File No: LTD/1816

August 2015

**NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)**

# PUBLIC REPORT

## Cyclopentaneacetic acid, 3 hydroxy-2-pentyl-, sodium salt (1:1) (INCI Name: Sodium Tetrahydrojasmonate)

This Assessment has been compiled in accordance with the provisions of the *Industrial Chemicals (Notification and Assessment) Act 1989* (the Act) and Regulations. This legislation is an Act of the Commonwealth of Australia. The National Industrial Chemicals Notification and Assessment Scheme (NICNAS) is administered by the Department of Health, and conducts the risk assessment for public health and occupational health and safety. The assessment of environmental risk is conducted by the Department of the Environment.

For the purposes of subsection 78(1) of the Act, this Public Report may be inspected at our NICNAS office by appointment only at Level 7, 260 Elizabeth Street, Surry Hills NSW 2010.

This Public Report is also available for viewing and downloading from the NICNAS website or available on request, free of charge, by contacting NICNAS. For requests and enquiries please contact the NICNAS Administration Coordinator at:


| | |
|---|---|
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**Director**
**NICNAS**

# TABLE OF CONTENTS

# SUMMARY

The following details will be published in the NICNAS *Chemical Gazette:*

| ASSESSMENT REFERENCE | APPLICANT(S) | CHEMICAL OR TRADE NAME | HAZARDOUS CHEMICAL | INTRODUCTION VOLUME | USE |
|---|---|---|---|---|---|
| LTD/1816 | L'Oreal Australia Pty Ltd | Cyclopentaneacetic acid, 3 hydroxy-2-pentyl-, sodium salt (1:1) (INCI Name: Sodium Tetrahydrojasmonate) | Yes | ≤ 1 tonne per annum | Cosmetic ingredient |

# CONCLUSIONS AND REGULATORY OBLIGATIONS

**Hazard classification**
Based on the available information, the notified chemical is recommended for hazard classification according to the *Globally Harmonised System for the Classification and Labelling of Chemicals (GHS),* as adopted for industrial chemicals in Australia. The recommended hazard classification is presented in the table below.

| *Hazard classification* | *Hazard statement* |
|---|---|
| Eye Damage (Category 1) | H318 – Causes serious eye damage |

Based on the available information, the notified chemical is recommended for hazard classification according to the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004) with the following risk phrase:

R41: risk of serious damage to eyes

**Human health risk assessment**
Under the conditions of the occupational settings described, the notified chemical is not considered to pose an unreasonable risk to the health of workers.

Based on the available information, when used at ≤ 6.5% concentration in face cream, ≤ 4% concentration in other leave-on cosmetic products and ≤ 3% concentration in make-up products, the notified chemical is not considered to pose an unreasonable risk to public health.

**Environmental risk assessment**
On the basis of the PEC/PNEC ratio and the reported use pattern, the notified chemical is not considered to pose an unreasonable risk to the environment.

**Recommendations**

REGULATORY CONTROLS

Hazard Classification and Labelling

- The notified chemical should be classified as follows:
  - H318 – Causes serious eye damage

The above should be used for products/mixtures containing the notified chemical, if applicable, based on the concentration of the notified chemical present and the intended use/exposure scenario.

CONTROL MEASURES

Occupational Health and Safety

- A person conducting a business or undertaking at a workplace should implement the following engineering controls to minimise occupational exposure to the notified chemical as introduced in the raw material Mexoryl SBO:
  - Enclosed and automated processes
  - Exhaust ventilation

- A person conducting a business or undertaking at a workplace should implement the following safe work practices to minimise occupational exposure during handling of the notified chemical as introduced in the raw material Mexoryl SBO:
  - Avoid contact with eyes

- A person conducting a business or undertaking at a workplace should ensure that the following personal protective equipment is used by workers to minimise occupational exposure to the notified chemical as introduced in the raw material Mexoryl SBO:
  - Eye protection

  Guidance in selection of personal protective equipment can be obtained from Australian, Australian/New Zealand or other approved standards.

- A copy of the (M)SDS should be easily accessible to employees.

- If products and mixtures containing the notified chemical are classified as hazardous to health in accordance with the *Globally Harmonised System for the Classification and Labelling of Chemicals (GHS)* as adopted for industrial chemicals in Australia, workplace practices and control procedures consistent with provisions of State and Territory hazardous substances legislation should be in operation.

Disposal

- Where reuse or recycling are not appropriate, dispose of the notified chemical in an environmentally sound manner in accordance with relevant Commonwealth, state, territory and local government legislation.

Storage

- The handling and storage of the notified chemical should be in accordance with the Safe Work Australia Code of Practice for *Managing Risks of Hazardous Chemicals in the Workplace* (SWA, 2012) or relevant State or Territory Code of Practice.

- Spills or accidental release of the notified chemical should be handled by physical containment, collection and safe disposal.

**Regulatory Obligations**

*Secondary Notification*
This risk assessment is based on the information available at the time of notification. The Director may call for the reassessment of the chemical under secondary notification provisions based on changes in certain circumstances. Under Section 64 of the *Industrial Chemicals (Notification and Assessment) Act (1989)* the notifier, as well as any other importer or manufacturer of the notified chemical, have post-assessment regulatory obligations to notify NICNAS when any of these circumstances change. These obligations apply even when the notified chemical is listed on the Australian Inventory of Chemical Substances (AICS).

Therefore, the Director of NICNAS must be notified in writing within 28 days by the notifier, other importer or manufacturer:

(1) Under Section 64(1) of the Act; if

- the importation volume exceeds one tonne per annum notified chemical;
- the concentration of the notified chemical exceeds or is intended to exceed 6.5% in face cream, 4% in other leave-on cosmetic products and 3% in make-up products;

or

(2) Under Section 64(2) of the Act; if

- the function or use of the chemical has changed from a cosmetic ingredient, or is likely to change significantly;
- the amount of chemical being introduced has increased, or is likely to increase, significantly;
- the chemical has begun to be manufactured in Australia;
- additional information has become available to the person as to an adverse effect of the chemical on occupational health and safety, public health, or the environment.

The Director will then decide whether a reassessment (i.e. a secondary notification and assessment) is required.

*(Material) Safety Data Sheet*

The (M)SDS of the raw material containing the notified chemical (Mexoryl SBO) provided by the notifier was reviewed by NICNAS. The accuracy of the information on the (M)SDS remains the responsibility of the applicant.

# ASSESSMENT DETAILS

## 1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)
L'Oreal Australia Pty Ltd (ABN: 40 004 191 673)
564 St Kilda Road
Melbourne VIC 3004

NOTIFICATION CATEGORY
Limited-small volume: Chemical other than polymer (1 tonne or less per year).

EXEMPT INFORMATION (SECTION 75 OF THE ACT)
Data items and details claimed exempt from publication: analytical data, degree of purity, residual monomers, impurities, additives/adjuvants and use details.

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
Variation to the schedule of data requirements is claimed as follows: water solubility, hydrolysis as a function of pH, dissociation constant, and flammability.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
ECHA (2012)

## 2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
Mexoryl SBO (contains the notified chemical at < 30% concentration)

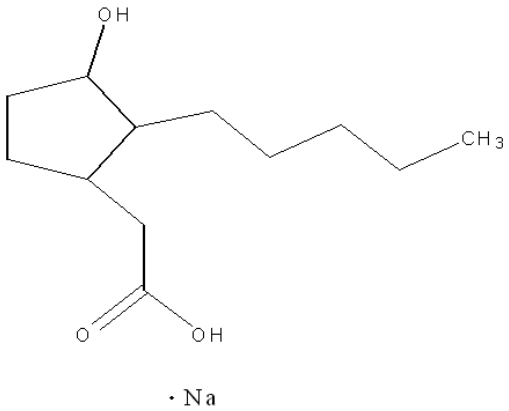
CAS NUMBER
1175006-92-4

CHEMICAL NAME
Cyclopentaneacetic acid, 3 hydroxy-2-pentyl-, sodium salt (1:1)

OTHER NAME(S)
Sodium Tetrahydrojasmonate (INCI)

MOLECULAR FORMULA
$C_{12}H_{22}O_3.Na$

STRUCTURAL FORMULA



MOLECULAR WEIGHT
236.29 Da

ANALYTICAL DATA
Reference IR spectra were provided.

## 3. COMPOSITION

DEGREE OF PURITY
> 75%

## 4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20 °C AND 101.3 kPa: pale yellow liquid*

| Property | Value | Data Source/Justification |
|---|---|---|
| Melting Point/Freezing Point* | -25 °C | Measured |
| Boiling Point* | 102.2 °C at 101.3 kPa | Measured |
| Density* | 1,082.2 kg/m$^3$ at 20 °C | Measured |
| Vapour Pressure* | 0.988 kPa at 25 °C | Measured |
| Water Solubility | Not determined | Notified chemical is a salt, expected to be soluble |
| Hydrolysis as a Function of pH | Not determined | Contains no hydrolysable functionalities |
| Partition Coefficient (n-octanol/water) | log Pow = -0.61 | Calculated using KOWWIN v1.68 (US EPA, 2011) |
| Surface Tension* | 59.4 mN/m at 20.5 °C | Measured |
| Adsorption/Desorption | log $K_{oc}$ = -0.593 | Calculated using KOCWIN v2.00 (US EPA, 2011) |
| Dissociation Constant | Not determined | Expected to be ionised under environmental pH (4-9) |
| Flash Point* | > 102 °C at 100.2 kPa | Measured |
| Flammability | Not determined | Introduced as an aqueous solution |
| Autoignition Temperature | Not determined | Introduced as an aqueous solution |
| Explosive Properties | Not determined | Not expected to be explosive based on chemical structure |
| Oxidising Properties | Not determined | Not expected to be an oxidiser based on chemical structure |

*For the imported raw material Mexoryl SBO containing the notified chemical at < 30% concentration in aqueous solution

DISCUSSION OF PROPERTIES
For full details of tests on physical and chemical properties, refer to Appendix A.

*Reactivity*
The notified chemical is expected to be stable under normal conditions of use.

***Physical hazard classification***
Based on the submitted physico-chemical data depicted in the above table, the notified chemical is not recommended for hazard classification according to the *Globally Harmonised System for the Classification and Labelling of Chemicals (GHS),* as adopted for industrial chemicals in Australia.

## 5. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS
The notified chemical will be imported as a component of a raw material Mexoryl SBO at < 30% concentration in aqueous solution for formulation of cosmetic products, or as a component of finished cosmetic products at ≤ 6.5% concentration.

MAXIMUM INTRODUCTION VOLUME OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS

| *Year* | *1* | *2* | *3* | *4* | *5* |
|---|---|---|---|---|---|
| *Tonnes* | 1 | 1 | 1 | 1 | 1 |

PORT OF ENTRY
Melbourne and Sydney

IDENTITY OF RECIPIENTS
L'Oreal Australia Pty Ltd

TRANSPORTATION AND PACKAGING
The notified chemical will be transported as a component of a raw material Mexoryl SBO at < 30% concentration in 30 kg plastic containers. The containers will be packed on pallets and transported by sea and rail. The notified chemical may also be imported as a component of finished cosmetic products at ≤ 6.5% concentration. Finished cosmetic products containing the notified chemical will be packaged in ≤ 500 mL plastic bottles or tubes for retail sale. These containers will be packaged in cartons and pallets for transport by sea and rail.

USE
The notified chemical will be used as an ingredient in cosmetic products (face cream at ≤ 6.5% concentration other leave-on cosmetic products at ≤ 4% concentration and make-up products at ≤ 3% concentration).

OPERATION DESCRIPTION
The notified chemical will be imported as a component of a raw material Mexoryl SBO at < 30% concentration for formulation of cosmetic products, or as a component of finished cosmetic products at ≤ 6.5% concentration which will be sold to the public in the same form in which they are imported.

*Reformulation*
When reformulated, the raw material Mexoryl SBO containing the notified chemical at < 30% concentration will be blended into end-use consumer products at customer sites. Procedures will vary depending on the nature of the cosmetic product being formulated. Both manual and automated steps will likely be involved. For example, a chemist will sample and test the notified chemical for QA purposes manually, a compounder will weigh an appropriate amount of the notified chemical into a container then add the amount directly into a flame proof mixing tank, with periodic sampling for quality control purposes also carried out during the manufacturing process. Automated processes may include mixing and filling of end-use containers with products.

