dividing the NOAEL from a reproductive toxicity study of **70,000** μg/kg bw/day and the Threshold of Toxicological Concern of **30** μg/kg bw/day by the systemic dose of **9.3 x 10^{-5}** μg/kg bw/day estimated as exposure from hand washing detergents.

MOE$_{\text{from NOAEL}}$ = **7 x 10^4 / 9.3 x 10^{-5}= 7.5 x 10^8**

MOE$_{\text{from TTC}}$ = **30 / 9.3 x 10^{-5}= 3.2 x 10^{5*}**

5.4.1.3.2 Exposure scenario: Direct skin contact from pre-treatment of clothes

The MOE was calculated by dividing the NOAEL from a reproductive toxicity study of **70,000** μg/kg bw/day and the Threshold of Toxicological Concern of **30** μg/kg bw/day by the systemic dose of **9.3 x 10^{-4}** μg/kg bw/day estimated as exposure from pre-treatment of clothes using a paste detergent.

MOE$_{\text{from NOAEL}}$ = **7 x 10^4 /9.3 x 10^{-4} = 7.5 x 10^7**

MOE$_{\text{from TTC}}$ = **30 /9.3 x 10^{-4} = 2.1 x 10^{4*}**

5.4.1.3.3 Exposure scenario: Direct skin contact from hand dish-washing

The MOE was calculated by dividing the NOAEL from a reproductive toxicity study of **70,000** μg/kg bw/day and the Threshold of Toxicological Concern of **30** μg/kg bw/day by the systemic dose of **1.2 x 10^{-5}** μg/kg bw/day estimated as exposure from hand dish-washing.

MOE$_{\text{from NOAEL}}$ = **7 x 10^4 /1.2 x 10^{-5} = 5.8 x 10^9**

MOE$_{\text{from TTC}}$ = **30 /1.2 x 10^{-5} = 2.5 x 10^{6*}**

5.4.1.3.4 Exposure scenario: Direct skin contact from hard surface cleaning

The MOE was calculated by dividing the NOAEL from a reproductive toxicity study of **70,000** µg/kg bw/day and the Threshold of Toxicological Concern of **30** µg/kg bw/day by the systemic dose of **1.8×10^{-5}** µg/kg bw/day estimated as exposure from hard surface cleaning.

$MOE_{\text{from NOAEL}} = 7 \times 10^{4} / 1.8 \times 10^{-5} = 3.9 \times 10^{9}$

$MOE_{\text{from TTC}} = 30 / 1.8 \times 10^{-5} = 1.7 \times 10^{6*}$

5.4.1.3.5 Exposure scenario: Indirect skin contact from wearing clothes

The MOE was calculated by dividing the NOAEL from a reproductive toxicity study of **70,000** µg/kg bw/day and the Threshold of Toxicological Concern of **30** µg/kg bw/day by the systemic dose of **1.5×10^{-7}** µg/kg bw/day estimated as exposure from wearing clothes.

$MOE_{\text{from NOAEL}} = 7 \times 10^{4} / 1.5 \times 10^{-7} = 4.7 \times 10^{11}$

$MOE_{\text{from TTC}} = 30 / 1.5 \times 10^{-7} = 2 \times 10^{8*}$

5.4.1.3.6 Exposure scenario: Aggregate Direct & Indirect skin contact

In a worst-case scenario, the aggregate consumer exposure from dermal penetration after all of the above scenarios does not exceed **1.06×10^{-3}** µg/kg bw/day**.** The MOE was calculated by dividing the NOAEL from a reproductive toxicity study of **70,000** µg/kg bw/day and the Threshold of Toxicological Concern of **30** µg/kg bw/day by this aggregate dose.

$MOE_{\text{from NOAEL}} = 7 \times 10^{4} / 1.06 \times 10^{-3} = 6.6 \times 10^{7}$

$MOE_{\text{from TTC}} = 30 / 1.06 \times 10^{-3} = 2.8 \times 10^{4*}$

5.4.1.3.7 Exposure scenario: Indirect exposure by oral route from food and drinking water

The MOE was calculated by dividing the NOAEL from a reproductive toxicity study of **70,000** µg/kg bw/day and the Threshold of Toxicological Concern of **30** µg/kg bw/day by the systemic dose of **2.87×10^{-4}** µg/kg bw/day estimated as exposure from drinking water.

$MOE_{\text{from NOAEL}} = 7 \times 10^{4} / 2.87 \times 10^{-4} = 2.4 \times 10^{8}$

$MOE_{\text{from TTC}} = 30 / 2.87 \times 10^{-4} = 1 \times 10^{5*}$

5.4.1.3.8 Exposure scenario: Indirect exposure by oral route from dishwashing residues

The MOE was calculated by dividing the NOAEL from a reproductive toxicity study of **70,000** µg/kg bw/day and the Threshold of Toxicological Concern of **30** µg/kg bw/day by the daily systemic dose of **5×10^{-5}** µg/kg bw/day estimated as exposure from dishwashing residues.

