File No: LTD/1731

May 2014

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

Bicyclo[2.2.1]hept-5-ene-2-carboxylic acid, ethyl ester

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

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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/1731	International Flavours & Fragrances (Australia) P/L	Bicyclo[2.2.1]hept-5-ene-2-carboxylic acid, ethyl ester	Yes	≤ 1 tonne/s per annum	Fragrance 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*
Skin Sensitisation (Category 1B)	H317 – May cause an allergic skin reaction

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:

R43: May cause sensitisation by skin contact

The environmental hazard classification according to the *Globally Harmonised System for the Classification and Labelling of Chemicals* (GHS) is presented below. Environmental classification under the GHS is not mandated in Australia and carries no legal status but is presented for information purposes.

Hazard classification	*Hazard statement*
Acute Category 2	H401 – Toxic to aquatic life
Chronic Category 2	H411 – Toxic to aquatic life with long lasting effects

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.

When used at concentrations ≤0.25% in cosmetics, personal care and household cleaning 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:
  - Skin Sensitisation (Category 1B): H317 – May cause an allergic skin reaction

Health Surveillance

- As the notified chemical is a skin sensitiser, employers should carry out health surveillance for any worker who has been identified in the workplace risk assessment as having a significant risk of sensitisation.

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:
  - Enclosed, automated processes, where possible

- 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:
  - Avoid contact with skin and 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:
  - Coveralls
  - Impervious gloves
  - 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.

Public Health

- The following measures should be taken by formulators to minimise public exposure to the notified chemical:
  - The notified chemical should only be used at ≤ 0.25% in cosmetics, personal care, air care and household cleaning products.
  - Take account of the skin sensitisation potential of the notified chemical.

Disposal

- The notified chemical should be disposed of to landfill.

Emergency procedures

- Spills or accidental release of the notified chemical should be handled by containment, physical collection and subsequent 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 in cosmetics, personal care, air care and household cleaning products exceeds or is intended to exceed 0.25%;

or

(2) Under Section 64(2) of the Act; if
- the function or use of the chemical has changed from a fragrance ingredient, or is likely to change significantly;
- the amount of chemical being introduced has increased, or is likely to increase from one tonne per annum, 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 notified chemical 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)
International Flavours and Fragrances (Australia) Pty Ltd. (ABN: 77 004 269 658)
310 Frankston-Dandenong Rd
DANDENONG VIC 3175

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

EXEMPT INFORMATION (SECTION 75 OF THE ACT)
No details are claimed exempt from publication.

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
Variation to the schedule of data requirements is claimed as follows: particle size, dissociation constant, flammability, acute inhalation toxicity

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None.

NOTIFICATION IN OTHER COUNTRIES
US (2013)

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
Tropicalia Toco
Tropicate

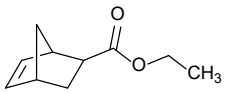
CAS NUMBER
10138-32-6

CHEMICAL NAME
Bicyclo[2.2.1]hept-5-ene-2-carboxylic acid, ethyl ester

OTHER NAME(S)
Ethyl bicycle]2.2.1]hept-5-ene-2-carboxylate

MOLECULAR FORMULA
$C_{10}H_{14}O_2$

STRUCTURAL FORMULA



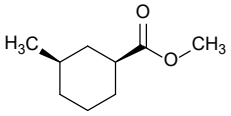
MOLECULAR WEIGHT
166.2 Da

ANALYTICAL DATA
Reference NMR, IR, HPLC, GC, GPC, UV spectra were provided.

3. IDENTITY OF ANALOGUE

An analogue chemical was provided for the human health effects assessment of the notified chemical and was tested as a mixture of stereoisomers.

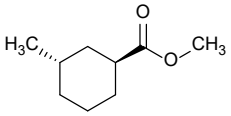
ISOMER 1



CAS NUMBER
7605-52-9

CHEMICAL NAME
Cyclohexanecarboxylic acid, 3-methyl-, methyl ester, (1*R*,3*S*)-*rel*-
Cyclohexanecarboxylic acid, 3-methyl-, methyl ester, cis-

ISOMER 2



CAS NUMBER
7605-53-0

CHEMICAL NAME
Cyclohexanecarboxylic acid, 3-methyl-, methyl ester, (1*R*,3*R*)-*rel*-
Cyclohexanecarboxylic acid, 3-methyl-, methyl ester, trans-

3. COMPOSITION

DEGREE OF PURITY
>99%

HAZARDOUS IMPURITIES/RESIDUAL MONOMERS
None

NON HAZARDOUS IMPURITIES/RESIDUAL MONOMERS (> 1% BY WEIGHT)
None

ADDITIVES/ADJUVANTS
None

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20 °C AND 101.3 kPa: Clear liquid

Property	Value	Data Source/Justification
Melting Point/Freezing Point	6.5 °C	Estimated (using EPISuite V4.11)
Melting Point/Freezing Point	< -25 °C	Analogue data
Boiling Point	216 °C at 101.3 kPa	Estimated
Boiling Point	200 °C at 101.3 kPa	Analogue data
Density	1.02×10^{-3} kg/m^3 at 22 °C	Measured
Vapour Pressure	2.7×10^{-1} kPa at 22 °C	Measured. Analogue data
Water Solubility	0.84 g/L at 20 °C	Measured
Hydrolysis as a Function of pH	t½ > 4.3 year at 25 °C at pH 7 t½ = 158 days at pH 8 t½ > 1 year at 25 °C (pH 4 & 7) t½ = 21 days at pH 9	Calculated (using HYDROWIN v2.00; US EPA, 2009) Measured. Analogue data
Partition Coefficient	log Pow = 3.4 and 3.7 at 25 °C log Pow = 3.7 at 25 °C	Measured Measured. Analogue data

(n-octanol/water)		
Surface tension	52.5 mN/m at 20 °C	Measured. Analogue data
Adsorption/Desorption	log K_{oc} = 2.9 at 25 °C	Calculated (using KOCWIN v2.00; US EPA, 2009)
	log K_{oc} = 2.6 at 25 °C	Measured. Analogue data
Dissociation Constant	Not determined	No dissociable functionality
Autoignition Temperature	324 °C	Analogue data
Explosive Properties	Predicted negative	Does not contain explosophores
Oxidising Properties	Predicted negative	Does not contain oxidising groups

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. It is not explosive, non-oxidising and not auto-ignitable under normal conditions. The notified chemical presents no significant reactivity hazard by itself or in contact with water. However, direct sources of heat and contact with strong acids, alkali or oxidising agents should be avoided.