*End-use*
Finished products containing the notified chemical at ≤ 6.5% concentration will be used by the public and may also be used by professionals such as hairdressers and workers in beauty salons. Depending on the nature of the product, these could be applied by hand or by using an applicator.

## 6. HUMAN HEALTH IMPLICATIONS

### 6.1. Exposure Assessment

#### 6.1.1. Occupational Exposure

CATEGORY OF WORKERS

| *Category of Worker* | *Exposure Duration (hours/day)* | *Exposure Frequency (days/year)* |
|---|---|---|
| Transport and Storage | 4 | 12 |
| Professional Compounder | 8 | 12 |
| Chemist | 3 | 12 |
| Packers | 8 | 12 |
| End Users (workers) | 8 | 365 |

EXPOSURE DETAILS
Transport, storage and retail workers may come into contact with the notified chemical, as a component of the raw material Mexoryl SBO at < 30% concentration or at various concentrations in cosmetic products (≤ 6.5%), only in the event of accidental rupture of packages.

*Reformulation*
During reformulation into cosmetic products, dermal, ocular and inhalation exposure of workers to the notified chemical at < 30% concentration may occur. Exposure is expected to be minimised through the use of exhaust ventilation and/or automated/enclosed systems as well as through the use of personal protective equipment (PPE) such as coveralls, eye protection, impervious gloves and respiratory protection (as appropriate).

*End-use*
Exposure to the notified chemical in end-use products at ≤ 6.5% concentration may occur in professions where the services provided involve the application of cosmetic products to clients (e.g. workers in beauty salons). The principal route of exposure will be dermal, while ocular exposure is also possible. Such professionals may use some PPE to minimise repeated exposure, but this is not expected to occur in all workplaces. However, good hygiene practices are expected to be in place. If PPE is used, exposure of such workers is expected to be of a similar or lesser extent than that experienced by consumers using products containing the notified chemical.

### 6.1.2. Public Exposure
There will be widespread and repeated exposure of the public to the notified chemical at ≤ 6.5% concentration through the use of cosmetic products. The principal route of exposure will be dermal. Accidental ocular and oral exposure (from the use of lip products) is also possible. Inhalation exposure is not expected based on the use pattern and low vapour pressure of the notified chemical.

A combined internal dose of 0.261 mg/kg bw/day was estimated using data on typical use patterns of cosmetic product categories in which the notified chemical may be used (SCCS, 2012; specific use details of the notified chemical are considered as exempt information). This estimation assumed a worst case scenario and is for a person who is a simultaneous user of a selection of cosmetic products that may contain the notified chemical.

## 6.2. Human Health Effects Assessment
The results from toxicological investigations conducted on the raw material Mexoryl SBO containing the notified chemical at < 30% concentration are summarised in the following table. For full details of the studies, refer to Appendix B.

Water and an organic solvent make up the balance of the raw material Mexoryl SBO. The organic solvent has no known toxic effects, hence the toxicity profile of Mexoryl SBO is considered to reflect the notified chemical. For some endpoints the dose was adjusted for the concentration of the notified chemical in Mexoryl SBO and is indicated in the table.

| *Endpoint* | *Result and Assessment Conclusion** |
|---|---|
| Rat, acute oral toxicity | LD50 > 2000 mg/kg bw; low toxicity** |
| Skin irritation (in vitro) | non-irritating |
| Rabbit, skin irritation | non-irritating |
| Eye irritation (in vitro) (x2) | severely irritating |
| Rabbit, eye irritation | severely irritating<br>slightly irritating (10% dilution) |
| Mouse, skin sensitisation – LLNA (x2) | no evidence of sensitisation |
| Rat, repeat dose oral toxicity – 28 days. | NOAEL 300 mg/kg bw/day |
| Rat, repeat dose oral toxicity – 90 days. | NOAEL 300 mg/kg bw/day |
| Rat, repeat dose dermal toxicity – 28 days. | NOAEL > 100 mg/kg bw/day |
| Rat, repeat dose dermal toxicity – 14 days. | NOEL(males) 1000 mg/kg bw/day<br>NOEL(females) 500 mg/kg bw/day |
| Mutagenicity – bacterial reverse mutation | non mutagenic** |
| Genotoxicity – in vitro Mammalian Chromosome Aberration Test | non genotoxic |
| Genotoxicity – in vitro Mammalian Cell Micronucleus Test | non genotoxic** |
| Rat, reproductive and developmental toxicity | $NOAEL_{(maternal)}$ 100 mg/kg bw/day<br>$NOAEL_{(developmental)}$ 100 mg/kg bw/day |

* For the raw material Mexoryl SBO containing the notified chemical at < 30% concentration (unless otherwise stated)
** Dose was adjusted for the concentration of the notified chemical in the raw material Mexoryl SBO

*Toxicokinetics.*
No toxicokinetic data on the notified chemical were submitted.

Dermal absorption is expected to be limited given the high water solubility and low partition coefficient of the notified chemical. This is supported by an *in vitro* dermal penetration study on the notified chemical.

The *in vitro* skin penetration of the notified chemical using human skin was tested in a formulation designed to represent a typical cosmetic product (containing the notified chemical at a concentration of 4%) in compliance with OECD TG 428. The mean total systemically available dose was determined as 2.92% of the applied dose with a mean recovery of 99.7%. Based on this study, a dermal absorption value of 2.92% was used for exposure calculation purposes (see Section 6.1.2).

*Acute toxicity.*
The notified chemical is of low acute oral toxicity based on a study conducted in rats with Mexoryl SBO (containing the notified chemical at < 30% concentration), where the dose was adjusted for the concentration of notified chemical in the test substance.

*Irritation.*
Mexoryl SBO (containing the notified chemical at < 30% concentration) is not irritating to the skin based on a study conducted in rabbits and in an *in vitro* study conducted using a reconstituted Human Epidermis model (EpiSkin$^{SM}$).

Mexoryl SBO (containing the notified chemical at < 30% concentration) is severely irritating to the eye based on a study conducted in rabbits and two separate *in vitro* Bovine Corneal Opacity and Permeability (BCOP) tests. When Mexoryl SBO was tested as a 10% dilution in rabbits (corresponding to < 3% notified chemical), only slight irritating effects were observed.

*Sensitisation.*
Mexoryl SBO (containing the notified chemical at < 30% concentration) was not a sensitiser when tested at up to 100% concentration in two separate mouse local lymph node assays (LLNA).

*Repeated dose toxicity.*
A NOAEL of 300 mg/kg bw/day was established for Mexoryl SBO (containing the notified chemical at < 30% concentration) in a 28-day repeated dose oral gavage toxicity study in rats based on liver effects, including hepatocellular hypertrophy, higher liver weights and liver enlargement at the highest dose tested of 1000 mg/kg bw/day. A NOAEL of 300 mg/kg bw/day was also established for Mexoryl SBO in a 90-day repeated dose oral gavage toxicity study in rats based on similar effects on the liver.

A NOAEL of 100 mg/kg bw/day was established for Mexoryl SBO (containing the notified chemical at < 30% concentration) in a 28-day repeated dose dermal toxicity study in rats based on the absence of treatment related adverse effects at the highest dose tested. When Mexoryl SBO (containing the notified chemical at < 30% concentration) was tested in a 14-day repeated dose dermal toxicity study, a NOEL of 1000 mg/kg bw/day was established for males based on the absence of treatment related adverse effects, while a NOEL of 500 mg/kg bw/day was established for females based on skin irritation effects and an increase in urea and creatinine levels.

*Mutagenicity/Genotoxicity.*
Mexoryl SBO (containing the notified chemical at < 30% concentration), with dose adjusted for concentration of notified chemical in the test substance, was found to be non-mutagenic in a bacterial reverse mutation assay and non-genotoxic in an *in vitro* mammalian cell micronucleus test. Mexoryl SBO (containing the notified chemical at < 30% concentration) was non-genotoxic in an *in vitro* mammalian chromosome aberration test.

*Reproductive and developmental toxicity*
In a prenatal developmental (oral gavage) toxicity study in rats, the NOAEL for developmental toxicity was established as 100 mg/kg bw/day for Mexoryl SBO (containing the notified chemical at < 30% concentration), based on slightly lower foetal weights and higher foetal and litter incidence of a skeletal variation (short

supernumerary rib) at the higher doses of 300 mg/kg bw/day and 1000 mg/kg bw/day. The NOAEL for maternal toxicity was established as 100 mg/kg bw/day for Mexoryl SBO in this study, based on lower body weight gains.

***Health hazard classification***
Based on the available information, the notified chemical is recommended for hazard classification according to the *Globally Harmonised System for the Classification and Labelling of Chemicals (GHS),* as adopted for industrial chemicals in Australia. The recommended hazard classification is presented in the following table.

| *Hazard classification* | *Hazard statement* |
|---|---|
| Eye Damage (Category 1) | H318 – Causes serious eye damage |

Based on the available information, the notified chemical is recommended for hazard classification according to the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004), with the following risk phrase(s):

R41: risk of serious damage to eyes

### 6.3. Human Health Risk Characterisation

#### 6.3.1. Occupational Health and Safety
Based on the available information the critical health effect of the notified chemical is as a severe eye irritant.

*Reformulation*
During reformulation workers may be at risk of eye irritation effects when handling the notified chemical at < 30% concentration. This risk should be reduced through the expected use of enclosed processes and personal protective equipment (PPE) including eye protection.

Therefore, under the occupational settings described, the risk to the health of workers from use of the notified chemical is not considered to be unreasonable.

*End-use*
Workers involved in professions where the services provided involve the application of cosmetic products containing the notified chemical to clients (*e.g.*, hairdressers and beauty salon workers) may be exposed to the notified chemical. If PPE is used, the risk to these workers is expected to be of a similar or lesser extent than that experienced by consumers using products containing the notified chemical (for details of the public health risk assessment, see Section 6.3.2).

#### 6.3.2. Public Health
Cosmetic products containing the notified chemical will be available to the public at ≤ 6.5% concentration. The main route of exposure is expected to be dermal with some potential for accidental ocular or oral exposure.

*Local effects*
The notified chemical is severely irritating to the eye. The greatest risk of eye irritation effects are through the use of make-up products containing the notified chemical. However based on animal studies only slight irritating effects are expected at the proposed use concentration in make-up products (≤ 3%).

*Systemic effects*
The repeat dose toxicity potential was estimated by calculation of the margin of exposure (MoE) of the notified chemical using the worst case scenario from use of multiple products of 0.261 mg/kg bw/day (see Section 6.1.2) and the NOAEL of < 30 mg/kg bw/day which was established in a prenatal developmental toxicity study on the raw material Mexoryl SBO (containing the notified chemical at < 30% concentration). A MoE value ≥ 100 is considered acceptable to account for intra- and inter-species differences. Using the abovementioned NOAEL corrected for the concentration of the notified chemical in Mexoryl SBO, a MoE of 99 was estimated. This is considered to be acceptable given the estimate is based on the worst case scenario of a simultaneous user of all products.

Therefore, based on the information available, the risk to the public associated with the use of the notified chemical at ≤ 6.5% in face cream, at ≤ 4% concentration in other leave-on cosmetic products and ≤ 3% concentration in make-up products is not considered to be unreasonable.

# 7. ENVIRONMENTAL IMPLICATIONS

## 7.1. Environmental Exposure & Fate Assessment

### 7.1.1. Environmental Exposure

RELEASE OF CHEMICAL AT SITE

The notified chemical will not be manufactured in Australia. The notified chemical will be imported as a component of a raw material for reformulation into cosmetic products, or as a component of finished cosmetic products in end-use packaging. Therefore, there is unlikely to be any significant release to the environment from transport and storage, except in the case of accidental spills or leaks. In the event of spills, the product containing the notified chemical is expected to be collected by inert absorbent material, and disposed of to landfill in accordance with local government regulations.

The reformulation process will involve blending operations that will be highly automated, and are expected to occur within a fully enclosed environment. Therefore, significant release of the notified chemical from this process to the environment is not expected. The process will be followed by automated filling of the formulated products into containers of various sizes suitable for retail. Wastes containing the notified chemical generated during reformulation include equipment wash water, empty import containers, and spilt materials. Wastes may be collected and released to sewers in a worst case scenario, or disposed of to landfill in accordance with local government regulations.

RELEASE OF CHEMICAL FROM USE

The notified chemical is a component of cosmetic products. The formulated products will be applied to the body, and will either be removed with tissues and disposed of to domestic garbage, or washed off the body with ultimate release to the sewer.