$MOE_{\text{from NOAEL}} = 7 \times 10^{4} / 5 \times 10^{-5} = 1.4 \times 10^{9}$

$MOE_{\text{from TTC}} = 30 / 5 \times 10^{-5} = 6 \times 10^{5*}$

5.4.1.3.9 Aggregate of exposure by the oral route

The MOE was calculated by dividing the NOAEL from a reproductive toxicity study of **70,000** μg/kg bw/day and the Threshold of Toxicological Concern of **30** μg/kg bw/day by the daily aggregate oral exposure (from 5.4.1.3.7 and 5.4.1.3.8) of **3.37 x 10^{-4}** μg/kg bw/day estimated as exposure from dishwashing residues.

MOE$_{\text{from NOAEL}}$ = **7 x 10^{4} /3.37 x 10^{-4} = 2.1 x 10^{8}**

MOE$_{\text{from TTC}}$ = **30 /3.37 x 10^{-4} = 8.9 x 10^{4*}**

5.4.1.3.10 Exposure scenario: Indirect inhalation

The exposure estimates were **9.5 x 10^{-7}** μg/ kg bw/day from the inhalation of detergent dust and **6 x 10^{-7}** μg/kg bw/day from the inhalation of aerosols giving an aggregate inhalation exposure of **1.55 x 10^{-6}** μg/kg bw/day.

MOE$_{\text{from NOAEL}}$ = **7 x 10^{4} /1.55 x 10^{-6} = 4.5 x 10^{10}**

MOE$_{\text{from TTC}}$ = **30 /1.55 x 10^{-6} = 1.9 x 10^{7*}**

5.4.1.3.11 Exposure scenario: dermal route from accidental or intentional over-exposure

As this type of exposure would not be repeated for a significant number of times, the systemic MOE is meaningless.

5.4.1.3.12 Total Consumer Exposure

In a worst-case scenario, the aggregate consumer exposure from all of the above scenarios would be unlikely to exceed **1.7 x 10^{-1} μg/kg bw/day.** The MOE was calculated by dividing the NOAEL from a reproductive toxicity study of **70,000** μg/kg bw/day and the Threshold of Toxicological Concern of **30** μg/kg bw/day by the daily systemic dose of **1.4 x 10^{-3}** μg/kg bw/day estimated as exposure from all sources.

MOE$_{\text{from NOEL}}$ = **7 x 10^{4} /1.4 x 10^{-3} μg/kg = 5 x 10^{7}**

MOE$_{\text{from TTC}}$ = **30 /1.4 x 10^{-3} μg/kg = 2 x 10^{4}***

***** *Threshold of Toxicological Concern already incorporates a 300-fold safety factor.*

5.4.2 Risk characterization

5.4.2.1 Contact allergy

Cell-mediated (Type IV) contact allergy results from dermal exposure. It may be induced after a single exposure episode, although the likelihood of its acquisition is increased by multiple exposures and is dependent on the concentration of the allergen in the different products to which the skin is exposed (Marzulli and Maibach, 1974;Basketter *et al.*, 1997) although the true measure of "dose" for this effect is the quantity applied per unit area (Boukhman and Maibach,

2001;Roggeband *et al.*, 2001). For this reason, it is necessary to consider each individual exposure scenario as a separate occasion for inducing allergy to isoeugenol.

The skin of consumers will be exposed to isoeugenol in a repetitive fashion due to its presence in household laundry and cleaning products. All potential dermal exposure scenarios arising from the use and accidental miss-use of these products have been identified, quantified and assessed by comparing the estimated dermal exposures with the non-induction threshold doses determined from studies in human subjects and reinforced by studies carried out on animals. The Margin of Exposure (MOE_{sens}) for this sensitization induction dose resulting from the worst case of potential allergy-inducting exposure (accidental or intentional over-exposure) is still over 350.

This $MOE_{sens}$is large enough to account for the inherent uncertainty and variability of the hazard data on which it is based. The $MOE_{sens}$is based on worst-case exposure assumptions and a value for the No Expected Sensitisation Level is taken from studies that have exaggerated exposure conditions (e.g. use and duration of occlusion) relative to the anticipated econsumer exposure. The true maximum dermal exposure is probably significantly lower in real life than is presented here for a number of reasons. The material has significant solubility in water and will be even more soluble in aqueous solutions of cleaning products containing surfactants. It will generally be rinsed off the skin under normal conditions of anticipated use thereby reducing exposure to levels considerably below those used in the exposure calculations in section 5.1.

There are experimentally observable threshold doses below which the allergic state is not induced and also, once subjects have been sensitised, there are certainly threshold doses below which an allergic response is not elicited to the degree of producing clinically recognisable symptoms. As explained in **Appendix 3** however, the exposure level is only one of a multitude of factors that predispose prior-sensitised subjects to producing the clinical manifestations of allergic reactions. Furthermore, methods used to determine this critical threshold can be criticised (**Appendix 3**). For this reason, empirically observed non-elicitation levels are not reliable indicators for risk assessment.