Dangerous Goods classification
Based on the submitted physical-chemical data in the above table the notified chemical is not classified according to the Australian Dangerous Goods Code (NTC, 2007). However, the data above do not address all Dangerous Goods endpoints. Therefore, consideration of all endpoints should be undertaken before a final decision on the Dangerous Goods classification is made by the introducer of the chemical.

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 manufactured outside of Australia and then imported as a component of fragrance oils (at ≤5% concentration), encased in polypropylene-lined steel drums delivered to the notifier facility.

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

IDENTITY OF MANUFACTURER/RECIPIENTS
International Flavours & Fragrances (Australia) Pty Ltd. (IFF)

TRANSPORTATION AND PACKAGING
The notified chemical (at 5% concentration) will be imported as a component of finished fragrance oils in 205 L polypropylene-lined steel drums. The imported and formulated products containing the notified chemical will be transported within Australia by road. The end-use products (up to 0.25% concentration notified chemical) will be packaged in containers suitable for retail sale.

USE
The notified chemical will be used as a fragrance ingredient and will be sold to industrial and commercial customers in finished fragrance oils to be incorporated into cosmetic, personal care and household consumer products. Product categories include fragrances, deodorant, hand cream, facial cleanser, hair spray, body lotion, cosmetic rinse-off products (shower gel, shampoo, conditioner, hand wash soap), air care (aerosol and candles), laundry detergents, fabric conditioners, and household cleaners. The notified chemical will be reformulated from finished fragrance oils at ≤ 5% concentration into end-use products at a final concentration of ≤ 0.25%.

OPERATION DESCRIPTION
The notified chemical will not be manufactured within Australia. The notified chemical will be imported in finished fragrance oils at ≤5% concentration for reformulation into cosmetic, personal care and household products containing the notified chemical at a concentration of ≤0.25%.

No manufacturing, processing, reformulating or repackaging of the notified chemical will occur at the notifier facility. The finished fragrance oil containing the notified chemical will be stored at this facility until it is sold and shipped to customer facilities.

Reformulation
The procedures for incorporating the notified chemical (at ≤5% concentration) into end-use products will likely vary depending on the nature of the formulated products and may involve both automated and manual transfer steps. However, in general, it is expected that for the reformulation process, the notified chemical will be weighed and added to the mixing tank where it will be blended with additional additives to form the finished cosmetic and household products. This is followed by automated filling of the reformulated products into containers of various sizes. The notifier states that the mixing facilities are expected to be highly automated, well ventilated (local exhaust ventilation) and use closed systems. After being reformulated, the finished products containing the notified chemical will be transferred into the retail packaging. During the formulation process, samples of the notified chemical and the finished cosmetic products will be taken for quality control testing.

Cleaning and washing products.
Cleaning and washing agents containing the notified chemical (≤0.25% concentration) may be used by consumers and professional workers. The cleaning and washing agents may be used in either closed systems with episodes of controlled exposure, for example automatic washing machines, or open processes and manually by rolling, brushing, spraying and dipping. The cleaning and washing liquids are completely discharged into industrial sewerage systems after use.

Cosmetics
The finished cosmetic products containing the notified polymer at ≤0.25% concentration will be used by consumers and professionals (such as beauticians and hairdressers). Depending on the nature of the product, application of products could be by hand, sprayed or through the use of an applicator.

Air care products
The finished aerosol products containing the notified polymer at ≤0.25% concentration will be used by consumers. Depending on the nature of the product, application of products could be by pump or pressurised spray (manual or automatic) or through burning of candles.

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 warehouse workers	None	Incidental exposure only
Plant operators – mixing/compounding (customer site)	4	250
Plant operators – drum handling (customer site)	1	250
Plant operators – drum cleaning/washing (customer site)	2	200
Plant operators – equipment cleaning/washing	2	250

(customer site)		
Plant operators – quality control (customer site)	1	250
Professional users – hairdressers, beauty salon artists, cleaners	Not specified	Not specified

EXPOSURE DETAILS

Transport and storage

Transport and storage workers may come into contact with the notified chemical as a component of fragrance oils (at ≤ 5% concentration) only in the event of accidental rupture of the drum containers.

At the notifier facility, the primary work activity undertaken by transport and warehouse workers will include the handling, loading and off-loading of drums containing fragrance oils formulated with the notified chemical at up to 5% concentration. Exposures of these workers will be limited to situations involving products sampling for quality control or, in the event of a discharge, clean up from a spill or leaking drum. If such an event occurs, a worker may be exposed through dermal or ocular contact. Such exposures will be minimised to the extent possible through the use of personal protective equipment (PPE) including protective overalls, hard hats, chemical resistant gloves and safety glasses.

Formulation of end products

During reformulation, dermal, ocular and perhaps inhalation exposure of workers to the notified chemical (at 5% concentration) may occur during weighing and transfer stages, blending, quality control analysis and cleaning and maintenance of equipment. The notifier states that exposure is expected to be minimised through the use of mechanical ventilation, local exhaust ventilation and/or enclosed systems, and through the use of PPE such as coveralls, goggles and impervious gloves. Due to the vapour pressure of the notified chemical, inhalation exposure may be expected especially where mists or aerosols may be generated. Self-contained breathing apparatus will be used if ventilation is inadequate.