RELEASE OF CHEMICAL FROM DISPOSAL

It is estimated that a maximum of 1%, or up to 10 kg of the notified chemical, may remain in import containers, and a maximum of 3%, or up to 30 kg, in end-use containers once the consumer products are used up. Wastes and residue of the notified chemical in empty containers are likely either to share the fate of the container and be disposed of to landfill, or to be released to sewer when containers are rinsed, before recycling through an approved waste management facility.

### 7.1.2. Environmental Fate

Following its use in cosmetic products, the majority of the notified chemical is expected to enter the sewer system, before potential release to surface waters nationwide. The notified chemical is not considered readily biodegradable, but shows inherent biodegradability (74.6% in 28 days). For details of the environmental fate studies, please refer to Appendix C.

Based on its expected high water solubility and calculated adsorption coefficient (log $K_{OC}$ = -0.593), release to surface waters may occur as limited partitioning to sludge and sediment is expected under environmental pH. The notified chemical is not expected to bioaccumulate due to its low calculated n-octanol/water partition coefficient (log $P_{OW}$ = -0.61) and inherent biodegradability. This is supported by a low bioconcentration factor (BCF = 3.162), calculated using EPI Suite v 4.10 (US EPA, 2011). Therefore, in surface waters the notified chemical is expected to disperse and degrade through biotic and abiotic processes to form water and oxides of carbon.

The majority of the notified chemical will be released to sewer after use. A small proportion of the notified chemical may be applied to land when effluent is used for irrigation, or when sewage sludge is used for soil remediation, or disposed of to landfill as collected spills and empty container residue. The notified chemical residues in landfill, soil and sludge are expected to eventually degrade to form water and oxides of carbon.

### 7.1.3. Predicted Environmental Concentration (PEC)

The predicted environmental concentration (PEC) has been calculated to assume a worst case scenario, with 100% release of the notified chemical into sewer systems nationwide and no removal within sewage treatment plants (STPs).

| Predicted Environmental Concentration (PEC) for the Aquatic Compartment | | |
|---|---|---|
| Total Annual Import/Manufactured Volume | 1,000 | kg/year |
| Proportion expected to be released to sewer | 100% | |
| Annual quantity of chemical released to sewer | 1,000 | kg/year |
| Days per year where release occurs | 365 | days/year |
| Daily chemical release: | 2.74 | kg/day |
| Water use | 200.0 | L/person/day |
| Population of Australia (Millions) | 22.613 | million |
| Removal within STP | 0% | |
| Daily effluent production: | 4,523 | ML |
| Dilution Factor - River | 1.0 | |
| Dilution Factor - Ocean | 10.0 | |
| PEC - River: | 0.606 | µg/L |
| PEC - Ocean: | 0.061 | µg/L |

STP effluent re-use for irrigation occurs throughout Australia. The agricultural irrigation application rate is assumed to be 1000 L/$m^2$/year (10 ML/ha/year). The notified chemical in this volume is assumed to infiltrate and accumulate in the top 10 cm of soil (density 1500 kg/$m^3$). Using these assumptions, irrigation with a concentration of 0.606 µg/L may potentially result in a soil concentration of approximately 4.039 µg/kg. Assuming accumulation of the notified chemical in soil for 5 and 10 years under repeated irrigation, the concentration of the notified chemical in the applied soil in 5 and 10 years may be approximately 20.19 µg/kg and 40.39 µg/kg, respectively.

## 7.2. Environmental Effects Assessment

The results from ecotoxicological investigations conducted on the raw material Mexoryl SBO containing the notified chemical at < 30% concentration are summarised in the table below. Details of these studies can be found in Appendix C.

| *Endpoint* | *Result** | *Assessment Conclusion* |
|---|---|---|
| Fish Toxicity | 96 h LC50 > 100 mg/L | Not harmful to fish |
| Daphnia Toxicity | 48 h EC50 > 100 mg/L | Not harmful to *Daphnia* |
| Algal Toxicity | 72 h $E_rC50$ > 100 mg/L | Not harmful to algae |
| Inhibition of Bacterial Respiration | 3 h IC50 > 1000 mg/L | Not inhibitory to bacterial respiration |

* Based on nominal test substance concentrations (100%) corrected for water content (~45%).

Based on the above ecotoxicological endpoints, the notified chemical is not considered to be harmful to fish, daphnids, and algae. Therefore, under the Globally Harmonised System of Classification and Labelling of Chemicals (GHS) (United Nations, 2009), the notified chemical is not formally classified for acute and chronic toxicities.

### 7.2.1. Predicted No-Effect Concentration

The predicted no-effects concentration (PNEC) has been calculated from the most sensitive endpoint for fish. A safety factor of 100 was used given acute endpoints for three trophic levels are available.

| Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment | | |
|---|---|---|
| LC50 (Fish, 96 h) | > 100 | mg/L |
| Assessment Factor | 100 | |
| Mitigation Factor | 1.00 | |
| PNEC: | > 1000 | µg/L |

### 7.3. Environmental Risk Assessment

The Risk Quotient (Q = PEC/PNEC) has been calculated based on the predicted PEC and PNEC.

| Risk Assessment | PEC µg/L | PNEC µg/L | Q |
|---|---|---|---|
| Q - River | 0.606 | > 1000 | **< 0.001** |
| Q - Ocean | 0.061 | > 1000 | **<< 0.001** |

The risk quotient for discharge of treated effluents containing the notified chemical to the aquatic environment indicates that the notified chemical is unlikely to reach ecotoxicologically significant concentrations in surface waters (Q << 1), based on its maximum annual importation quantity. Whilst the notified chemical is not readily biodegradable, it is considered inherently biodegradable and is expected to have a low potential for bioaccumulation. On the basis of the PEC/PNEC ratio, maximum annual importation volume and assessed use pattern in cosmetic products, the notified chemical is not expected to pose an unreasonable risk to the environment.

## APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

**Melting Point/Freezing Point** -25 °C

| | |
|---|---|
| Method | OECD TG 102 Melting Point/Melting Range.<br>EC Council Regulation No 440/2008 A.1 Melting/Freezing Temperature. |
| Remarks | Test performed on the imported raw material Mexoryl SBO containing the notified chemical at < 30% concentration. |
| Test Facility | Harlan (2010a) |

**Boiling Point** 102.2 °C at 99.4 kPa

| | |
|---|---|
| Method | OECD TG 103 Boiling Point.<br>EC Council Regulation No 440/2008 A.2 Boiling Temperature. |
| Remarks | Test performed on the imported raw material Mexoryl SBO containing the notified chemical at < 30% concentration. Test performed using differential scanning calorimeter. |
| Test Facility | Harlan (2010a) |

**Density** 998.2 kg/$m^3$ at 20 °C

| | |
|---|---|
| Method | OECD TG 109 Density of Liquids and Solids.<br>EC Council Regulation No 440/2008 A.3 Relative Density. |
| Remarks | Test performed on the imported raw material Mexoryl SBO containing the notified chemical at < 30% concentration. Test performed using oscillating densitimeter. |
| Test Facility | Harlan (2010b) |

**Vapour Pressure** 0.988 kPa at 25 °C

| | |
|---|---|
| Method | OECD TG 104 Vapour Pressure.<br>EC Council Regulation No 440/2008 A.4 Vapour Pressure. |
| Remarks | Test performed on the imported raw material Mexoryl SBO containing the notified chemical at < 30% concentration. Test performed using the static method. |
| Test Facility | Harlan (2010c) |

**Surface Tension** 59.4 mN/m at 20.5 °C

| | |
|---|---|
| Method | OECD TG 115 Surface Tension of Aqueous Solutions.<br>EC Council Regulation No 440/2008 A.5 Surface Tension. |
| Remarks | Test performed on the imported raw material Mexoryl SBO containing the notified chemical at < 30% concentration. Test performed using ring tensiometer.<br>Concentration: 0.1% (substance considered as a surface active substance) |
| Test Facility | Harlan (2010d) |

**Flash Point** > 102 °C at 100.2 kPa

| | |
|---|---|
| Method | EC Council Regulation No 440/2008 A.9 Flash Point. |
| Remarks | Test performed on Mexoryl SBO containing the notified chemical at < 30% concentration.<br>Test performed using a Pensky-Martens closed flash point tester.<br>Test was stopped when the test item started boiling. |
| Test Facility | Harlan (2010e) |

# APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

## B.1. Acute toxicity – oral

| | |
|---|---|
| TEST SUBSTANCE | Mexoryl SBO (contains the notified chemical at < 30% concentration) |
| METHOD | OECD TG 420 Acute Oral Toxicity – Fixed Dose Procedure.<br>EC Council Regulation No 440/2008 B.1 bis Acute toxicity (oral) fixed dose method. |
| Species/Strain | Rat/Sprague-Dawley Rj:SD (IOPS Han) |
| Vehicle | Water |
| Remarks - Method | No significant protocol deviations. The dose was adjusted for the concentration of the notified chemical. |

RESULTS

| *Group* | *Number and Sex of Animals* | *Dose mg/kg bw* | *Mortality* |
|---|---|---|---|
| 1 | 4 F | 2000 | 1/4 |

| | |
|---|---|
| LD50 | > 2000 mg/kg bw |
| Signs of Toxicity | Hypoactivity, piloerection and shortness of breath was recorded in all animals on Day 1.<br><br>One unscheduled death occurred on Day 2. Of the surviving animals, one showed slightly lower body weights gains in the first half of the study (days 1 to 8) which returned to normal in the second half of the study (days 8 to 15). |
| Effects in Organs | No apparent abnormalities were observed in all surviving animals. Advanced autolysis was observed in the animal that died on Day 2. |
| Remarks - Results | None. |
| CONCLUSION | The notified chemical is of low toxicity via the oral route. |
| TEST FACILITY | CIT (2009a) |

## B.2. Irritation – skin (in vitro)

| | |
|---|---|
| TEST SUBSTANCE | Mexoryl SBO (contains the notified chemical at < 30% concentration) |
| METHOD | Similar to OECD TG 439 In vitro Skin Irritation: Reconstructed Human *Epidermis* Test Method<br>EpiSkin$^{SM}$ Reconstituted Human Epidermis Model |
| Vehicle | None |
| Remarks - Method | The test substance (10 µL) was applied to the tissues. Tests were performed in triplicate on three different batches of reconstituted epidermis model (total of 9 tests). After a 15 minute exposure period at room temperature, the tissues were rinsed with PBS and incubated at ~37 °C in fresh medium for 42 hours. The tissues were then treated with MTT and incubated at ~37 °C for 3 hours.<br><br>Cell viability was determined using the MTT test. IL-1α released was determined by the ELISA (enzyme-linked immunosorbent assay) method.<br><br>The study authors indicated that a preliminary test had been conducted, which indicated that the test substance does not directly reduce MTT.<br><br>The test substance was considered by the study authors to be an irritant if the relative mean tissue viability was ≤ 50%, or the relative mean viability |

value was > 50% and the final [IL-1α RM release] ≥ 50 ρg/mL.

Positive and negative controls were run in parallel with the test substance:
Negative control: PBS
Positive control: sodium dodecyl sulfate (5%)

RESULTS

| *Test material* | *Mean $OD_{570}$ of triplicate tissues* | *Relative mean Viability (%)* | *SD of relative mean viability* | *Mean IL-1α (ρg/mL)* |
|---|---|---|---|---|
| *Negative control* | 0.87 | 100 | - | 3.8 ± 0.2 |
| *Test substance* | 0.88 | 98.6 | 2.5 | 12.2 ± 11.6 |
| *Positive control* | 0.08 | 9.4 | 0.72 | - |

OD = optical density; SD = standard deviation

Remarks - Results No significant adverse findings.

CONCLUSION The test substance was non-irritating to the skin under the conditions of the test.

TEST FACILITY Episkin (2010)

**B.3. Irritation – skin**

TEST SUBSTANCE Mexoryl SBO (contains the notified chemical at < 30% concentration)

METHOD OECD TG 404 Acute Dermal Irritation/Corrosion.
EC Directive 2004/73/EC B.4 Acute Toxicity (Skin Irritation).

Species/Strain Rabbit/New Zealand White
Number of Animals 3
Vehicle None
Observation Period 72 h
Type of Dressing Semi-occlusive.
Remarks - Method An initial sighting test (animal number 1) was performed prior to the main tests (animal numbers 2 and 3). The animal in the sighting test was exposed to the test substance for exposure periods of 3 min, 1 h and 4 h.