Although, numerous cases of positive patch test reactions to isoeugenol in dermatitic patients are recorded, none have been specifically linked to the use of laundry or cleaning products. In a multi-centre study involving 738 patients suffering from contact dermatitis, little evidence that aqueous solutions of 0.1% granular or liquid laundry detergents were able (even after occlusion for 48 hours in special occlusive chambers) were able to elicit the patients' contact allergies (Belsito *et al.*, 2002). None-the-less, allergy to isoeugenol is not uncommon (**Section 5.2.4.4: Tables 20-24**) but is probably at a “sub-clinically” low level with the result that allergic reactions will only be manifested at exposure levels that are higher than those that result from ordinary daily exposure to consumer goods (Hostynek and Maibach, 2004). A significant number of consumers using these types of products can therefore be expected to be already sensitized to isoeugenol due to other causes. It has been shown that some of these may react to doses of isoeugenol as low as 80 $\mu g/cm^2$ in open non-occluded exposure situations as found with the use of these products (**Appendix 2**). Even if this was to be a reasonable no-effect level for elicitation, there would still be a $MOE_{sens}$of greater than one between this and the exposure dose resulting from direct skin contactresulting from accidental or intentional over-exposure (hardly a regular twice-daily event). However, for various reasons (see **Appendix 3**), there is still insufficient knowledge relating to thresholds for elicitation, to be able to make proper risk assessments for this effect. It is also clear that if the risk of induction is adequately managed, the assessment of the risk of elicitation becomes unnecessary.

In summary, the use of isoeugenol in consumer products such as laundry and other household cleaning products does not raise any safety concerns with regard to the induction of contact allergy. Although it is not possible in theory to exclude the likelihood that pre-existing allergies to

isoeugenol may be elicited in exquisitely sensitive subjects as a result of the use of some of these laundry and cleaning products, these cases should be extremely rare and would be obviated by adequate risk management of induction.

5.4.2.2 Systemic effects

Inadequate data exist for establishing a reliable No Observed Adverse Effect Level (NOAEL) for long-term systemic exposure to isoeugenol. Two measures have been employed as substitutes for this. One is a conservative No Effect Level obtained from a reproductive toxicity study. The other is a base-line Toxicological Threshold of Concern (TTC) based on the classification of the chemical structure of isoeugenol with regard to a large body of NOAELs for similarly-classified chemicals.

Consumers are exposed to isoeugenol through its use in laundry and cleaning products. All significant potential exposure scenarios were identified and quantified and assessed by comparing theses estimated maximum exposures with these two measures (NOEL and TTC). The Margin of Exposure (MOE) for an aggregate of all possible routes of consumer exposure is above ten million for the NOAEL from a reproductive toxicity study and over ten thousand for the TTC (which incorporates a safety factor of 300). This MOE calculation represents the total of all possible exposure scenarios using worst-case assumptions, an exposure situation that is very unlikely to occur in real life.

The determined MOEs using both measures are certainly large enough to account for the inherent uncertainty and variability of the hazard data on which it is based. The MOE derived from the NOEL from an old gavage study which itself may have produced effects due to its manner of exposing sensitive rodent gastric tissues to a bolus of isoeugenol; a material with known irritant properties. The true consumer exposure is probably significantly lower than presented here particularly as isoeugenol has significant solubility in water and will be even more soluble in cleaning products containing surfactants. It will generally be rinsed off the skin under normal conditions of anticipated use thereby reducing exposure to levels considerably below those used here in the calculating the MOEs. Even under reasonably foreseeable conditions of miss-use, it is unlikely that these conditions will be repeated with the same daily rhythm as in the referenced repeated dose studies in rodents.

The available toxicological information indicates that isoeugenol is not mutagenic or genotoxic. There was no evidence of reproductive toxicity, developmental or teratogenic effects at doses that did not already cause lethal embryotoxicity.

An overwhelmingly large proportion of the total systemic isoeugenol exposure results from the percutaneous absorption of isoeugenol in applications involving transient skin contact. The percutaneous absorption of isoeugenol was measured in studies carried out *in vitro* on excised rat skin. This system is known to over-estimate absorption into and through human skin. The measures used to estimate probable penetration of isoeugenol assume conservatively but incorrectly that all material bound to or within the skin will eventually become bioavailable.

In summary, the use of isoeugenol in consumer products such as laundry and cleaning products does not raise any safety concerns with regard to systemic toxicity.

5.4.2.3 Other local effects

The irritation potential (and possible phototoxic potential) of isoeugenol are concentration dependent. Under normal use conditions of all of these products, these effects are not likely to be manifested. For this reason, these endpoints were not identified as being critical. The same argument applies for acute effects resulting from the accidental ingestion of isoeugenol containing detergent products.