Beauty care and cleaning professionals

Exposure to the notified chemical in end-use products (at ≤ 0.25% concentration) may occur in professions where the services provided involve the application of cosmetic and personal care products to clients (e.g. hair dressers, workers in beauty salons) or the use of household products in the cleaning industry. The principal route of exposure will be dermal, while ocular and inhalation exposure is also possible. Such professionals may use some PPE to minimise repeated exposure, but use is not expected. 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 up to 0.25% concentration) through the use of a wide range of cosmetic, personal care and household products. The principal routes of exposure will be dermal, while ocular, oral (during facial use), and inhalation exposures (through the use of spray products) are also possible.

Data on typical use patterns of product categories in which the notified chemical may be used are shown in the following table (SCCS, 2012; Cadby *et al.*, 2002). For the purposes of the exposure assessment, Australian use patterns for the various product categories are assumed to be similar to those in Europe. In the absence of dermal absorption data, the default dermal absorption of 100% was assumed for calculation purposes (European Commission, 2003). Although, the actual level of dermal absorption may be lower than 100%, it may vary with the formulation type. Considering that there may be penetration enhancers in some cosmetic formulations, 100% was used in the estimation of the systemic dose. An adult bodyweight of 60 kg has been used for calculation purposes.

Cosmetic products (dermal exposure)

Product type	Amount (mg/day)	C (%)	RF	Daily systemic exposure (mg/kg bw/day)
Body lotion	7,820	0.25	1	0.326
Face cream	1,540	0.25	1	0.064
Hand cream	2,160	0.25	1	0.090
Deodorant (aerosol/ethanol)	1,430	0.25	1	0.060

Fragrances	750	0.25	1	0.031
Hair styling products	4,000	0.25	0.1	0.017
Shower gel	18,670	0.25	0.01	0.008
Hand wash soap	20,000	0.25	0.01	0.008
Shampoo	10,460	0.25	0.01	0.004
Hair conditioner	3,920	0.25	0.01	0.002
Facial cleanser	800	0.25	0.01	0.0003
Total				**0.610**

C = concentration; RF = retention factor based on 100% dermal absorption.
Daily exposure = mg/day × C (%) × RF; Daily systemic exposure = daily exposure × dermal absorption (%) /body weight (60 kg)

Household products (Indirect dermal exposure - from wearing clothes)

Product type	Amount (g/use)	C (%)	Product Retained (PR) (%)	Percent Transfer (PT) (%)	Daily systemic exposure (mg/kg bw/day)
Laundry liquid	230	0.25	0.95	10	0.009
Fabric softener	90	0.25	0.95	10	0.004
Total					**0.013**

Daily systemic exposure = (Amount × C × PR × PT × dermal absorption)/body weight

Household products (Direct dermal exposure)

Product type	Frequency (use/day)	C (%)	Contact Area (cm^2)	Product Use C (g/cm^3)	Film Thickness (cm)	Time Scale Factor	Daily systemic exposure (mg/kg bw/day)
Laundry liquid	1.43	0.25	1980	0.01	0.01	0.007	0.00008
Dishwashing liquid	3	0.25	1980	0.0093	0.01	0.03	0.0007
All-purpose cleaner	1	0.25	1980	1	0.01	0.007	0.006
Total							**0.007**

Daily systemic exposure = (Frequency × C × Contact area × Product Use Concentration × Film Thickness on skin × Time Scale Factor x dermal absorption)/body weight

Aerosol products (Inhalation exposure)

Product type	Frequency (use/day)	Amount (g/use)	C (%)	Inhalation rate (m^3/day)	Exposure duration (mins)	Airspace volume (m^3)	Daily systemic exposure (mg/kg bw/day)
Hairspray	2	10	0.25	23	15	2	0.10
Air-care products	4	10	0.25	23	15	20	0.02
Total							**0.12**

Daily systemic exposure = (Frequency × Amount × C × Inhalation rate × Exposure duration × bioavailability via the inhalation route)/(body weight × Airspace volume)

The worst case scenario estimation using these assumptions is for a person who is a simultaneous user of all products listed in the above table that contain the notified chemical. This would result in a combined internal dose of 0.66 mg/kg bw/day.

6.2. Human Health Effects Assessment

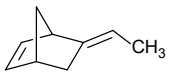
The results from toxicological investigations conducted on the notified chemical and an analogue chemical are summarised in the following table. For full details of the studies, refer to Appendix B.

Endpoint	*Result and Assessment Conclusion*
Rat, acute oral toxicity*	LD50 300–2,000 mg/kg bw; harmful*
Rat, acute dermal toxicity*	LD50 > 2,000 mg/kg bw; low toxicity*
Rabbit, skin irritation*	slightly irritating*

Rabbit, eye irritation*	slightly irritating*
Mouse, skin sensitisation – Local lymph node assay	Evidence of sensitisation at 50%
Human, skin sensitisation – RIPT (2.5%)	no evidence of sensitisation
Rat, repeat dose oral toxicity – 28 days*	NOAEL= 150 mg/kg bw*
Mutagenicity – bacterial reverse mutation	non mutagenic
Genotoxicity – in vitro mammalian cell gene mutation	non genotoxic
Genotoxicity – in vitro mammalian chromosome aberration	genotoxic
Genotoxicity – in vitro mammalian cell micronucleus	non genotoxic
Genotoxicity – in vivo mammalian bone marrow chromosome aberration	non clastogenic

* Tests conducted on the analogue of the notified chemical (M3MC-Carboxylate).

The analogue chemical provided by the notifier is not considered to be a suitable analogue for the interpretation of the possible human health effects of the notified chemical. Although the chemicals have some similar physicochemical properties, the analogue does not contain the bicyclic structure or alkene functional group of the notified chemical. Furthermore, scientific justification was not provided to establish how the structural features which are common are related to possible toxicological effects. Additionally, due to the non-planarity of the alkene in the bicyclic ring of the notified chemical, the alkene is likely to have a different reactivity than other alkenes (Steinmann, 2010). An alternate analogue chemical identified by NICNAS, 5-ethylidene-2-norbornene (CAS No. 16219-75-3) (ENB) is structurally and physico-chemically similar to the notified chemical (OECD SIDS, 2002). It is used only as a chemical intermediate (synthetic rubber). It does not have the ester functional group of the notified chemical and has an additional exocyclic alkene (see structure below).