RESULTS

| *Lesion* | *Mean Score* Animal No.* | | | *Maximum Value* | *Maximum Duration of Any Effect* | *Maximum Value at End of Observation Period* |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | | | |
| *Erythema/Eschar* | 0 | 0 | 0.3 | 1 | 24 h | 0 |
| *Oedema* | 0 | 0 | 0 | 0 | - | 0 |

* Calculated on the basis of the scores at 24, 48, and 72 hours for EACH animal.

Remarks - Results Very slight erythema observed in all animals at the 1 hr observation point for exposure periods of 3 min and 1 h (sighting test) and 4 hr (sighting and main test). This reaction persisted in one animal for at least 24 h. All animals had recovered by the 48 h observation.

CONCLUSION The test substance is non-irritating to the skin.

TEST FACILITY CIT (2009b)

**B.4. Irritation – eye (in vitro)**

TEST SUBSTANCE Mexoryl SBO (contains the notified chemical at < 30% concentration)

METHOD: Similar to OECD TG 437 Bovine Corneal Opacity and Permeability Test Method for Identifying Ocular Corrosives and Severe Irritants

Vehicle: None

Remarks - Method: Exposure periods of 10 min and 30 min were tested.

Positive and negative controls were run in parallel with the test substance:
Negative control: MEM
Positive control: 0.5 % cetyl trimethylammonium bromide (CTAB).

RESULTS

| *Test material* | *Exposure Period* | *Mean opacities of tissues (SD)* | *Mean permeabilities of triplicate tissues (SD)* | *IVIS (SD)* |
|---|---|---|---|---|
| *Vehicle control* | 10 min | 0 | 0.01 (0.006) | - |
| *Vehicle control* | 30 min | 0 | 0.008 (0.007) | - |
| *Test substance** | 10 min | 32.3 (2.1) | 5.954 (0.144) | 121.6 (2.5) |
| *Test substance** | 30 min | 33.3 (6.4) | 5.406 (0.033) | 114.4 (6.7) |

SD = Standard deviation; IVIS = in vitro irritancy score
*Corrected for background values

Remarks - Results: Measurements for the positive control not provided.

According to OECD TG 437 (2013), a substance that induces an *In Vitro* Irritancy Score (IVIS) > 55 is considered a severe eye irritant and is classified as a Category 1 Eye Irritant under the GHS. Substances that induce IVIS Scores ≤ 3 are considered non irritants. However, no prediction can be made for chemicals that produce an 3 < IVIS ≤ 55 given the high false positive rate for the test.

Therefore according to the criteria in OECD TG 437 (2013), the test substance (undiluted) is considered a severe eye irritant.

CONCLUSION: The test substance is a severe eye irritant under the conditions of the test.

TEST FACILITY: IEC (2009)

**B.5. Irritation – eye (in vitro)**

TEST SUBSTANCE: Mexoryl SBO (contains the notified chemical at < 30% concentration)

METHOD: Similar to OECD TG 437 Bovine Corneal Opacity and Permeability Test Method for Identifying Ocular Corrosives and Severe Irritants

Vehicle: Distilled water

Remarks - Method: Exposure periods of 10 min, 30 min and 4 h were tested.

Positive and negative controls were run in parallel with the test substance:
Negative control: MEM
Positive control: 0.5 % cetyl trimethylammonium bromide (CTAB).

Test substance was tested as supplied and at 10% concentration.

RESULTS

| *Test material* | *Exposure Period* | *Mean opacities of tissues (SD)* | *Mean permeabilities of triplicate tissues (SD)* | *IVIS (SD)* |
|---|---|---|---|---|
| | 10 min | 1.3 (1.2) | 0.008 (0.001) | - |
| *Vehicle control* | 30 min | 1 (1) | 0.011 (0.002) | - |
| | 4 hr | 1.3 (1.5) | 0.018 (0.012) | - |
| *Test substance* | 10 min | 33 (1.2) | 3.469 (0.033) | 85 (1.6) |
| *(as supplied)** | 30 min | 26 (2) | 6.178 (0.129) | 118.7 (1) |

| | | | | |
|---|---|---|---|---|
| *Test substance (10%)** | 30 min | 2 (1) | 0.187 (0.057) | 4.8 (0.3) |
| | 4 hr | 2.7 (1) | 3.686 (0.024) | 58 (0.6) |
| *Positive control** | 10 min | 18.3 (2.1) | 1.044 (0.06) | 34 (2.9) |
| | 30 min | 34 (2.6) | 3.974 (0.256) | 93.6 (6.3) |

SD = Standard deviation; IVIS = in vitro irritancy score
*Corrected for background values

Remarks - Results

According to OECD TG 437 (2013), a substance that induces an *In Vitro* Irritancy Score (IVIS) > 55 is considered a severe eye irritant and is classified as a Category 1 Eye Irritant under the GHS. Substances that induce IVIS Scores ≤ 3 are considered non irritants. However, no prediction can be made for chemicals that produce an 3 < IVIS ≤ 55 given the high false positive rate for the test.

Therefore according to the criteria in OECD TG 437 (2013), the test substance (undiluted) is considered a severe eye irritant. No prediction can be made on the irritation level for the test substance at 10% concentration.

CONCLUSION

The test substance is a severe eye irritant under the conditions of the test.

TEST FACILITY

IEC (2010)

**B.6. Irritation – eye**

TEST SUBSTANCE

Mexoryl SBO (contains the notified chemical at < 30% concentration)

METHOD

OECD TG 405 Acute Eye Irritation/Corrosion.
EC Directive 2004/73/EC B.5 Acute Toxicity (Eye Irritation).

Species/Strain: Rabbit/New Zealand White
Number of Animals: 3
Observation Period: 72 h

Remarks - Method

An initial sighting test was performed on an undiluted sample of the test substance. This animal exhibited a severe irritant reaction with conjunctival swelling, diffuse redness and moderate to slight discharge over time. At the 24 and 48 h observations, moderate iridial inflammation and moderate to severe corneal opacity was observed. This animal was euthanised on Day 3 (72 h after exposure) based on the severe reactions to the test substance.

A second sighting test on a 10% dilution of the test substance was then performed prior to the main test (results described below).

RESULTS

| *Lesion* | *Mean Score** *Animal No.* | | | *Maximum Value* | *Maximum Duration of Any Effect* | *Maximum Value at End of Observation Period* |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | | | |
| *Conjunctiva: redness* | 0 | 0.3 | 0 | 1 | < 48 h | 0 |
| *Conjunctiva: chemosis* | 0 | 0 | 0 | - | - | 0 |
| *Conjunctiva: discharge* | 0.3 | 0 | 0 | 1 | < 48 h | 0 |
| *Corneal opacity* | 0 | 0 | 0 | - | - | 0 |
| *Iridial inflammation* | 0 | 0 | 0 | - | - | 0 |

* Calculated on the basis of the scores at 24, 48, and 72 hours for EACH animal.

Remarks - Results

On initial exposure, all animals exposed to a 10% concentration of the test substance exhibited slight conjunctival chemosis and/or redness. At the 24 h observation, one animal showed conjunctival discharge, while another animal exhibited slight conjunctival redness. All effects were reversed by the 48 h observation point.

CONCLUSION

The test substance at 10% concentration is slightly irritating to the eye.

TEST FACILITY CIT (2009c)

**B.7. Skin sensitisation – mouse local lymph node assay (LLNA)**

TEST SUBSTANCE Mexoryl SBO (contains the notified chemical at < 30% concentration)

METHOD OECD TG 429 Skin Sensitisation: Local Lymph Node Assay
EC Directive 2004/73/EC B.42 Skin Sensitisation (Local Lymph Node Assay)

Species/Strain Mouse/CBA/J

Vehicle Dimethylformamide

Remarks - Method Test substance tested in two batches: No. 022 D 001 and No. 023 D 001.

All test animals female.

Positive and negative controls were run in parallel with the test substance:
Negative control: dimethylformamide
Positive control: α-hexyl cinnamaldehyde

RESULTS

| *Concentration (% w/w)* | *Proliferative response (DPM/lymph node)* | *Stimulation Index (Test/Control Ratio)* |
|---|---|---|
| 0 (vehicle control) | 81.44 | - |
| *Test Substance (No. 022 D 001)* | | |
| 10 | 68.65 | 0.84 |
| 25 | 109.52 | 1.34 |
| 50 | 42.79 | 0.53 |
| 75 | 64.92 | 0.80 |
| 100 | 104.37 | 1.28 |
| *Test Substance (No. 023 D 001)* | | |
| 10 | 84.22 | 1.03 |
| 25 | 87.75 | 1.08 |
| 50 | 76.64 | 0.94 |
| 75 | 123.28 | 1.51 |
| 100 | 128.13 | 1.57 |
| *Positive Control* | | |
| 25 | 450.91 | 5.54 |

Remarks - Results All animals gained body weight as expected. No clinical signs of toxicity or mortality were observed. No skin reactions or significant changes in ear thickness were observed in any animals.

Positive and negative controls performed as expected.

No clear dose-response relationship was observed between the concentration of the test substance and the stimulation index. All stimulation indices were < 3.

CONCLUSION There was no evidence of induction of a lymphocyte proliferative response indicative of skin sensitisation to the test substance.

TEST FACILITY CIT (2008)

**B.8. Skin sensitisation – mouse local lymph node assay (LLNA)**

TEST SUBSTANCE Mexoryl SBO (contains the notified chemical at < 30% concentration)

METHOD OECD TG 429 Skin Sensitisation: Local Lymph Node Assay

| | |
|---|---|
| | EC Directive 2004/73/EC B.42 Skin Sensitisation (Local Lymph Node Assay) |
| Species/Strain | Mouse/CBA/J |
| Vehicle | Dimethylformamide |
| Remarks - Method | All test animals female.<br><br>Positive and negative controls were run in parallel with the test substance:<br>Negative control: dimethylformamide<br>Positive control: α-hexyl cinnamaldehyde |

RESULTS

| *Concentration (% w/w)* | *Proliferative response (DPM/lymph node)* | *Stimulation Index (Test/Control Ratio)* |
|---|---|---|
| *Test Substance* | | |
| 0 (vehicle control) | 96.72 | - |
| 10 | 136.34 | 1.41 |
| 25 | 110.85 | 1.15 |
| 50 | 109.34 | 1.13 |
| 75 | 59.61 | 0.62 |
| 100 | 107.84 | 1.11 |
| *Positive Control* | | |
| 25 | 481.03 | 4.97 |

| | |
|---|---|
| Remarks - Results | All animals gained body weight as expected. No clinical signs of toxicity or mortality were observed. On day 6, skin dryness of the ears was recorded in one animal treated with 75% of the test substance. All animals were sacrificed on Day 6 so it is unknown if this was a transitory effect. No other skin reactions were observed in any of the other animals. No significant changes in ear thickness were observed in any animals.<br><br>Positive and negative controls performed as expected.<br><br>No clear dose-response relationship was observed between the concentration of the test substance and the stimulation index. All stimulation indices were < 3. |
| CONCLUSION | There was no evidence of induction of a lymphocyte proliferative response indicative of skin sensitisation to the test substance. |
| TEST FACILITY | CIT(2009d) |

**B.9. Repeat dose toxicity**

| | |
|---|---|
| TEST SUBSTANCE | Mexoryl SBO (contains the notified chemical at < 30% concentration) |
| METHOD | Based on OECD TG 407 Repeated Dose 28-day Oral Toxicity Study in Rodents.<br>OECD TG 410 Repeated Dose Dermal Toxicity: 28-day Study. |
| Species/Strain | Rat/ Sprague-Dawley/Crl CD® |
| Route of Administration | Oral – gavage<br>Dermal –non-occluded |
| Exposure Information | Total exposure days: 28 days<br>Dose regimen: 7 days per week<br>Duration of exposure (dermal): 24 hours/day<br>Post-exposure observation period: None |
| Vehicle | Aqueous carboxymethylcellulose (0.5%) |
| Remarks - Method | Only one dose level was applied in the repeated dose dermal toxicity study. No other significant protocol deviations were recorded. |

RESULTS

*Oral Administration*

| *Group* | *Number and Sex of Animals* | *Dose mg/kg bw/day* | *Mortality* |
|---|---|---|---|
| control | 10 M, 10 F | - | 0/20 |
| low dose | 10 M, 10 F | 100 | 0/20 |
| mid dose | 10 M, 10 F | 300 | 0/20 |
| high dose | 10 M, 10 F | 1000 | 0/20 |

*Dermal Administration*

| *Group* | *Number and Sex of Animals* | *Dose mg/kg bw/day* | *Mortality* |
|---|---|---|---|
| control | 10 M, 10 F | - | 0/20 |
| Test substance | 10 M, 10 F | 100 | 0/20 |

*Mortality and Time to Death*

No unscheduled deaths occurred during the study.