5.4.3 Summary and Conclusion

Exposure to isoeugenol due to its presence in laundry and cleaning products occurs overwhelmingly by the dermal route. Skin exposure occurs mainly in hand-washed laundry, laundry pre-treatment and hand dishwashing. Some dermal exposure will result from the use of other products or from indirect exposures such as through contact with isoeugenol residues in fabrics after the washing cycle and skin contact during hard surface cleaning. Oral exposure occurs from the possible environmental presence of isoeugenol resulting in residues being consumed in drinking water and in food. Oral exposure can also arise from residues on eating utensils and dishes after hand washing. Isoeugenol is also used in spray cleaners that may give rise to inhalation exposure via the aerosols generated during spraying. Inhalation of isoeugenol will also arise from detergent dusts. However, these routes give rise to extremely minor exposure levels compared to direct dermal exposure from the use of a few specific laundry products. The consumer aggregate exposure (body burden) has been estimated to be less than 1.4×10^{-3} μg /kg/day. Maximum dermal exposure expressed as the dose that is critical to the induction and elicitation of contact allergy is 0.7 $\mu g/cm^2$ from hand pretreatment of clothes using undiluted detergent and from accidental or intentional over-exposure.

From the available toxicological data and information *in vivo* and *in vitro*, only two end-points: contact allergy and systemic toxicity were identified as being critical.

There is a large body of data in man and animals to show that isoeugenol is a skin sensitizer. A No Expected Sensitisation Level (NESL) of 250 $\mu g/cm^2$ was determined by a “weight of evidence” approach. Less certain data exist for the threshold dose for elicitation of a previously acquired allergy to isoeugenol. Available evidence shows that “threshold” is only one of many factors that determine if an allergy will be elicited. This complicates the determination of these “thresholds”, a determination that is rendered more complicated by the fact that the method used to measure this threshold is known to affect the measured threshold. None-the-less, from limited tests carried out under maximized conditions, it appears that exquisitely sensitive individuals may react under open conditions to levels down to 80 $\mu g/cm^2$. This is still higher than the highest estimate of likely exposure.

On the basis of the worst-case exposure scenarios resulting from the use and miss-use of these laundry and cleaning products, a $MOE_{sens}$of more than 350 was obtained for the induction of contact allergy to isoeugenol.

For systemic toxicity, an old multiple dose study on isoeugenol was judged to be sub-optimal for the determination of a reliable NOAEL. Instead, two conservative measures were taken: a NOAEL determined on the basis of a reproductive toxicity study and the Threshold of Toxicological Concern based on a large data set of NOAELs for substances having a similar structure classification as that of isoeugenol. Comparison with aggregate exposure results in a MOE of over one hundred million for the NOEL and over ten thousand for the TTC. These large margins of exposure are large enough to account for the inherent uncertainty and variability of the available hazard data and also for inter- and intra-species extrapolations.

Human experience has shown that neat isoeugenol may be irritating to the eye. The irritation potential of this substance depends on concentration. Local dermal and

Ocular effects due to direct or indirect contact with isoeugenol containing solutions in hand-washed laundry or hand dishwashing are not of concern because isoeugenol is not expected to be irritating at the extremely low concentrations of use in these products.

In summary, this human health risk assessment has demonstrated that the use of isoeugenol in household laundry and cleaning products is safe and does not cause concern with regard to consumer use.

APPENDIX 1

NO EXPECTED SENSITISATION LEVELS (NESLs)

5.4.4 At Induction

On the basis of a weight of evidence approach, a No Expected Sensitisation Level (NESL) of 250 μg/cm^2 has been chosen for isoeugenol.

Non-induction levels from animal tests.

Studies using adjuvants and/or intradermal injection indicate that isoeugenol has a clear sensitization potential. These methods are not particularly appropriate for determining sensitization induction thresholds.

Studies in non-adjuvant predictive tests in animals gives a better opportunity for estimating induction threshold doses of isoeugenol. However, we have no reliable information on what the doses were in terms of quantity per unit area. As a result, the Buehler Guinea pig tests and Open Epicutaneous Testsdo not contribute to our knowledge of the NESL. In order to estimate an induction threshold for isoeugenol, EC3 values have been obtained from over forty Local Lymph Node Assays. A weighted mean value based on the number of dose levels has been calculated to be 2% (500 μg/cm^2) (RIFM/COLIPA, 2004) EC3 values can provide a quantitative estimate of the relative skin sensitising potency that has been shown to correlate well with NOELs established from human studies (Gerberick *et al.*, 2001b;Griem *et al.*, 2003;Schneider and Akkan, 2004)

Non-induction levels in human tests.

In human studies 19 maximization tests gave an aggregate of 101/484 reactions at an induction concentration of 8% (c. 4000 μg/cm^2)in petrolatum but because high applied dose levels were used, little information on possible no effect levels was obtained from these tests. In 10 Human Repeated Insult Patch Tests (HRIPT) carried out under different conditions, negative reactions were obtained when induction and challenge concentrations were 0.5% (260 μg/cm^2) and in one test when the concentration was 1.25% (970 μg/cm^2) but one other tests carried out at this exposure level (970 μg/cm^2) gave marginally positive scores.