ENB was reported in OECD SIDS (2002) to be of low acute toxicity by the oral (LD50 = 2,276–5,071 mg/kg), dermal (LD50 > 7,168 mg/kg) and inhalation (LC50 = 13.3–14.8 mg/L) routes. ENB was reported to be a mild irritant to skin and a slight eye irritant in rabbits. There was no data on skin sensitisation. The oral NOAEL for systemic effects was report as 20 mg/kg bw/day based on a 28-day repeated dose study in rats. Adverse effects reported in the kidneys at 4 mg/kg bw/day for males were consistent with α-2-microglobulin nephropathy and therefore not relevant to humans. Adverse effects reported in the thyroid in males at 4 mg/kg bw/day and females at 100 mg/kg bw/day were stated to be of little or no relevance to humans. In the most recent rat study the inhalation NOAEL was reported to be 5 ppm based on thyroid effects. The inhalation NOAEL based on effects other than thyroid was reported to be 25 ppm. In an oral reproductive/developmental study in rats that were administered ENB up to 100 mg/kg/ bw/day, the oral NOAEL was reported to be 20 mg/kg bw/day based on significantly lower implantation and deliveries in the 100 mg/kg bw/day group.

Toxicokinetics, metabolism and distribution.
No information on the toxicokinetics of the notified chemical was provided. Based on the low molecular weight (<500 Da) of the notified chemical, the potential to cross the gastrointestinal (GI) tract by passive diffusion or to be dermally absorbed after exposure is possible. The notified chemical may be absorbed across the respiratory tract. The low molecular weight and a log Pow > 0 suggest that the notified chemical is likely to be widely distributed within the body. The potential for dermal absorption is supported by the observed sensitisation effects seen with application of the notified chemical in the local lymph node assay.

Acute toxicity.
No studies on the acute toxicity endpoints were available for the notified chemical. Acute toxicity studies via the oral and dermal route were conducted on the analogue chemical M3MC-Carboxylate.

In the acute oral study, two test animals died on day 2 and various clinical signs of toxicity were observed at the highest (2,000 mg/kg bw/day) dose level. These reactions included hunched posture, unsteady gait, piloerection, increased and irregular breathing, reduced body tone, underactivity, partially closed eyelids and reduced body weight. Some clinical signs were noted at the 300 mg/kg bw/day dose level, including unsteady gait (seen in five animals) and loose faeces (seen in two animals). In the two deceased animals, macroscopic examination revealed congestion of the subcutaneous tissue, heart, lungs, spleen, kidneys and duodenum and enlarged stomach. Inspection of the stomach and small intestine contents showed yellow fluid and red fluid in the large intestines. One surviving animal from the highest dose group terminated at the end of the study showed stomach atrophy. No abnormalities were noted in the other surviving animals.

No acute inhalation toxicity data on the notified or analogue chemicals were provided.

Irritation and sensitisation.
No studies on acute irritation and corrosion were available for the notified chemical. Acute irritation/corrosion studies via the dermal and ocular routes were conducted on the analogue chemical M3MC-Carboxylate.

The analogue chemical was found to be slightly irritating to rabbit skin in an acute dermal irritation study. Very slight to well-defined erythema was evident at the treated site of all animals from 1 hour post application and sustained for the entire study period. Loss of elasticity was noted in one animal during the 48 hour observations but had subsided by day 15. Exfoliation was noted in all three animals during the second week of observations. The analogue was classified with the recommendation for labelling with the risk phrase R38 – Irritating to skin.

A rabbit eye irritation study was conducted for the analogue chemical on three female rabbits. The test material caused redness of the conjunctiva, slight to moderate discharge, very slight chemosis and diffuse areas of opacity. Two animals appeared normal by the day 8 observations and all signs had cleared by day 15 in the remaining animal.

Based on the limited data available, potential irritation effects from dermal or ocular exposure to the notified chemical cannot be ruled out.

The notified chemical was a potential skin sensitiser in a local lymph node assay (LLNA) in mice, with reported stimulation indices of 1.3, 3.0 and 3.6 at 25%, 50% and 'neat' concentration, respectively. The reported EC3 value was 50% v/v. The notified chemical was not a skin sensitiser at 2.5% concentration in a human repeat insult patch test (HRIPT) with 102 subjects. No clinical symptoms were noted during the study period in either the induction or challenge phases.

Repeated dose toxicity.
Repeated dose toxicity information on the notified chemical was not provided. In a 28 day oral toxicity study conducted with the analogue M3MC-Carboxylate, rats were administered the test substance at 0, 15, 150 and 1,000 mg/kg bw/day. Various treatment-related adverse effects were seen at the highest dosing level. The NOEL was determined by forestomach oedema and depression, elevated liver weight and decreased body weights, which were seen in the 1,000 mg/kg bw/day dose group animals. Some treatment related disturbances in urinary pH in male animals and forelimb grip strength in female animals were noted in the 150 mg/kg/day group, however these changes were considered by the study authors to be not toxicologically significant. The No Observed Effect Level (NOEL) was established by the study authors at 150 mg/kg bw/day.

As the analogue chemical M3MC-Carboxylate is not considered a suitable analogue of the notified chemical there is uncertainty in attributing the NOEL of 150 mg/kg bw/day to calculating the MOE for the notified chemical. However, the notified chemical is expected to be less toxic than the alternate analogue ENB with an oral NOAEL of 20 mg/kg bw/day, as it does not possess the highly reactive exocyclic alkene functional group (OECD SIDS, 2002). In a 45 day reproductive/developmental screening test and a 28 day oral toxicity study conducted with the analogue ENB, rats were administered the test substance at 0, 4, 20 and 100 mg/kg bw/day. In the reproductive/developmental screening test, treatment-related adverse effects were seen at the highest dose level and included reduced body weight gain and decreased food consumption observed in both sexes. The relative liver weights were increased in males and histopathological examination revealed hypertrophy and vacuolation of hepatocytes. There was a decrease in the number of implantation and delivery indices observed in dams in the high dose group. The NOAEL for reproductive and developmental toxicity was considered to be 20 mg/kg bw/day for the parental animals and offspring. In the 28 day study, treatment-related adverse effects were seen at the highest dose level and included reduced mean body weight in females, protein-positive urine and a decrease in water consumption in males, and increased brain/body weight and kidney/body weight ratios in males. The NOAEL for repeated dose oral toxicity was considered to be 20 mg/kg bw/day.