*Clinical Observations*

*Oral administration:*

Where the test substance was administered orally, excessive salivation was observed in 6/10 M and 7/10 F in the high-dose group during the last week of treatment. This was not considered an adverse effect of the test substance as the effect is commonly observed where a substance is delivered by oral gavage. Loud breathing was observed in 1/10 M in the low-dose group (from day 24) and 2/10 F exhibited an emaciated appearance (one female also exhibited a round back) from days 22 to 23. These effects were not considered to be treatment related as they were transient and occurred at a low incidence.

All males exhibited slightly higher body weights (compared to controls), with males in the high-dose group gaining a statistically significantly increase in weight during the last week of the study. A dose-response relationship was not observed so the weight gain was not considered to be an adverse effect of the test substance. No significant differences in mean body weight gains in females across all dose-groups and controls were observed.

*Dermal administration:*

Scabs (2/10 F), associated with very slight or well-defined erythema (before treatment), and flaking (1/10 F) were observed between days 6 and 9 in control group animals. Two females (2/10) exposed to the test substance exhibited well-defined to severe erythema between days 4 and 7 (one animal also exhibited scabs and vocalization during dosing) and flaking on day 8. These effects were attributed to the vehicle and not the test substance based on their transient, nature and similar levels of occurrence in control and test-substance groups.

Mean body weight gains were similar between control and test-substance exposed animals.

*Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis*

*Oral administration:*

Males in the high-dose group exhibited slightly lower erythrocyte count, haemoglobin and mean cell haemoglobin concentrations and a higher mean cell volume. While these differences were within historical background data, they were considered to be related to treatment with the test item. Differences observed in haemoglobin concentration in mid- and high-dose group females were not considered toxicologically significant as they were slight and not associated with changes in other red blood cell parameters. No other significant or dose-related differences were observed.

Statistically significant higher triglyceride levels and lower mean chloride levels were observed in males and females in the high dose group. Lower mean calcium levels and higher mean alkaline phosphatase activity was recorded in females and lower mean inorganic phosphorous levels in males was recorded across all dosages. Differences in potassium, sodium, total protein, albumin and cholesterol levels remained within expected physiological ranges. Higher triglyceride levels were attributed to the test substance as the other effects

observed were slight or a clear dose-response relationship could not be established.

The lower mean urinary pH observed in males and females in mid- and high-dose groups was attributed to the test substance. However, no other related effects were observed and the authors considered the effect to be non-adverse. Any other urinary effects were not dose-related and not considered to be toxicologically important.

*Dermal administration:*
Males exhibited a statistically significant lower mean erythrocyte count (compared to control). However, no other changes in red blood cell parameters were recorded and the change was not considered toxicologically significant. Any other differences were not significant or fell within the range of historical background data.

Differences observed in the albumin/globulin ratio and in chloride, urea, glucose and triglyceride levels remained within expected physiological ranges and were not considered to be treatment related.

*Effects in Organs*
No treatment-related ophthalmological observations were recorded in any animals.

Vacuolated hepatocytes were recorded in control and animals exposed to the test substance, with treated males exhibiting a slightly higher incidence. This effect is observed spontaneously in untreated rats of this strain and age and the higher incidence in males was not considered toxicologically important,

*Oral administration:*
Higher liver weights were recorded in males and females in the high-dose group with liver enlargement (2/10 M and 1/10 F) and minimal to slight hepatocellular hypertrophy (10/10 M and 8/10 F) also observed. No other effects were attributed to the test substance,

*Dermal administration:*
No adverse toxic effects were attributed to the test substance. Minimal thickening of the skin was recorded in the majority of treated sites in both control and test substance groups. This was attributed to the treatment technique and not the test substance.

Remarks – Results
Where the test substance was administered by oral gavage, males and females in the high-dose group exhibited excessive saliva secretion, lower erythrocyte count, lower haemoglobin and mean cell haemoglobin concentrations, higher mean cell volume and higher triglyceride levels. Males and females in the high-dose group exhibited adverse liver effects including hepatocellular hypertrophy, higher liver weights and liver enlargement.

No adverse effects were recorded for those animals exposed to the test substance dermally, or in those animals in the low- and mid-dose groups exposed to the test substance by oral gavage.

CONCLUSION
The oral No Observed (Adverse) Effect Level (NO(A)EL) was established as 300 mg/kg bw/day in this study, based on liver effects.

The dermal No Observed (Adverse) Effect Level (NO(A)EL) was established as 100 mg/kg bw/day in this study, based on no adverse effects at the highest dose tested.

| | |
|---|---|
| TEST FACILITY | CIT (2004a) |

**B.10. Repeat dose toxicity**

| | |
|---|---|
| TEST SUBSTANCE | Mexoryl SBO (contains the notified chemical at < 30% concentration) |
| METHOD | Similar to OECD TG 410 Repeated Dose Dermal Toxicity: 21/28-day Study |
| Species/Strain | Rat/ Sprague-Dawley/Rj Han:SD |
| Route of Administration | Dermal –non-occluded |
| Exposure Information | Total exposure days: 14 days |

| | |
|---|---|
| | Dose regimen: 7 days per week<br>Duration of exposure (dermal): 6 hours/day<br>Post-exposure observation period: 0 |
| Vehicle | Purified water |
| Remarks - Method | Study was conducted over 14-days and not the 21/28 days as described in the test guideline. No other significant protocol deviations.<br><br>Hair was clipped using clippers and not a chemical depilatory. Doses were selected based on results of a previous study (CIT 2004a). The test substance was supplied as "ready-to-use" to avoid the occurrence of effects related to the vehicle rather than the test substance. Composition of formulation and concentration of test substance was not stated.<br><br>Technical issues with the material administered to mid-dose males and high-dose males and females required an additional control group and high-dose group (phase II) to be tested. Only a high-dose group was introduced in phase II as an absence of toxic effects had been recorded in high-dose males during phase I,<br><br>Results from both control groups (phase I and Phase II) were reported while only the results from the phase II high-dose group were reported. |

RESULTS

| *Group* | *Number and Sex of Animals* | *Dose mg/kg bw/day* | *Mortality* |
|---|---|---|---|
| Control (phase I) | 5 M, 5 F | - | 0/10 |
| Control (phase II) | 5 M, 5 F | - | 0/10 |
| low dose | 5 M, 5 F | 250 | 0/10 |
| mid dose | 5 M, 5 F | 500 | 0/10 |
| high dose (phase II) | 5 M, 5 F | 1000 | 0/10 |

*Mortality and Time to Death*

No unscheduled deaths occurred during the study.

*Clinical Observations*

There was no evidence of systemic toxicity in any of the animals tested.

Within the high-dose group, males and females exhibited a slightly lower mean body weight gain between days 11 and 14 which was only statistically significant in males. This effect was transient and the study authors did not consider it to be related to the test substance.

Dermal irritation was not observed in any male animals. Females in the low- and mid-dose groups (1/5 animals in each group) exhibited very slight erythema. This effect was also observed in the phase I control group and the effect may be considered to be unrelated to the test substance. Well-defined erythema (3/5) and scab formation (1/5) were observed in females in the high-dose group.

*Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis*

No haematology or urinalysis effects were attributed to the test-substance. Increased levels of fibrinogen in males in the high-dose group were also observed in the control group. Increased urea and creatinine levels in high-dose females were attributed to the presence of the test-substance as the effect was not observed in controls. No other blood biochemistry effects were recorded for males and females in the low- and mid-dose groups, or males in the high-dose group.

*Effects in Organs*

Males in phase I exhibited higher kidney and liver weighs (high-dose) and higher mean absolute and relative thyroid weights (mid-dose). The higher kidney and liver weights in the high-dose group were not reproduced in phase II animals. The weight changes were not attributed to the test substance by the authors as the changes were not significant, did not exhibit a dose-response relationship, were not seen in females and had no macroscopic correlation. Within the high-dose phase II animals, lower absolute thymus weights in males and

higher ovaries weights in females were recorded. There was no macroscopic correlate, and the increase in ovaries weights was attributed to cycling rather than the test substance.

Macroscopic examination revealed a sore on the treated skin of 1/5 females in the high-dose group. However this was not attributed to the test substance given the low incidence.

Thickening of the skin was observed in control and treated animals and was considered by the authors to be a secondary effect of clipping hair for treatment rather than an adverse effect of the test substance.

Remarks – Results
No treatment related findings were recorded for the low- and mid-dose animals. Females in the high-dose group (phase II) exhibited very slight to well-defined erythema and scab formations at the application site as well as increased urea and creatinine levels. Based on these results, the No Observed Effect Level (NOEL) was considered by the study authors to be 1000 mg/kg bw/day for males and 500 mg/kg bw/day for females.

CONCLUSION
A No Observed Effect Level (NOEL) of 1000 mg/kg bw/day was established for males based on the absence of treatment related adverse effects. A NOEL of 500 mg/kg bw/day was established for females based on skin irritation effects and an increase in urea and creatinine levels.

TEST FACILITY CIT (2009e)

**B.11. Repeat dose toxicity**

TEST SUBSTANCE Mexoryl SBO (contains the notified chemical at < 30% concentration)

METHOD OECD TG 408 Repeated Dose 90-Day Oral Toxicity Study in Rodents.
Species/Strain Rat/Wistar (RccHan: WIST)
Route of Administration Oral – gavage
Exposure Information Total exposure days: 90 days
Dose regimen: 7 days per week
Post-exposure observation period: None
Vehicle Aqueous carboxy methyl cellulose (0.5%)
Remarks - Method No significant protocol deviations

RESULTS

| *Group* | *Number and Sex of Animals* | *Dose mg/kg bw/day* | *Mortality* |
|---|---|---|---|
| control | 10 M, 10 F | - | 0/20 |
| low dose | 10 M, 10 F | 100 | 0/20 |
| mid dose | 10 M, 10 F | 300 | 0/20 |
| high dose | 10 M, 10 F | 1000 | 0/20 |

*Mortality and Time to Death*
No unscheduled deaths occurred during the study.

*Clinical Observations*
No treatment-related clinical signs were observed in low-and mid-dose groups. Mild to moderate salivation was observed in animals in the high-dose group.

*Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis*
No treatment related changes were observed in haematology and urinalysis parameters. Males and females in the high-dose group exhibited changes in alkaline phosphatase, total bilirubin and triglyceride levels.

*Effects in Organs*
Higher mean liver weight (absolute and relative) and microscopic lesions in the liver (micro- and macro-vesicular hepatocellular vacuolation, mostly periportal) were observed in the high dose group.

Remarks – Results

No toxicologically significant effects related to exposure to the test substance on body weight, body weight gain, food consumption, ophthalmological examination, neurobehavioural observations or functional observational battery testing were observed.

No treatment related clinical signs were observed in the low- and mid-dose groups. Animals in the high-dose group exhibited mild to moderation salivation intermittently from week 9 to the end of the treatment period. While the increase in salivation was considered related to the test substance there were no other supporting observations or findings so the effect was not considered adverse.

Changes observed in blood clinical parameters (alkaline phosphatase, total bilirubin and triglyceride levels) of males and females in the high-dose group were associated with higher mean liver weight (absolute and relative) and microscopic lesions in the liver (micro- and macro-vesicular hepatocellular vacuolation, mostly periportal).

CONCLUSION
The No Observed (Adverse) Effect Level (NO(A)EL) was established as 300 mg/kg bw/day in this study, based on liver effects at the highest dose tested.