Weight-of-Evidence No Expected Sensitization Levels (NESL)

In view of the particularities of allergic contact dermatitis, it is not appropriate to use terms like No Effect Levels. The No Expected Sensitisation Levels (NESLs) are applied doses (expressed as quantities retained on unit areas of skin) that are not expected to give rise to the induction of sensitisation in subjects under exaggerated test conditions. The non-inducing levels seen in the different test systems are:

- in animal tests (LLNA EC3): 500 μg/cm^2

- in studies on humans (HRIPTs): 250 μg/cm^2

Giving precedence to the lower value obtained from studies carried out on human volunteers, a NESL of 250 μg/cm^2 has recently been chosen

5.4.5 At Elicitation

See **Appendix 2** for studies carried out on human subjects and **Appendix 3** for considerations concerning non-elicitation levels.

ISOEUGENOL – ANNEX 2

APPENDIX 2

ELICITATION STUDIES ON SUBJECTS ALREADY SENSITIZED TO ISOEUGENOL

1. Elicitation studies on subjects who had been sensitised to isoeugenol in Repeat Insult Patch Testing

One of the earliest studies in this area was a rechallenge on two subjects who had been previously sensitized to isoeugenol in human repeated insult patch testing reported above (RIFM, 1964). One patient gave a similarly intense allergic reaction to 1.25% isoeugenol as he had in the original challenge. The other however, showed only mild erythema when rechallenged at 1.25% (c. 970 μg/cm^2). Other studies that showed dose response effects but also failed to reveal clear non-elicitation thresholds were also reported (Ishihara *et al.*, 1979) in which the number of reactions seen after 48 hour occlusion (out of 133 cosmetic-sensitive patients) decreased from 5 at 5% to 4 at 2% and then to 3 at 1%. Similar effects were also seen in another study (Itoh, 1982) in which the number of reactions seen in 8 isoeugenol-positive patients diminished from 8/8 at 5% to 5/8 at 1% and 4/8 at 0.5%.

In another study,12 isoeugenol-sensitive patients were subjected to patch testing and use testing (Epstein, 1982). In the patch testing part they were exposed to serial dilutions of isoeugenol from 8% to 0.008% in petrolatum under occlusive patches. In this study, all responded to the 8% patch and 6 reacted to the patch at 0.8%. None reacted at lower concentrations than this although one patient developed a rash in the second week of exposure to 0.008% (approximately 6 μg/cm^2).

In the use test they were asked to apply a small amount of isoeugenol (3-5 mg as a 0.008% mix with petrolatum) to one of the anticubital fossae 2-3 times per day, on a daily basis for 2 weeks. Only one of the 12 patients reacted in this phase and this occurred only on the last (14th) day. This patient was one of the 6 who had reacted to both the 8% and 0.8% patches. After a rest period, these use tests were continued on the remaining 11 patients using 0.08% isoeugenol instead of 0.008%. In this phase, 3 patients reacted (one on day 2, one on day 3 and one on day 7). These three patients had also reacted to both patches at 8% and 0.8% and one of these had also shown a rash when patch tested at 0.08%. Yet another phase at 0.8% produced an additional reaction in one of the 8 remaining patients (on day 11). Once again, this patient had also reacted to patches at 8% and 0.8%. The remaining 7 patients failed to react to 6 weeks of use testing (2 weeks at 0.008%, 2 weeks at 0.08% and 2 weeks at 0.8%). This included one patient who had reacted to the patch test at 0.8%.

The author of this study concluded that the use test was not particularly reliable and did not provide any further information in addition to that obtained from traditional patch testing.

In another key study (Johansen *et al.*, 1996c), 20 patients participated. None of these had active eczema at the time of the testing but had given clear positive or doubtful patch test reactions to isoeugenol at 1 or 2% (300 or 600 μg/cm^2) subsequent to having shown clear reactions to the Fragrance Mix at 8 or 16%. This number was reduced to 19 after a confirmatory patch test at 2% (600 μg/cm^2) failed to produce any reaction in one of these. In this test, 3 patients gave +++ reactions, 6 gave ++ reactions, one gave a single + reaction and the remaining 9 gave reactions judged as +?. Sequential dilution patch testing (48 hours under Scanpore of Finn Chamber occlusion) of the remaining 19 gave reactions in 17 of these patients at 1% (400 μg/cm^2), 14 of

them at 0.5% and 0.2% (200 µg/cm^{2}and80 µg/cm^2), in 13 patients at 0.1% (40 µg/cm^2), in 8 at 0.05% (20 µg/cm^2), in 4 at 0.02% (8 µg/cm^2) and 0.01% (4 µg/cm^2). Patch testing was not performed at levels below this and as a consequence, the authors conclude that with 4 patients still reacting at the lowest patch test dose (0.01% (4 µg/cm^2), the potentially lowest threshold for elicitation to isoeugenol had not been determined.

Interestingly, of the 4 patients who continued reacting to all patch test concentrations including the lowest of 0.01% (4 µg/cm^2), one had originally showed a +++ reaction in the first test at 2% and two had shown ++ reactions but one had shown a +? reaction. The 5 patients who did not give any patch test reactions below 1% were logically, all from the group of 9 who had given +? reactions in this first patch test. The 10 patients who had given clear positives (+ to +++), all continued to give reactions down to at least 0.1% (40 µg/cm^2).