A NOAEL range of 20–150 mg/kg bw/day was therefore considered in the risk assessment.

Mutagenicity/Genotoxicity.
The notified chemical was not mutagenic in an in vitro bacterial mutation test, although some cytotoxicity was observed following exposure at 5,000 µg/plate. The notified chemical did not demonstrate mutagenic potential to mouse lymphoma L5178Y cells treated in vitro. In the in vitro mammalian cell micronucleus assay the notified chemical was considered not clastogenic.

The notified chemical was shown to be clastogenic in an in vitro chromosome aberration assay in human lymphocytes. In metaphase analysis, there were statistically significant increases in the frequency of structural

chromosome aberrations, following 21 hour continuous exposure only, without metabolic activation. Under all other experimental conditions, there was no evidence of causing an increase in the proportion of cells with aberrations.

Following these tests, an in vivo mammalian bone marrow chromosome aberration test was performed with the notified chemical and it was considered not clastogenic. Based on the weight of evidence of the available studies the notified chemical is not considered to be genotoxic.

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***
Skin Sensitisation (Category 1B)	H317- May cause an allergic skin reaction

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):
R43: May cause sensitisation by skin contact

6.3. Human Health Risk Characterisation

6.3.1. Occupational Health and Safety

Transport and Storage
Workers may experience dermal and accidental ocular exposure to the notified chemical (at up to 5% concentration) where the fragrance oils are sampled for quality control purposes or in the event of a discharge via spill or drum leakage. The use of PPE (impervious gloves, goggles, coveralls, hard hats) should minimise the potential for exposure.

Therefore, provided adequate control measures are in place to minimise worker exposure, including PPE, the risk to workers from use of the notified chemical is not considered to be unreasonable.

Reformulation
Workers may experience dermal and accidental ocular and perhaps inhalation exposure to the notified polymer (at up to 5% concentration) during formulation processes. This exposure may occur during handling of the drums, cleaning and/or maintenance of the equipment. At these facilities, exposure may also extend to compounders and laboratory staff involved in the formulation of the end products containing the notified chemical and the sampling and quality control testing of these products.

The use of enclosed, automated processes and PPE (impervious gloves, goggles, coveralls and respiratory protection, if significant inhalation exposure is expected) should minimise the potential for exposure. Occupational surveillance programs should be in place for workers which may be at a significant risk of sensitisation.

Therefore, provided that adequate control measures are in place to minimise worker exposure, including the use of automated processes and PPE, the risk to workers from use of the notified polymer is not considered to be unreasonable.

End-use
Workers involved in professions where the services provided involve the application of cosmetic products to clients (e.g. beauty salon workers) may be exposed to the notified chemical. Hairdressers may also be repetitively exposed to the notified chemical in their application of shampoo and hairspray to salon clients. 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 on a regular basis (for details of the public health risk assessment, see Section 6.3.2.).

Such professionals may use PPE to minimise repeated exposure, and good hygiene practices are expected to be in place. For hairdressing salons, good ventilation would be recommended if hair spray is routinely used in a confined space. If PPE is used, the exposure of such workers is expected to be of a similar or lesser extent than

that experienced by consumers using the various cosmetic and household products containing the notified chemical. Based on the information available, the risk to workers associated with use of the notified chemical at ≤ 0.25% concentration in cosmetic products is not considered to be unreasonable.

6.3.2. Public Health

Members of the public may be repeatedly exposed to the notified chemical during the use of cosmetic, hair care, personal care, air-care and household products containing the notified chemical at the proposed concentration up to 0.25%.

Local effects
Based on the limited information available, the potential for skin and eye irritation cannot be ruled out.

Systemic effects
The potential systemic exposure to the public from the use of the notified chemical in cosmetic and household products was estimated to be 0.66 mg/kg bw/day (see Section 6.1.2). Using a NOEL of 150 mg/kg bw/day, which was derived by the study authors from a 28 day repeated dose toxicity study on the analogue chemical M3MC-Carboxylate, the margin of exposure (MOE) was estimated to be 227. Using the conservative NOAEL of 20 mg/kg bw/day derived from a 28 day repeated dose toxicity study on the alternate analogue ENB, the MOE was estimated to be 33. A MOE value greater than or equal to 100 is considered acceptable to account for intra- and inter-species differences. Based on the exposure assumptions in this assessment (refer Section 6.1), the MOE of 100 corresponds to a NOAEL of 66 mg/kg bw/day. Considering that 150 mg/kg bw/day corresponds to a NOEL, rather than a NOAEL; the conservativeness of the hazard of the ENB analogue compared to the notified chemical, and the conservative exposure assumptions used in estimating the MOE (daily exposure through the use of many cosmetic, personal care and household products) the MOE range of 33–227 is considered to be acceptable.

The risk to the public of systemic toxicity associated with the use of the notified chemical in cosmetics, air-care and household products at up to 0.25% concentration is not considered to be unreasonable.

Sensitisation
There is a risk of potential skin sensitisation associated with the use of the notified chemical in cosmetics, personal care and household cleaning products at the proposed usage concentrations (up to 0.25%).

Proposed methods for the quantitative risk assessment of dermal sensitisation have been the subject of significant discussion (see for example, Api *et al.*, 2008 and RIVM, 2010). As is shown in the table below, the Consumer Exposure Level (CEL) from use of the notified chemical in a number of different cosmetic and cleaning products may be estimated (SCCS, 2010 and RIVM, 2006). When tested at 2.5% concentration in a human repeat insult patch study (0.2 mL applied to 3.63 cm^2 patches), the notified chemical was determined by the study authors to not be a skin sensitiser. When tested in an LLNA study, the notified chemical was a potential skin sensitiser with and EC3 value of 50%.