TEST FACILITY JRF (2013)

**B.12. Genotoxicity – bacteria**

TEST SUBSTANCE Mexoryl SBO (contains the notified chemical at < 30% concentration)

METHOD OECD TG 471 Bacterial Reverse Mutation Test.
Test 1: Plate incorporation procedure
Test 2: Pre-incubation procedure

| | |
|---|---|
| Species/Strain | *S. typhimurium*: TA1535, TA1537, TA98, TA100, TA102 |
| Metabolic Activation System | S9 mix from Aroclor 1254 induced rat liver |
| Concentration Range in Test 1 | a) With metabolic activation: 1.6, 8, 40, 200, 1000, 5000 µg/plate<br>b) Without metabolic activation: 1.6, 8, 40, 200, 1000 5000 µg/plate |
| Concentration Range in Test 2 | a) With metabolic activation: 156.25, 312.5, 625, 1250, 2500, 5000 µg/plate<br>b) Without metabolic activation: 156.25, 312.5, 625, 1250, 2500, 5000 µg/plate |
| Vehicle | Dimethyl sulphoxide |
| Remarks - Method | The dose was adjusted for the concentration of the notified chemical.<br><br>Positive and vehicle controls were run concurrently with the test substance.<br>Positive controls: i) with metabolic activation: benzo[a]pyrene (TA98), 2-aminoanthracene (TA100, TA1535, TA1537, TA102; ii) without metabolic activation: 2-Nitrofluorene (TA98), sodium azide (TA100, TA1535), 9-aminoacridine (TA1537), mitomycin C (TA102).<br><br>Range finding test performed using the test substance at concentrations 1.6, 8, 40, 200, 1000 and 5000 µg/plate on TA100 in the presence and absence of metabolic activation. No evidence of toxicity was observed. This test was considered as part of the results for test 1 by the authors. |

RESULTS

| *Metabolic Activation* | *Test Substance Concentration (µg/plate) Resulting in:* | | | |
|---|---|---|---|---|
| | *Cytotoxicity in Preliminary Test* | *Cytotoxicity in Main Test* | *Precipitation* | *Genotoxic Effect* |
| *Absent* | | | | |
| Test 1 | > 5000 | ≥ 5000 | > 5000 | negative |
| Test 2 | | ≥ 1250 | > 5000 | negative |
| *Present* | | | | |
| Test 1 | > 5000 | ≥ 1000 | > 5000 | negative |
| Test 2 | | ≥ 5000 | > 5000 | negative |

| | |
|---|---|
| Remarks - Results | Positive and vehicle controls performed as expected.<br><br>In test 1, toxicity was observed at ≥ 1000 µg/plate in strain TA102 in the presence of metabolic activation, and at 5000 µg/plate in strain TA102 in the absence of metabolic activation.<br><br>In test 2, a reduction in the number of revertants was observed in strain TA102 at 5000 µg/plate in the presence of metabolic activation and at ≥ 1250 µg/plate in the absence of metabolic activation.<br><br>No evidence of toxicity was observed in the strains TA1535, TA1537, TA98 or TA100 in the presence or absence of metabolic activation in either test 1 or test 2.<br><br>No biologically relevant increases in the frequency of revertant colonies were observed for any of the strains tested, in the presence or absence of metabolic activation in either test 1 or test 2. |
| CONCLUSION | The notified chemical was not mutagenic to bacteria under the conditions of the test. |
| TEST FACILITY | Covance (2009 a) |

**B.13. Genotoxicity – in vitro**

| | |
|---|---|
| TEST SUBSTANCE | Mexoryl SBO (contains the notified chemical at < 30% concentration) |
| METHOD | OECD TG 473 In vitro Mammalian Chromosome Aberration Test.<br>EC Directive 2000/32/EC B.10 Mutagenicity - In vitro Mammalian Chromosome Aberration Test. |
| Species/Strain | Human |
| Cell Type/Cell Line | Lymphocytes |
| Metabolic Activation System | S9 mix from Aroclor 1254 induced rat liver |
| Vehicle | Culture medium |
| Remarks - Method | Positive (mitomycin C and cyclophosphamide) and vehicle controls were run concurrently with the test substance except for Test 2b (in the presence and absence of metabolic activation).<br><br>A third confirmatory test was run in the absence of metabolic activation as part of the experimental design. |

| *Metabolic Activation* | *Test Substance Concentration (µg/mL)* | *Exposure Period* | *Harvest Time* |
|---|---|---|---|
| *Absent* | | | |
| Test 1 | 39.06, 78.13, 156.3, 312.5, 625, 1250*, 2500*, 5000* | 3 h | 20 h |
| Test 2a | 156.3, 312.5, 625, 1250*, 2500*, 5000* | 20 h | 20 h |
| Test 2b | 156.3, 312.5, 625, 1250*, 2500, 5000 | 44 h | 44 h |
| Test 3 | 1000, 2000, 2500, 3750*, 4167*, 5000* | 20 h | 20 h |
| *Present* | | | |
| Test 1 | 39.06, 78.13, 156.3, 312.5, 625, 1250*, 2500*, 5000* | 3 h | 20 h |
| Test 2a | 156.3, 312.5, 625, 1250*, 2500*, 5000* | 3 h | 20 h |
| Test 2b | 156.3, 312.5, 625, 1250, 2500, 5000* | 3 h | 44 h |

*Cultures selected for metaphase analysis.

RESULTS

| *Metabolic Activation* | *Test Substance Concentration (µg/mL) Resulting in:* | | | |
|---|---|---|---|---|
| | *Cytotoxicity in Preliminary Test* | *Cytotoxicity in Main Test* | *Precipitation* | *Genotoxic Effect* |

| | | | | |
|---|---|---|---|---|
| *Absent* | | | | |
| Test 1 | - | > 5000 | > 5000 | negative |
| Test 2a | | ≥ 2500 | > 5000 | negative |
| Test 2b | | ≥ 2500 | > 5000 | negative |
| Test 3 | | > 5000 | > 5000 | negative |
| *Present* | | | | |
| Test 1 | - | > 5000 | > 5000 | negative |
| Test 2a | | > 5000 | > 5000 | negative |
| Test 2b | | > 5000 | > 5000 | negative |

Remarks - Results

In the absence of metabolic activation, the cytotoxicity observed following a 20 h exposure period (test 2a) was not reproducible (test 3). However cytotoxicity was observed at dose levels ≥ 2500 µg/mL following a 44 h exposure period (test 2b), with a clear dose-response relationship observed in the decrease in mitotic index.

No significant increase in the frequency of cells with structural chromosomal aberrations was recorded for cells exposed for 3 h or 44 h (tests 1 and 2b). A slight significant increase in aberrations was observed for cells exposed for 20 h (tests 2a and 3) at the highest exposure dose only (5000 µg/mL). However, the increases recorded were not considered to be biologically relevant by the study authors as the aberrations were observed at concentrations higher than 10 mM which can induce artefactual increases in aberrations.

In the presence of metabolic activation, cytotoxicity was not observed and there was no significant increase in the frequency of cells with structural chromosomal aberrations recorded.

Positive and negative controls performed as expected.

CONCLUSION

The test substance was not clastogenic to human lymphocytes treated in vitro under the conditions of the test.

TEST FACILITY

CIT (2004b)

**B.14. Genotoxicity – in vitro**

TEST SUBSTANCE

Mexoryl SBO (contains the notified chemical at < 30% concentration)

METHOD

Similar to OECD TG 487 In vitro Mammalian Cell Micronucleus Test.

Species/Strain: Human

Cell Type/Cell Line: Lymphocytes

Metabolic Activation System: S9 mix from Aroclor 1254 induced rat liver

Vehicle: Dimethyl sulphoxide

Remarks - Method

The dose was adjusted for the concentration of the notified chemical.

Phytohaemagglutinin (PHA) included in culture medium to stimulate cell division.

Cells treated in the absence of metabolic activation had Cytochalasin B added at the time of treatment.

Positive controls: i) presence of metabolic activation – cyclophosphamide; ii) absence of metabolic activation - mitomycin C (clastogenicity) and vinblastine (aneuploidy).

Positive and vehicle controls were run concurrently with the test substance (in the presence and absence of metabolic activation).

No significant protocol deviations.

| *Metabolic Activation* | *Test Substance Concentration (µg/mL)* | *Exposure Period* | *Recovery Period* | *Harvest Time* |
|---|---|---|---|---|
| *Absent* | | | | |
| Test 1 | 701.2, 876.6, 1096, 1370*, 1712*, 2140* | 3 h | 21 h | 72 h |
| Test 2 | 400*, 450, 500, 550*, 600, 650, 700, 750*, 800, 900, 1000, 1250, 1500, 1750, 2000 | 24 h | 0 h | 72 h |
| *Present* | | | | |
| Test 1 | 701.2, 876.6, 1096, 1370*, 1712*, 2140* | 3 h | 21 h | 72 h |

*Cultures selected for bi- and multinucleate cell analysis.

RESULTS

| *Metabolic Activation* | *Test Substance Concentration (µg/mL) Resulting in:* | | | |
|---|---|---|---|---|
| | *Cytotoxicity in Preliminary Test* | *Cytotoxicity in Main Test* | *Significant Increase in MNBN Cell Frequency* | *Genotoxic Effect* |
| *Absent* | | | | |
| Test 1 | - | > 2140 | > 2140 | negative |
| Test 2 | ≥ 770.6 | ≥ 750 | > 750 | negative |
| *Present* | | | | |
| Test 2 | > 2140 | > 2140 | > 2140 | negative |

MNBN – proportion of micronucleated binucleate cells

| | |
|---|---|
| Remarks - Results | There was no indication of cytotoxicity (in the presence or absence of metabolic activation) in cells exposed to the test substance for 3 h. Cytotoxic effects observed in cells exposed to the test substance for 24 h did not follow a clear dose-response relationship.<br><br>No significant increases in the frequencies of micronucleated binucleate cells were observed in the presence or absence of metabolic activation for all concentrations and exposure periods tested.<br><br>Positive and negative controls performed as expected. |
| CONCLUSION | The notified chemical was not clastogenic to human lymphocytes treated in vitro under the conditions of the test. |
| TEST FACILITY | Covance (2009b) |

**B.15. Developmental toxicity**

| | |
|---|---|
| TEST SUBSTANCE | Mexoryl SBO (contains the notified chemical at < 30% concentration) |
| METHOD | Similar to OECD TG 414 Prenatal Developmental Toxicity Study. |
| Species/Strain | Rat/Wistar |
| Route of Administration | Oral – gavage |
| Exposure Information | Exposure days: gestation days 6-19<br>Post-exposure observation period: None |
| Vehicle | 0.5% Carboxymethylcellulose and 0.1% Tween 80 |
| Remarks - Method | Animals were 13 weeks old at mating instead of 9 weeks old. No other significant protocol deviations. |

RESULTS

| *Group* | *Number of Animals* | *Dose mg/kg bw/day* | *Mortality* |
|---|---|---|---|
| control | 23 | - | 0/23 |
| low | 20 | 100 | 0/20 |

| | | | |
|---|---|---|---|
| mid | 22 | 300 | 0/22 |
| high | 23 | 1000 | 0/23 |

*Mortality and Time to Death*

No abnormal clinical findings or mortalities in any of the animals.

*Effects on Dams*

Dams in the high-dose group exhibited a statistically significant lower body weight gain from days 9 to 12 of gestation which was correlated with decreased food consumption during this period. Overall body weight gain was slightly lower across all treated animals with a dose-response relationship observed, although this difference did not reach statistical significance. However, dams in the mid- and high-dose groups exhibited a statistically significant lower "corrected" maternal body weight gain. The corrected bodyweight gain was calculated by carcass weight minus body weight on day 6 of gestation. The carcass weight was obtained by terminal body weight (body weight on day 20 of gestation) minus unopened uterine weight.

The test substance was not attributed to any reproduction parameters. Post-implantation loss and mean number of live foetuses was similar among all groups. A single animal in the mid-dose group (1/22) had a single late resorption and no other implantation sites (post-implantation loss of 100%).

No test-substance related effects were observed at necropsy.

*Effects on Foetus*

No clear dose-response relationship was observed for differences in placental weight so the effect was not considered to be biologically relevant. Foetal weight (male, female and total) was statistically lower in mid- and high-dose groups, but no biologically relevant alterations were observed in the low-dose group.

No malformations or variations were observed on external and soft tissue examinations of foetuses. All groups showed similar numbers of foetuses and litters with soft tissue alterations. No skeletal malformations were recorded in foetuses from the low-, mid-, and high-dose groups. Absent and fused sternebrae were observed in 2 foetuses of 2 litters in the control group. These were considered to be spontaneous malformations.

Foetal and litter incidence of short supernumerary ($14^{th}$) rib was statistically significantly higher in the high-dose group (50.5% of the foetuses affected and 69.6% of the litters affected when compared to the control group (7.4% of the foetuses affected and 26.1% of the litters affected). In the mid- and low-dose groups, foetal incidence of short supernumerary ribs was moderately higher than in the control group (23.2% and 21.7% versus 7.4% of the foetuses affected, respectively). While significant, these observations are within the historical range and litter incidence was generally unaffected.