This study also subjected the same 19 isoeugenol-sensitive patients to Repeated Open Application Testing (ROAT) (Hannuksela and Salo, 1986). In this, the 19 patients applied a solution of 0.2% isoeugenol in ethanol to a 25 cm^2 surface of the upper arm twice daily. From weighing the solutions before and after application, it was estimated that applied doses were in the –5.8-5.5 µg/cm^{2}range. No patients reacted to the first application at this dose. The first reactions appeared after 7 days (14 applications). After 14 days, 12 patients had reacted but 7 had not. These 7 patients then continued with ROATesting as before but also with application to an additional 5 cm^2 area on the neck. This was continued for another 14 days. Of these 7, one gave a false positive reaction to the area on the neck (a similar reaction was obtained to an equal area receiving only ethanol on the other side of the neck).The other 6 patients showed no reaction even though on average they had been applying higher doses of isoeugenol (6.5 µg/cm^2).

Of the 7 patients who failed to react in the ROAT, all had shown +? reactions in the first patch test at 2%. However, two had shown low thresholds in the serial dilution patch tests. One had reacted down to 0.05% (200 µg/cm^2) and the other reacted down to 0.1% (400 µg/cm^2).

On the face of it, these studies would indicate that a significant proportion of isoeugenol-sensitive patients will show elicitation thresholds below 0.01% (4 µg/cm^2) in closed patch testing and below 6 µg/cm^2 in open application tests. The possibility of active sensitization occuring under the conditions of the sequential closed and open exposures to isoeugenol that were experienced by each patient would seem to be ruled out by the fact that an equivalent sized group of patients who were not sensitive to isoeugenol, completed the same ROAT regime without acquiring a sensitivity to isoeugenol.

In another study by some of the same authors (Andersen *et al.*, 2001), 27 isoeugenol-positive patients who all gave reactions greater than +? when patch tested at 1% or 2% were subjected to a slightly different variant of the closed patch testing and ROAT protocol described in the previous study. In the closed patch testing phase using 48-hour exposure to different concentrations of isoeugenol in ethanol in Finn Chambers. Progressively smaller doses were patch tested were applied starting from 2% (118 μg/cm^2) for those who had shown + reactions and from 1% (59 μg/cm^2) for those who had shown ++ or +++ reactions. A total of 16 different concentrations were chosen, going down to 0.00006% (0.0035 μg/cm^2). No patients gave reactions at these extreme doses and 3 of the 27 patients failed to shown any positive patch test reactions at all. The results for the remaining 24 are given below.

In the ROAT phase of testing, each subject applied two doses, one to each arm, of isoeugenol in ethanol, twice daily for up to 28 days. If a reaction was observed with one of the doses, application of this would be stopped but the other would continue to be applied until it too produced a positive reaction. The concentrations applied in this phase were 0.2% and 0.05%. On the basis of the weight of solutions used and the approximate areas, the doses were estimated to be in the range of 80 μg/cm^2 (0.2%) and 20 μg/cm^2 (0.05%).

According to the authors, only 16 patients gave positive reactions to the 0.2% dose (yet positive ROAT readings were apparently recorded for all 24 patients. On average, patients reacted to the 0.2% dose after 7 days and after about 15 days to 0.05%.

It is difficult to extract exact results from this publication. None-the-less, it appears that:

1 patient reacted to the closed patch at 2% but not at 1.32% (78 μg/cm^2). This patient reacted in the ROAT at 0.2% (80 μg/cm^2) only on day 15 but did not react at all to the ROAT at 0.05% (20 μg/cm^2).

1 patient reacted to closed patches down to 0.25% but not at 0.125% (7.4 μg/cm^2). This patient reacted in the ROAT at 0.2% (80 μg/cm^2) only on day 14 but also did not react at all to the ROAT at 0.05% (20 μg/cm^2).

4 patients reacted to closed patches at 0.125% but not at 0.063% (3.7 μg/cm^2). These patients reacted in the ROAT at 0.2% (80 μg/cm^2) at days 4, 7, 14 & 26. Two of these reacted to the ROAT at 0.05% (20 μg/cm^2) on days 27 & 28.

3 patients reacted to closed patches at 0.063% but not at 0.031% (1.9 μg/cm^2). These patients reacted in the ROAT at 0.2% (80 μg/cm^2) at days 7, 8 & 9. Only one of these reacted to the ROAT at 0.05% (20 μg/cm^2) (on day 18).

1 patient reacted to closed patches at 0.031% but not at 0.016% (0.9 μg/cm^2). This patient reacted in the ROAT at 0.2% (80 μg/cm^2) on day 3 and to the ROAT at 0.05% (20 μg/cm^2) on day 9.

2 patients reacted to closed patches at 0.16% but not at 0.008% (0.47 μg/cm^2). These patients reacted in the ROAT at 0.2% (80 μg/cm^2) at days 8 & 7 and to the ROAT at 0.05% (20 μg/cm^2) on days at days14 & 17.

1 patient reacted to closed patches at 0.008% but not at 0.004% (0. 24 μg/cm^2). This patient reacted in the ROAT at 0.2% (80 μg/cm^2) on days 4 and to the ROAT at 0.05% (20 μg/cm^2) on day 10.