Although this value has been used for the purposes of quantitative risk assessment of the notified chemical given the standard protocol followed in the LLNA study, the availability of additional information on the sensitisation potential of the notified chemical (i.e., the HRIPT) was taken into account when determining the safety assessment factors that should be applied. Thus, consideration of the details of the studies, and application of appropriate safety factors, allowed the derivation of an Acceptable Exposure Level (AEL) of 127.5 μg/cm^2 (derived from the LLNA study). In this instance, the factors employed included an interspecies factory (1), intraspecies factor (10), a matrix factor (3.16), and a use and time factor (3.16), giving an overall safety factor of >100 (100 used for calculations).

Product type	Proposed maximum usage concentration (%)	CEL chemical (μg/cm^2)	AEL chemical (μg/cm^2)	Recommended maximum usage concentration (%)
Deodorant spray	0.25	17.88	127.5	≤1.78
Fine fragrances	0.25	1.30	127.5	≤24.48
Other leave-on cosmetics (assumed: face cream)	0.25	6.81	127.5	≤4.68
Rinse-off cosmetics	0.25	0.58	127.5	≤54.83

(assumed: hand wash soap)				
Household products (assumed: cleaning liquid)	0.25	0.43	127.5	≤74.34

As the AEL > CEL, the risk to the public of the induction of sensitisation that is associated with the use of the notified chemical in cosmetics and cleaning products at up to 0.25% concentration is not considered to be unreasonable. The CEL for air-care products is expected to be significantly lower than that for deodorant spray (which represents the highest exposure to the notified chemical), and therefore, risk to the public of the induction of sensitisation that is associated with the use of the notified chemical in air-care products at up to 0.25% concentration is not considered to be unreasonable. It is acknowledged that consumers may be exposed to multiple products containing the notified chemical, and a quantitative assessment based on the aggregate exposure has not been conducted.

Therefore, based on the information available, the risk to the public associated with use of the notified chemical at ≤0.25% in cosmetics, air-care products and household cleaning 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 be imported as a component of fragrance preparations for local reformulation into a variety of consumer products (cosmetics, household products, fragrance oil). Release during reformulation in Australia is expected to arise from spills, formulation equipment cleaning and residues in import containers. These residues are likely to be discharged to an on-site waste water treatment plant.

RELEASE OF CHEMICAL FROM USE

The notified chemical is expected to be released to sewers in domestic situations across Australia as a result of its use in cosmetic and domestic products, which are either washed off the hair and skin of consumers, or disposed of following cleaning activities.

RELEASE OF CHEMICAL FROM DISPOSAL

It is anticipated that most of the spilled material containing the notified chemical is expected to be collected and sent to landfill. Some spilled material is likely to be discharged to sewer.

7.1.2. Environmental Fate

Following its use in Australia, the majority of the notified chemical is expected to enter the sewer system before potential release to surface waters on a nationwide basis. The submitted biodegradation study indicates that the notified chemical is not expected to be rapidly degraded in sewage treatment plants (STPs). In STPs the notified chemical is expected to be efficiently removed (based on its adsorption and partition coefficients) from influent by adsorption to sludge and only a small portion may be released to surface waters. The notified chemical is not likely to bioaccumulate based on its moderate water solubility and low n-octanol/water partition coefficient (Pow = 3.4–3.7). A proportion of notified chemical may be applied to land when effluent is used for irrigation, or disposed of to landfill as waste. Notified chemical residues in landfill and soils are expected to have low mobility based on its calculated soil adsorption coefficient (log Koc = 2.9). In the aquatic and soil compartments, the notified chemical is expected to slowly degrade through biotic and abiotic processes to form water and oxides of carbon.

7.1.3. Predicted Environmental Concentration (PEC)

Since most of the notified chemical will be washed into the sewer, under a worst case scenario, assuming no removal of the notified chemical in sewage treatment plants (STPs), the resultant Predicted Environmental Concentration (PEC) in sewage effluent on a nationwide basis is estimated as follows:

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	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:	0.61	µg/L
PEC - Ocean:	0.06	µg/L

STP effluent re-use for irrigation occurs throughout Australia. The agricultural irrigation application rate is assumed to be 1,000 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 1,500 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 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 analogue chemical (3-methy cyclohexyl ester, M3MC-Carboxylate) which contains one of the same reactive functional groups as the notified chemical are summarised in the table below. Details of these studies can be found in Appendix C.

The notified chemical is structurally similar to the analogue and there is not expected to be significant difference between the physico-chemical properties and ecotoxicity. Therefore, it is considered to be scientifically reasonable to predict the ecotoxicity endpoints for fish, aquatic invertebrates and algae using the analogue data. The ecotoxicity endpoints of the analogue chemical are compared with those calculated by ECOSAR v1.11 (US EPA 2011) using the class specific to the notified chemical and user entered log Kow of 3.7, and are tabulated below.

Endpoint	*Result*	*Assessment Conclusion*
Analogue data		
Fish Toxicity	LC50 (96 h) = 6.73 mg/L	Toxic to fish
Daphnia Toxicity	EC50 (48 h) = 21.3 mg/L	Harmful to aquatic invertebrates
Algal Toxicity	E_rC50 (72 h) = 27.7 mg/L	Harmful to algae
Inhibition of Bacterial Respiration	EC50 (3 h) = 210 mg/L	Not inhibitory to bacterial respiration
ECOSAR (v1.11) data for the notified chemical		
Fish Toxicity	LC50 (96 h) = 2.26 mg/L	Toxic to fish
Daphnia Toxicity	EC50 (48 h) = 3.91 mg/L	Toxic to aquatic invertebrates
Algal Toxicity	EC50 (96 h) = 1.27 mg /L	Toxic to algae

Under the Globally Harmonised System of Classification and Labelling of Chemicals (GHS; United Nations, 2009) the notified chemical is predicted to be acutely toxic to fish, aquatic invertebrates and algae. Based on both the toxicity to aquatic biota and analogue read across data, the notified chemical is formally classified under the GHS as 'Acute category 2; Toxic to aquatic life'. Based on the acute toxicity of the notified chemical, and its lack of ready biodegradability, it is formally classified under the GHS as 'Chronic category 2; Toxic to aquatic life with long lasting effects'.