Foetuses in the low- and high-dose groups showed higher incidences of dumbbell-shaped thoracic vertebrae (14.5% and 13.4 % respectively). As no statistical difference was observed on the litter data, and a dose-response relationship was not observed, the effects were not considered to be an adverse effect of the test substance. The incidence of foetuses affected by wavy ribs was significantly lower in the high-dose group. Other variations which occurred were considered to be spontaneous.

Foetal incidence of unossified sternebra was statistically significant increased in the low-, mid- and high-dose groups (17.4%, 17.2% and 28.9% of affected foetuses respectively), but still within the historical range data. The effect was not considered related to the test-substance as the exposed groups showed no alterations in the litter incidence of sternebra not ossified, and the incidence of foetal and litter retardation affecting the sternebrae (including all retardation of the ossification process) was similar among controls and test substance exposed groups.

Other observations or retardations which were statistically different from the control (such as a higher incidence of incomplete ossification of the supraoccipital bone of foetuses in the mid-dose group) did not show a dose-response relationship and were either considered to be spontaneous or not biologically relevant.

Remarks - Results

Signs of maternal toxicity were recorded for animals exposed to mid- and high-doses of the test substance including lower body weight gain (gestation days 9 to 12) correlated to lower food consumption during this period (high-dose group), and lower corrected maternal body weight gain (mid- and high-dose groups). Slightly lower foetal weight was recorded for dams in the mid- and high-dose groups. A higher incidence of foetuses

and litters affected by short supernumerary ribs was observed at the high-dose level.

CONCLUSION
The No Observed (Adverse) Effect Level (NO(A)EL) for maternal toxicity was established as 100 mg/kg bw/day in this study, based on lower body weight gains.

The No Observed (Adverse) Effect Level (NO(A)EL) for developmental toxicity was established as 100 mg/kg bw/day in this study, based on slightly lower foetal weights and higher foetal and litter incidence of a skeletal variation (short supernumerary rib).

TEST FACILITY BIOAGRI (2010)

**B.16. Dermal penetration – in vitro**

| | |
|---|---|
| TEST SUBSTANCE | Formulation containing the notified chemical at 4% |
| METHOD | OECD TG 428 Skin Absorption: *in vitro* Method |
| Species/strain | Human skin (dermatomed) |
| Membrane integrity | Electrical resistance barrier integrity |
| Group size | 12 skin membranes from 4 human donors |
| Purity | 95% (RHPLC) radio-labelled test substance |
| Dose(s) applied | 80 µg/ $cm^2$ of test substance in 2 mg/$cm^2$ of the test formulation |
| Dose volume/amount | 203 µg |
| Receptor Fluid | Phosphate buffered saline (PBS) |
| Method of Analysis | Liquid scintillation counting (LSC) |
| Remarks - Method | The test substance was formulated to represent a typical cosmetic product containing the notified chemical at a concentration of 4%. The penetration of the notified chemical out of this formulation through human dermatomed skin over 24 hr (un-occluded) was then determined *in vitro* using static glass diffusion cells.<br><br>An electrical resistance barrier integrity test was performed and any human skin sample exhibiting a resistance < 10 kΩ was excluded from penetration measurements. Discs of intact human dermatomed skin membranes (12 skin membranes from 4 human donors) were then mounted, dermal side down (exposed membrane area of 2.54 $cm^2$), in diffusion cells and maintained at a constant temperature (32 ± $1^0$C). The receptor chambers of the diffusion cells were filled with a recorded volume of PBS which ensured free partition of the tests substance into the receptor fluid (adequate sink conditions).<br><br>The dose was applied at a nominal rate of 80 µg/$cm^2$ (nominal total dose of 203 µg) in terms of test substance. At the end of the exposure period, the skin was washed twice with aqueous 2% (v/v) sodium dodecyl sulfate (SDS) solution and twice with water. Between each set of washes the washing fluid was aspirated and residual radioactivity levels on the skin surface assessed. All wash steps were repeated until decontamination was complete. After the final washes which were performed with water only, the skin surface was dried using cotton wool swabs. Once the donor chamber was removed, the surface of the skin was allowed to dry naturally. The stratum corneum was removed by a tape stripping process removing up to a maximum of 20 strips from each skin membrane. The flange skin was cut away from the dermis and the epidermis on the remaining skin disk, and the dermis was separated from the dermis using a heat separation technique.<br><br>The penetration process was monitored using [$^{14}$C]-radiolabelled test substance, which was incorporated into the formulation prior to application. The distribution of notified chemical within the test system was measured and a 24 hr penetration profile was determined by collecting receptor fluid samples (0.5, 1, 2, 4, 8, 12, 24 hr after application). Receptor fluid samples, donor chamber wash, skin wash, stratum corneum, remaining epidermis (following tape stripping), |

dermis, and flange were analysed for radioactivity contents by means of LSC.

RESULTS

| *Test compartment* | *Recovery (mean ± SD, n =9 )* | |
|---|---|---|
| | *[µg/cm²]* | *[% of applied dose]* |
| Donor chamber | 0.114 ± 0.183 | 0.140 ± 0.224 |
| Skin wash at 24 hr | 76.8 ± 4.33 | 94.1 ± 5.31 |
| *Stratum corneum* | 2.07 ± 1.07 | 2.54 ± 1.31 |
| Remaining epidermis | 1.88 ± 0.914 | 2.30 ± 1.12 |
| Dermis | 0.106 ± 0.080 | 0.130 ± 0.98 |
| Flange | 0.019 ± 0.035 | 0.024 ± 0.043 |
| Receptor fluid | 0.403 ± 0.424 | 0.494 ± 0.520 |
| Total non-absorbed[1] | 79.0 ± 4.73 | 96.8 ± 5.79 |
| Systemically available[2] | 2.39 ± 1.24 | 2.92 ± 1.52 |
| Total recovered | 81.4 ± 4.52 | 99.7 ± 5.54 |

*SD – standard deviation; n: number of samples*
*[1] Sum of the applied dose retrieved in the donor chamber, skin wash, stratum corneum and flange skin*
*[2] Sum of the applied dose retrieved in the remaining epidermis, dermis and the receptor fluid*

Remarks - Results

After a short lag phase of 2 hr, the mean penetration rate of the notified chemical was 0.018 µg/cm²/h between 2-24 hr. Between 0 – 24 hr, the overall penetration rate was 0.016 µg/cm²/h.

The amounts of notified chemical that penetrated through human skin at 4, 8 and 12 hr were 0.007 ± 0.008 µg/cm², 0.038 ± 0.045 µg/cm² and 0.098 ± 0.111 µg/cm², respectively. These respective amounts expressed as percentages of the applied dose were 0.008, 0.047 and 0.120%. The mean amount that penetrated over the entire 24 hr study period was 0.403 ± 0.424 µg/cm², corresponding to 0.494% of the applied dose.

Three of the 12 dosed cells either had a penetration profile that indicated membrane damage and were therefore rejected and not included in the evaluations.

Mean recovery of the applied test item was 99.7 ± 5.54% (n=9), with individual cell values ranging from 90.7 to 110%. The mean amount of remaining notified chemical removed by washing the skin surface 24 hr after application was 94.1 ± 5.31% (76.8 ± 4.33 µg/cm²). The mean amount of the dose present in the outer layers of the *stratum corneum* was 2.54 ± 1.31% of the applied dose (2.07 ± 1.07 µg/cm²) with 2.30 ± 1.12% of the dose (1.88 ± 0.914 µg/cm²) present in the remaining epidermis. The mean amount recovered for the dermis was 0.130 ± 0.098% of the applied dose (0.106 ± 0.080 µg/cm²).

The proportion of the applied dose of the notified chemical present in the receptor fluid after 24 hr was 0.494 ± 0.520% (0.403 ± 0.424 µg/cm²). The mean total non-absorbed dose (donor chamber, skin wash, *stratum corneum* and flange skin) represented 96.8 ± 5.79% (79.0 ± 4.73 µg/cm²) of the applied dose.

The mean total systemically available dose (remaining epidermis plus dermis and receptor fluid) of the notified chemical was 2.92 ± 1.52% of the applied dose (corresponding to 2.39 ± 1.24 µg/cm²).

CONCLUSION

Under the conditions of the study, the notified chemical penetrates through human dermatomed skin at a very slow rate. The extent of penetration through human skin was measured as 0.494 ± 0.520% (0.403 ± 0.424 µg/cm²) of the applied dose after 24 hr.

| | |
|---|---|
| | The mean total systemically available dose (remaining epidermis plus dermis and receptor fluid) of the notified chemical was 2.92 ± 1.52% of the applied dose (corresponding to 2.39 ± 1.24 µg/cm$^2$). |
| TEST FACILITY | DTL (2012) |

# APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

## C.1. Environmental Fate

### C.1.1. Ready biodegradability

| | |
|---|---|
| TEST SUBSTANCE | Mexoryl SBO (contains the notified chemical at < 30% concentration) |
| METHOD | OECD TG 301 B Ready Biodegradability: CO2 Evolution Test (modified Sturm Test). |
| Inoculum | Activated sludge from a local domestic wastewater treatment plant (Füllinsdorf, Switzerland). |
| Exposure Period | 28 days |
| Auxiliary Solvent | Dipropylene glycol |
| Analytical Monitoring | Total Organic Carbon Content (TOC) |
| Remarks - Method | No significant deviation in protocol. |

RESULTS

| *Test substance* | | *Solvent control* | | *Sodium benzoate* | |
|---|---|---|---|---|---|
| *Day* | *% Degradation* | *Day* | *% Degradation* | *Day* | *% Degradation* |
| 2 | 1.4 | 2 | 0.1 | 2 | 36.3 |
| 7 | 19.4 | 7 | 23.2 | 7 | 67.0 |
| 14 | 52.8 | 14 | 61.3 | 14 | 79.2 |
| 28 | 74.6 | 28 | 88.9 | 28 | 84.8 |

Remarks - Results

All validity criteria for the test were satisfied. The percentage degradation of the reference compound, sodium benzoate, surpassed the threshold level of 60% by 7 days (67%) and reached 84.8% degradation by 28 days. Therefore, the test indicates the suitability of the inoculums. The solvent control reached 88.9% degradation by 28 days.

The test substance attained 74.6% degradation by 28 days, but failed the 10-day window (< 60%). A degradation plateau was not achieved by 28 days. Therefore, the test substance cannot be classified as readily biodegradable according to the OECD (301B) guideline. However, the test substance exhibited inherent, primary biodegradability.

| | |
|---|---|
| CONCLUSION | The test substance is not readily biodegradable. |
| TEST FACILITY | Harlan (2009c) |

## C.2. Ecotoxicological Investigations

### C.2.1. Acute toxicity to fish

| | |
|---|---|
| TEST SUBSTANCE | Mexoryl SBO (the nominal concentration of the notified chemical was corrected to 100%) |
| METHOD | OECD TG 203 Fish, Acute Toxicity Test – Static. |
| Species | *Brachydanio rerio* (zebra fish) |
| Exposure Period | 96 hours |
| Auxiliary Solvent | None |
| Water Hardness | 125 mg $CaCO_3$/L |
| Analytical Monitoring | HPLC-MS/MS |
| Remarks – Method | No significant deviation in protocol. |

RESULTS

| *Concentration mg/L* | *Number of Fish* | *Mortality (%)* |
|---|---|---|

| *Nominal** | *Actual* | | *3 h* | *24 h* | *48 h* | *72 h* | *96 h* |
|---|---|---|---|---|---|---|---|
| Control | Control | 7 | 0 | 0 | 0 | 0 | 0 |
| 100 | 183.5 | 7 | 0 | 0 | 0 | 0 | 0 |

* After correcting for water content in the test substance

| | |
|---|---|
| LC50 | > 100 mg/L at 96 hours. |
| NOEC (or LOEC) | 100 mg/L at 96 hours. |
| Remarks – Results | All validity criteria for the test were satisfied. The test solutions were not renewed during the 96 h test period. No abnormalities in behaviour or appearance were observed. The 96 h LC50 and NOEC for fish were determined to be > 100 mg/L and 100 mg/L, respectively, based on nominal test substance concentration corrected for water content. |
| CONCLUSION | Under the conditions of the study, the test substance is not considered to be toxic to fish on an acute basis. |
| TEST FACILITY | Harlan (2013) |