2 patients reacted to closed patches at 0.004% but not at 0.002% (0.12 μg/cm^2). One of these patients reacted in the ROAT at 0.2% (80 μg/cm^2) at day 2. They reacted to the ROAT at 0.05% (20 μg/cm^2) on days 3 & 4.

1 patient reacted to closed patches down to 0.0005% but not at 0.00025% (0.015 μg/cm^2). This patient reacted in the ROAT at 0.2% (80 μg/cm^2) at day 4 and to the ROAT at 0.05% (20 μg/cm^2) on days at day 7.

These studies demonstrate that some isoeugenol-sensitive patients will, under the exaggerated conditions of a closed patch test, show elicitation thresholds down to 0.00025% or 0.015 μg/cm^2 (15 ng/cm^2). In the more realistic Repeated Open Application Test, a significant number of prior-sensitized patients (16 out of 27 reacted to a dose of 80 μg/cm^2 (80,000 ng/ cm^2) and of these, 10 reacted to a dose of 20 μg/cm^2 (20,000 ng/cm^2) after priming that may involve as little as only 6 applications over a 3-day period.

ISOEUGENOL - ANNEX 3

APPENDIX 3

CONSIDERATIONS REGARDING NON-ELICITATION LEVELS

A number of observations point to complications that prevent us from simply taking the lowest figures from the studies detailed in Appendix 2 as the "thresholds of elicitation". These relate to

(a) doubts over the "realism" of occlusive patch testing;

(b) lack of correlation between serial dilution patch tests and repeated open application testing;

(c) the influence of the severity of the induction regime on these thresholds;

(d) the influence of additional challenges on these thresholds.

(a) Is 48 hour occlusive patch testing relevant to transient open exposure ?

The potentiating effects of occlusion on dermal penetration of fragrance ingredients both *in vitro* (Ryatt *et al.*, 1988;Bronaugh *et al.*, 1985;Roper *et al.*, 1997) and *in vivo* (Bronaugh *et al.*, 1985;Bronaugh *et al.*, 1990), are well documented and appear to be without exception in chemicals spanning the range of molecular weights, volatility and lipophilicity of isoeugenol. Indeed, the only substances for which occlusion does not appear to enhance penetration would seem to be amphiphillic substances like nicotine (Ryatt *et al.*, 1988) and some high molecular weight steroids (Bucks *et al.*, 1988). The potentiating effects of occlusion on the intensity and frequency of allergic contact dermatitis have also been reported (numerous publications including (Kraus *et al.*, 1990;Ale and Maibach, 1995;Funk and Maibach, 1994;Zhai and Maibach, 2001). Furthermore, the duration of exposure (48 hours in patch testing compared to shorter periods to consumer products even when these are not immediately rinsed or wiped from the skin) also has a similar enhancing effect (McFadden *et al.*, 1998). As a result, it is extremely difficult to extrapolate to real-life scenarios from apparent thresholds obtained from studies using closed patches.

(b) Bad correlation between serial dilution patch testing and repeated open application testing

Studies that identify the performance of individual patients in both of these studies have revealed that often those that appear to be most sensitive in serial-dilution patch testing are found to be among the least sensitive in repeat open application tests. This has been demonstrated for studies on isoeugenol, formaldehyde and chromium (Villarama and Maibach, 2004). This lack of correlation between individual performances in these two test systems is also seen in a study on hydroxycitronellal (Svedman *et al.*, 2003b) in which for example, the patient who was the least sensitive in patch testing was among the most sensitive in the open test. As pointed out by Villarama and Maibach, there are many factors leading of elicitation that are not understood.

(c) Thresholds of elicitation vary according to the severity of the induction regime

Unlike most other toxicological thresholds, there is an increasing body of evidence to show that this *elicitation threshold* is not simply an intrinsic property of the allergenic substance. Indeed, there is now good evidence to show that it depends on a number of factors that are dependent on extraneous conditions. Recently published data show that the severity of the induction regime (i.e. the severity of the conditions under which allergy has been acquired) has an important influence over the no effect dose for elicitation products (Hostynek and Maibach, 2004).

There has for some time been evidence to show that reactions observed at challenge are more intense following more severe induction exposures. Early studies (Marzulli and Maibach, 1974) showed that dose response relationships exist for both induction and elicitation of sensitization in humans to a number of substances. Subsequently, this was taken a step further by demonstrating that the elicitation concentrations necessary to sensitize any given proportion of animals to the chloromethylisothiazolinone/ methylisothiazolinone biocide in a Buehler Guinea Pig Test, was inversely proportional to the induction concentration. (Chan *et al.*, 1983). Subsequently, (Friedmann and Moss, 1985) (although they did not go as far as determining thresholds), demonstrated that the induction dose determines not only the proportion of subjects sensitized but also the intensity of the allergic response at challenge. In studies on three groups of volunteers who were experimentally sensitized by exposure to three different doses of Dinitrochlorobenzene, the increase in skin-fold thickness at challenge to three increasing doses gave three parallel dose response curves. When all three groups were challenged to the same three doses, the curve for subjects sensitized to 62.5 µg/cm^{2}was lower in terms of skin-fold thickness, than that for the subjects sensitized by induction to 500 µg/cm^{2}that in turn was lower than (and parallel to) that for those sensitized to 1000µg/cm^2.