7.2.1. Predicted No-Effect Concentration

For the purpose of risk assessment, the Predicted No-Effect Concentration (PNEC) has been calculated using the lowest predicted endpoint for the notified chemical, which was for algae. An assessment factor of 100 was used since acute endpoints were available for 3 trophic levels.

Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment		
Algae (96 hr)	1.27	mg/L
Assessment Factor	100	
PNEC:	12.7	µg/L

7.3. Environmental Risk Assessment

Risk Assessment	PEC µg/L	PNEC µg/L	Q
Q – River	0.61	12.7	0.048
Q – Ocean	0.061	12.7	0.0048

The risk quotient for discharge of the notified chemical to the aquatic environment indicates that the notified chemical is unlikely to reach ecotoxicologically significant concentrations based on its annual importation quantity. The notified chemical has a low potential for bioaccumulation. Therefore, on the basis of the PEC/PNEC ratio, maximum annual importation volume and assessed use pattern in cosmetic and domestic products, the notified chemical is not expected to pose an unreasonable risk to the environment.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Freezing Point 6.5 °C

Method	OECD TG 102 Melting Point/Melting Range. EC Council Regulation No 440/2008 A.1 Melting/Freezing Temperature.
Remarks	Analogue chemical (M3MC-Carboxylate)
Test Facility	Huntingdon Life Sciences (2008)

Boiling Point 216 °C at 101.3 kPa

Method	OECD TG 103 Boiling Point. EC Council Regulation No 440/2008 A.2 Boiling Temperature.
Remarks	Analogue chemical (M3MC-Carboxylate)
Test Facility	Huntingdon Life Sciences (2008)

Relative Density 1.02×10^{-3} kg/m^3 at 22 °C

Method	OECD TG 109 Density of Liquids and Solids. EC Council Regulation No 440/2008 A.3 Relative Density.
Remarks	Analogue chemical (M3MC-Carboxylate)
Test Facility	Huntingdon Life Sciences (2008)

Vapour Pressure 2.7×10^{-1} kPa at 25 °C

Method	OECD TG 104 Vapour Pressure.
Remarks	Static method. Test conducted on analogue chemical (M3MC-Carboxylate).
Test Facility	Huntingdon (2008)

Water Solubility 0.84 g/L at 20 °C

Method	OECD TG 105 Water Solubility.
Remarks	Flask method. Test conducted on the notified chemical
Test Facility	Huntingdon (2010a)

Water Solubility 0.37 g/L at 20 °C

Method	OECD TG 105 Water Solubility.
Remarks	Flask Method. Test conducted on analogue chemical (M3MC-Carboxylate).
Test Facility	Huntingdon (2008)

Hydrolysis as a Function of pH

Method OECD TG 111 Hydrolysis as a Function of pH.

pH	*T (°C)*	*$t_{½}$*
4	25	> 1 year
7	25	> 1 year
9	25	21 days

Remarks In the hydrolysis test the change of the molecular weight of the test substance after 5 days at 50 °C and pH 4.0, 7.0 and 9.0 was determined. It was found that the change of the molecular weight of the test substance (M3MC-Carboxylate) was less than 10% at pH 4 and 7. However, at pH 9 greater than 10% but less than 50% hydrolysis was indicated. Therefore, a test was conducted at elevated temperatures of 60 and 70 °C and at pH 9 which indicated a half-life of 21 days. It can be concluded that the test substance can be considered as hydrolytically stable under acidic and neutral conditions, but hydrolysed at basic conditions.

The notified chemical is likely to hydrolyse more slowly than the analogue chemical because the ester group in the notified chemical is not freely available due to the complex structure.

Test Facility Huntingdon (2009b)

Partition Coefficient (n-octanol/water)	log Pow = 3.4 and 3.7 at 25 °C
Method	OECD TG 117 Partition Coefficient (n-octanol/water).
Remarks	HPLC Method. Test conducted on the notified chemical.
Test Facility	Huntingdon (2010a)

Partition Coefficient (n-octanol/water)	log Pow = 3.7 at 25 °C
Method	OECD TG 117 Partition Coefficient (n-octanol/water).
Remarks	HPLC Method. Test conducted on analogue chemical (M3MC-Carboxylate).
Test Facility	Huntingdon (2008)

SURFACE TENSION	52.5 mN/m at 20 °C
Method	OECD TG 115 Surface Tension of Aqueous Solutions.
Remarks	Concentration: 90% of the analogue chemical (M3MC-Carboxylate) saturation solubility in water.
Test Facility	Huntingdon (2008)

Adsorption/Desorption	log K_{oc} = 2.6 at 25 °C
Method	OECD TG 121 Estimation of the Adsorption Coefficient on Soil and on Sewage sludge.
Remarks	HPLC Method. Test conducted on analogue chemical (M3MC-Carboxylate).
Test Facility	Huntingdon (2009a)

Flash Point	83 °C
Method	EC Council Regulation No 440/2008 A.9 Flash Point.
Remarks	Analogue chemical (M3MC-Carboxylate)
Test Facility	Huntingdon Life Sciences (2008)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral

TEST SUBSTANCE	Analogue chemical (M3MC-Carboxylate)
METHOD	OECD TG 423 Acute Oral Toxicity – Acute Toxic Class Method. Annex to the Commission Directive 2004/73/EC B.1 tris Acute Oral Toxicity – Acute Toxic Class Method.
Species/Strain	Rat/CD (Crl:CD SD)
Vehicle	Corn oil
Remarks - Method	No significant protocol deviations.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
1	3F	300	0/3
2	3F	300	0/3
3	3F	2,000	0/3
4	3F	2,000	2/3