**C.2.2. Acute toxicity to aquatic invertebrates**

| | |
|---|---|
| TEST SUBSTANCE | Mexoryl SBO (contains the notified chemical at < 30% concentration) |
| METHOD | OECD TG 202 Daphnia sp. Acute Immobilisation Test and Reproduction Test – Static. |
| Species | *Daphnia magna* |
| Exposure Period | 48 hours |
| Auxiliary Solvent | None |
| Water Hardness | 250 mg $CaCO_3$/L |
| Analytical Monitoring | LC-MS/MS |
| Remarks - Method | Following the range finding test, the definitive test was conducted at a nominal concentration of 100 mg/L of the test substance (corrected for water content). No significant deviation in protocol. |

RESULTS

| *Concentration mg/L* | | *Number of D. magna* | *Cumulative Immobilised (%)* | |
|---|---|---|---|---|
| *Nominal** | *Actual* | | *24 h* | *48 h* |
| Control | Control | 20 | 0 | 0 |
| 100 | 184 | 20 | 0 | 0 |

* After correcting for water content in test substance

| | |
|---|---|
| EC50 | > 100 mg/L at 48 hours |
| NOEC (or LOEC) | ≥ 100 mg/L at 48 hours |
| Remarks - Results | All validity criteria for the test were satisfied. The test solutions were not renewed during the 48 h test period. No abnormalities in behaviour or appearance were observed. The 48 h EC50 and NOEC for daphnids were determined to be > 100 mg/L and ≥ 100 mg/L, respectively, based on nominal test substance concentration corrected for water content. |
| CONCLUSION | Under the conditions of the study, the test substance is not considered to be harmful to daphnids on an acute basis. |
| TEST FACILITY | Harlan (2009a) |

**C.2.3. Algal growth inhibition test**

| | |
|---|---|
| TEST SUBSTANCE | Mexoryl SBO (the nominal concentration of the notified chemical was corrected to 100%) |
| METHOD | OECD TG 201 Alga, Growth Inhibition Test – Static. |

| | |
|---|---|
| Species | *Pseudokirchneriella subcapitata* (green alga) |
| Exposure Period | 72 hours |
| Concentration Range | Nominal: 4.6-100 mg/L (corrected for water content in test substance)<br>Absolute: 8.4-183.8 mg/L (wet weight) |
| Auxiliary Solvent | None |
| Water Hardness | 24 mg $CaCO_3$/L |
| Analytical Monitoring | LC-MS/MS |
| Remarks - Method | The definitive test was conducted at nominal concentrations of 4.6, 10, 22, 46, and 100 mg/L of the test substance corrected for water content (absolute concentrations of 8.4, 18.2, 39.4, 85.1, and 183.8 mg/L of the test substance, respectively). No significant deviation in protocol. |

RESULTS

| *Biomass* | | *Growth* | |
|---|---|---|---|
| *$E_bC50$* | *$NOE_bC$* | *$E_rC50$* | *$NOE_rC$* |
| *mg/L at 72 h* | *mg/L* | *mg/L at 72 h* | *mg/L* |
| > 100 | 46 | > 100 | 46 |

| | |
|---|---|
| Remarks - Results | All validity criteria for the test were satisfied. No cellular abnormalities were observed. The test solutions were not renewed during the 72-hour test period. The $E_bC50$, $E_rC50$, and $NOE_rC$ were determined to be > 100 mg/L, > 100 mg/L, and 46 mg/L, respectively, based on nominal test substance concentrations corrected for water content. |

CONCLUSION Under the conditions of the study, the test substance is not considered to be harmful to algae on an acute basis.

TEST FACILITY Harlan (2009b)

**C.2.4. Inhibition of microbial activity**

TEST SUBSTANCE Mexoryl SBO (the nominal concentration of the notified chemical was corrected to 100%)

METHOD OECD TG 209 Activated Sludge, Respiration Inhibition Test.

| | |
|---|---|
| Inoculum | Aerated activated sludge from a local domestic wastewater treatment plant (Füllinsdorf, Switzerland), fed with a synthetic sewage feed. |
| Exposure Period | 3 hours |
| Concentration Range | Nominal: 1000 mg/L (corrected for water content in test substance)<br>Absolute: 1839 mg/L (wet weight) |
| Remarks – Method | No significant deviation in protocol. |

RESULTS

| | |
|---|---|
| IC50 | > 1000 mg/L at 3 hours |
| NOEC | 1000 mg/L at 3 hours |
| Remarks – Results | All validity criteria for the test were satisfied. No inhibitory effects were observed. The 3 h EC50 was determined to be > 1000 mg/L, based on nominal test substance concentration corrected for water content. |

CONCLUSION The test substance is not inhibitory to microbial activity

TEST FACILITY Harlan (2009d)

# BIBLIOGRAPHY

BIOAGRI (2010) Prenatal Developmental Toxicity Study in Wistar Rats for [Mexoryl SBO] (Study No. 9050.314.015.09, October, 2010). Planaltina/DF, Brazil, BIOAGRI Laboratórios (Unpublished report submitted by the notifier).

CIT (2004a) 4-Week Toxicity Study by Oral (Gavage) and Cutaneous Routes in Rats (Study No. 26883 TSR, September, 2004). Evreux, France, CIT Safety & Health Research Laboratories (Unpublished report submitted by the notifier).

CIT (2004b) *In Vitro* Mammalian Chromosome Aberration Test in Cultured Human Lymphocytes (Study No. 27076 MLH, July, 2004). Evreux, France, CIT Safety & Health Research Laboratories (Unpublished report submitted by the notifier).

CIT (2008) Evaluation of Skin Sensitisation Potential in Mice using the Local Lymph Node Assay (LLNA) (Study No. 34489 TSS, November, 2008). Evreux, France, CIT Safety & Health Research Laboratories (Unpublished report submitted by the notifier).

CIT (2009a) Acute Oral Toxicity in Rats "Fixed Dose Method" (Study No. 35527 TAR, November, 2009). Evreux, France, CIT Safety & Health Research Laboratories (Unpublished report submitted by the notifier).

CIT (2009b) Acute Dermal Irritation in Rabbits (Study No. 35478 TAL, May, 2009). Evreux, France, CIT Safety & Health Research Laboratories (Unpublished report submitted by the notifier).

CIT (2009c) Acute Eye Irritation in Rabbits (Study No. 35479 TAL, November, 2009). Evreux, France, CIT Safety & Health Research Laboratories (Unpublished report submitted by the notifier).

CIT (2009d) Evaluation of Skin Sensitisation Potential in Mice using the Local Lymph Node Assay (LLNA) (Study No. 35481 TSS, May, 2009). Evreux, France, CIT Safety & Health Research Laboratories (Unpublished report submitted by the notifier).

CIT (2009e) 2-Week Toxicity Study by Cutaneous Route in Rats (Study No. 35183 TSR, October, 2009). Evreux, France, CIT Safety & Health Research Laboratories (Unpublished report submitted by the notifier).

Covance (2009a) Reverse mutation in five histidine-requiring strains of Salmonella typhimurium (Study No. 8200469, April, 2009). Harrogate, North Yorkshire, England, Covance Laboratories Ltd (Unpublished report submitted by the notifier).

Covance (2009b) Induction of Micronuclei in Cultured Human Peripheral Blood Lymphocytes (Study No. 8200471, May, 2009). Harrogate, North Yorkshire, England, Covance Laboratories Ltd (Unpublished report submitted by the notifier).

DTL (2012) *In Vitro* Penetration of [$^{14}$C]-Tetrahydrojasmonate in a 'Leave-on' Skin Care Formulation through Human Dermatomed Skin (Study No. JV2191, June, 2012). Staffordshire, England, Dermal Technology Laboratory Ltd (Unpublished report submitted by the notifier).

Episkin (2010) Primary Cutaneous Tolerance. Prediction of the Acute Irritant Potential on Human Reconstructed Epidermis Episkin$^{SM}$ Model (Study No. 10-BPL-0521, November, 2009). Lyon Cedex, France, Episkin SNC (Unpublished report submitted by the notifier).

Harlan (2009a) Acute toxicity of Mexoryl SBO to *Daphnia magna* in a 48-Hour Immobilization Test (Study No. C39222, 12 October 2009). Itingen, Switzerland, Harlan Laboratories Ltd (Unpublished report submitted by the notifier).

Harlan (2009b) Toxicity of Mexoryl SBO to *Pseudokirchneriella subcapitata* in a 72-Hour Algal Growth Inhibition Test (Study No. C39233, 12 October 2009). Itingen, Switzerland, Harlan Laboratories Ltd (Unpublished report submitted by the notifier).

Harlan (2009c) Ready Biodegradability of Mexoryl SBO in a $CO_2$ Evolution (Modified Sturm) Test (Study No. C39244, 11 November 2009). Itingen, Switzerland, Harlan Laboratories Ltd (Unpublished report submitted by the notifier).

Harlan (2009d) Toxicity of Mexoryl SBO to Activated Sludge in a Respiration Inhibition Test (Study No. C39255, 14 August 2009). Itingen, Switzerland, Harlan Laboratories Ltd (Unpublished report submitted by the notifier).

Harlan (2010a) [Mexoryl SBO]: Determination of the Freezing Point/Freezing Range and of the Boiling Point/Boiling Range (Study No. C39154, June, 2010). Itingen, Switzerland, Harlan Laboratories Ltd (Unpublished report submitted by the notifier).

Harlan (2010b) [Mexoryl SBO]: Determination of the Relative Density (Study No. C39165, May, 2010). Itingen, Switzerland, Harlan Laboratories Ltd (Unpublished report submitted by the notifier).

Harlan (2010c) Mexoryl SBO: Determination of the Vapour Pressure (Study No. C39176, August, 2010). Itingen, Switzerland, Harlan Laboratories Ltd (Unpublished report submitted by the notifier).

Harlan (2010d) [Mexoryl SBO]: Determination of the Surface Tension of an Aqueous Solution (Study No. C51091, May, 2010). Itingen, Switzerland, Harlan Laboratories Ltd (Unpublished report submitted by the notifier).

Harlan (2010e) Mexoryl SBO: Determination of the Flash Point (Study No. C39200, May, 2010). Itingen, Switzerland, Harlan Laboratories Ltd (Unpublished report submitted by the notifier).

Harlan (2013) Mexoryl SBO Acute Toxicity to Zebra Fish (*Brachydanio rerio*) in a 96-Hour Test (Study No. D54040, 28 February 2013). Itingen, Switzerland, Harlan Laboratories Ltd (Unpublished report submitted by the notifier).

IEC (2009) Irritation Primaire Oculaire B.C.O.P (Study No. 082173RD, February, 2009). Lyon, France, Institut d'Expertise Clinique (Unpublished report submitted by the notifier).

IEC (2010) Ocular Primary Irritation B.C.O.P (Study No. 101225RD, September, 2010). Lyon, France, Institut d'Expertise Clinique (Unpublished report submitted by the notifier).

JRF (2013) Repeated Dose 90-Day Oral Toxicity Study of Mexoryl SBO in Wistar Rats (Study No. 443-1-03-3546, June, 2013). Gujarat, India, JAI Research Foundation (Unpublished report submitted by the notifier).

NOHSC (2004) Approved Criteria for Classifying Hazardous Substances, 3rd edition [NOHSC:1008(2004)]. National Occupational Health and Safety Commission, Canberra, AusInfo.

NTC (National Transport Commission) 2007 Australian Code for the Transport of Dangerous Goods by Road and Rail (ADG code), 7th Edition, Commonwealth of Australia

SCCS (2012) Notes of Guidance for Testing of Cosmetic Ingredients and Their Safety Evaluation (8th revision). European Commission – Scientific Committee on Consumer Safety.

SWA (2012) Code of Practice: Managing Risks of Hazardous Chemicals in the Workplace, Safe Work Australia, http://www.safeworkaustralia.gov.au/sites/swa/about/publications/pages/managing-risks-of-hazardous-chemicals-in-the-workplace.

United Nations (2009) Globally Harmonised System of Classification and Labelling of Chemicals (GHS), 3rd revised edition. United Nations Economic Commission for Europe (UN/ECE), <http://www.unece.org/trans/danger/publi/ghs/ghs_rev03/03files_e.html >.

US EPA (2011) Estimation Programs Interface (EPI) Suite™ for Microsoft® Windows, v 4.10. United States Environmental Protection Agency. Washington DC, USA.