Subsequent studies have shown how thresholds of elicitation vary with the severity of the induction regime. One of the earliest studies in this area (Jayjock and Lewis, 1992) was carried out on the chloromethylisothiazolinone/ methylisothiazolinone biocide. Although criticised for the low number of animals used (Basketter *et al.*, 1997) these Buehler studies are reinforced by the more detailed studies carried out after this. The work of (Nakamura *et al.*, 1999) gives a rare insight into the relationship between the induction dose and the observed threshold of elicitation. Although this study was primarily aimed at comparing the Guinea Pig Maximization Test, the Adjuvant and Patch Test and the Buehler Test, it provides valuable data on this relationship. The results of these three tests on four different substances (2,4-dinitrochlorobenzene, maleic anhydride, hexylcinnamic aldehyde and 2-Dodecen-1-yl succinic anhydride) show that as the induction dose increases, the threshold of elicitation decreases. (van Och *et al.*, 2001) also carried out similar studies on three chemicals (diethylamine, Tetramethyl thiuram disulfide & Zinc Dimethyl dithiocarbamate) using the Guinea Pig Maximization Test. Here too the same trend was seen in each case. (Scott *et al.*, 2002) have carried out studies on mice on two substances (2,4-dinitrochlorobenene and squaric acid dibutyl ester). Challenge was made on the flanks of the animals and despite the moderate degree of biological variation one would expect in this type of study, the threshold of elicitation (measured as the challenge dose which produced significant increase in flank fold thickness) also showed the same dependence on the induction concentration. Other studies using a modified Guinea Pig Maximization Test have also shown the same trend. Most notably these were the studies carried out on PTBS (p-t-butylphenylsalicylate) (Yamano *et al.*, 1995), on TPN (2,4,5,6-tetrachloroisophtalonitrile) and BIT (1,2-benzisothiazolin-3-one) (Noda *et al.*, 1998) and on IPBC (3-iodo-2-propynylbutylcarbamate) and CPIP (p-chlorophenyl-3-iodopropargylformyl) (Shimizu *et al.*, 2000).

With hydroxycitronellal in the Cumulative Contact Enhancement Test, the apparent elicitation threshold decreases from 0.1% (induction at 3% and 10%) to 0.03% (induction at 20%) and then to below 0.01% (induction at 100%) (Wahlkvist *et al.*, 1999).

Hence we see that studies using different protocols in guinea pigs, mice and even in human volunteers using 15 different test materials provide the necessary robustness to conclude that this trend is general: the threshold for elicitation decreases according to the severity of the induction regime.

(d) Thresholds of elicitation decrease progressively with each elicitation exposure

Thresholds of elicitation are lowered by sequential exposures. The rarity of reactions in the first days of the Repeat Open Application Test is empirical testimony to this “boosting” effect (Friedmann, 1990). This is true for cinnamic aldehyde sensitive patients (Johansen *et al.*, 1996a). Nearly half of these patients reacted in the same ROATesting as described here, after day 7 and some went up to day 14. Elicitation studies on hydroxycitronallal (Epstein, 1982;Johansen *et al.*, 1996c;Andersen *et al.*, 2001;Svedman *et al.*, 2003a) have also shown that in sensitized patients, the threshold of elicitation diminishes with successive exposures. In the ROATesting reported in the papers of Johansen et al., and Andersen et al., sensitized subjects failed to react to the test material until at least 14 applications. In the study by (Epstein, 1982), 2 patients only reacted on the 11 and 14 days of repeated exposure. It is difficult to quantify this "boosting" effect but this seems to be a general effect (Villarama and Maibach, 2004).

Exposure to isoeugenol present in a multitude of different consumer products will also be expected to "boost" the existing allergic sensitivity to this substance. However, the degree of exposure from consumer products is of a different order than exposure arising from the serial dilution closed patch testing that preceded these open use tests (ROATs) in the work described above (Epstein, 1982;Johansen *et al.*, 1996c;Andersen *et al.*, 2001;Svedman *et al.*, 2003b). The relative severity of patch testing can be seen by comparing doses. As we have seen in Section 5.1.3, exposure to isoeugenol in household products will lead to levels below1 µg/cm^2. If isoeugenol was used at 1% in a perfume spray (the product-type that produces the highest on-skin level of fragrance), it would give rise to a dermal loading of 26 µg/cm^2 (Gerberick *et al.*, 2001a). For shampoos the loading would be even lower (0.08 µg/cm^2 according to (Robinson *et al.*, 2000;Gerberick *et al.*, 2001a). Yet the use of diagnostic patch tests with 1% of the same ingredient in 8 mm Finn Chambers, will deliver a skin loading of 300 µg/cm^2 (Robinson *et al.*, 2000), a large increase over levels found in consumer products.

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7 CONTRIBUTORS TO THIS REPORT

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