LD50	300–2,000 mg/kg bw
Signs of Toxicity	Two test animals from Group 4 were found dead on day 2. Clinical signs noted prior to death in both animals included hunched posture, unsteady gait, piloerection, increased and irregular breathing, reduced body tone, underactivity, partially closed eyelids and reduced bodyweight. One animal showed red staining in urine, while the other animal showed yellow/brown staining of the perigenital area. Clinical signs in animals dosed at 300 mg/kg bw included unsteady gait (seen in five animals) and loose faeces (noted in two animals). These signs had resolved by day 2. Clinical signs of reaction to treatment in the surviving animals treated at 2,000 mg/kg bw included hunched posture, unsteady gait, piloerection, increased breathing, underactivity, muscle tremors, reduced body temperature, yellow/brown staining of the perigenital area, prominent eyes and thin build. All of these signs had resolved by day 10.
Effects in Organs	Macroscopic examination of the two deceased animals revealed congestion of the subcutaneous tissue, heart, lungs, spleen, kidneys and duodenum, enlarged stomach. Inspection of the stomach and small intestine contents showed yellow fluid and red fluid in the large intestines. One animal showed congestion if the liver and the other animal showed enlargement of the urinary bladder. The surviving animals were terminated on day 15. Macroscopy revealed stomach atrophy in 1 animal dosed at 2,000 mg/kg bw. No abnormalities were noted in the other surviving animals.
Remarks - Results	Three surviving animals dosed at 2,000 mg/kg bw and one animal dosed at 300 mg/kg bw were noted to have lower than expected bodyweight on day 15 observations. All other test animals showed the expected gains in bodyweight over the study period.

CONCLUSION	The analogue chemical was harmful via the oral route.
TEST FACILITY	Huntingdon Life Sciences (2008b)

B.2. Acute toxicity – dermal

TEST SUBSTANCE	Analogue chemical (M3MC-Carboxylate)
METHOD	OECD TG 402 Acute Dermal Toxicity.

	Annex to Directive 92/69/EEC B.3 Acute Toxicity (Dermal).
Species/Strain	Rat/CD (Crl:CD SD)
Vehicle	Test substance administered as supplied
Type of dressing	Occlusive
Remarks - Method	No significant protocol deviations.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
1	5 per sex	2,000	0/10

LD50	>2,000 mg/kg bw
Signs of Toxicity - Local	Very slight erythema was noted in one female on day 2. This effect had resolved by day 3.
Signs of Toxicity - Systemic	There were no unscheduled deaths or systemic responses observed during the study period.
Effects in Organs	Macroscopic examination at study termination on day 15 showed two males with small stomachs, one female with pale liver and kidneys. Another female animal had tinner tissue at the upper part of the stomach. There were no abnormalities noted for the other test animals.
Remarks - Results	The increases in body weights of the test animals over the test period were in the expected range, except 1 female noted on day 8.

CONCLUSION The analogue chemical was of low toxicity via the dermal route.

TEST FACILITY Huntingdon Life Sciences (2009b)

B.3. Irritation – skin

TEST SUBSTANCE Analogue chemical (M3MC-Carboxylate)

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	3F
Vehicle	Test substance administered as supplied
Observation Period	15 days
Type of Dressing	Semi-occlusive.
Remarks - Method	No significant protocol deviations. A single 4 hour application of the test material was made to the intact skin of 3 male rabbits. Test sites were observed for evidence of primary irritation at 1, 24, 48 and 72 hours post patch removal.

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	2	2	1.7	2	≥15 days	1
Oedema	0	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 to well-defined erythema was evident at the treated site of all animals from 1 hour post application and sustained for the entire study period. Loss of elasticity was noted in one animal during the 48 hour observations but had subsided by day 15. Exfoliation was noted in all three animals during the second week of observations. There were no signs of toxicity in any of the test animals during the study.

CONCLUSION	The analogue chemical was classified as mildly irritating to rabbit skin.
TEST FACILITY	Huntingdon Life Sciences (2010b)

B.4. Irritation – eye

TEST SUBSTANCE	Analogue chemical (M3MC-Carboxylate)
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	3F
Observation Period	15 days
Remarks - Method	No significant protocol deviations. A single application of 0.1 mL of the test material to the non-irrigated eye of three female rabbits.

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	1	0.7	1.3	2	<8 days	0
Conjunctiva: chemosis	0	0	0.7	1	<72 hours	0
Conjunctiva: discharge	0	0	0	2	<24 hours	0
Corneal opacity	1	0.7	1	1	<8 days	0
Iridial inflammation	0.3	0	0	1	<48 hours	0

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

Remarks - Results	Instillation of the analogue chemical gave rise to a moderate initial pain response in the test animals. Crimson red conjunctival appearance was apparent in all three animals during the first 48 hours post instillation, persisting in two animals at the 72 hour observations and in one animal on day 8. Slight to moderate discharge was evident in all three animals 1 hour post instillation. Very slight chemosis was noted in two animals from 1 to 24 hours post administration. Diffuse areas of opacity were evident in all animals 24 and 48 hours after instillation. Iritis was apparent in one animal 24 hours after instillation. Two animals appeared normal by the day 8 observations and all signs had cleared by day 15 in the remaining animal. There were no signs of toxicity in any of the test animals during the study.
CONCLUSION	The analogue chemical was slightly irritating to the eye.
TEST FACILITY	Huntingdon Life Sciences (2010c)

B.5. Skin sensitisation – mouse local lymph node assay (LLNA)

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 429 Skin Sensitisation: Local Lymph Node Assay EEC Commission Regulation No. 440/2008 B.42 Skin Sensitisation (Local Lymph Node Assay)
Species/Strain	Mouse/ CBA/Ca (CBA/CaCruBR)
Vehicle	Acetone:olive oil (4:1 v/v)
Positive Control	HCA (hexyl cinnamic aldehyde)

Remarks - Method	Pooled treatment group approach. No significant protocol deviations. Topical application was made to the dorsal surface of the ear. A concurrent positive control study was not run, but a previously conducted positive control data from the test laboratory was provided.

RESULTS

Concentration (% v/v)	*Proliferative response (DPM/lymph node)*	*Stimulation Index (Test/Control Ratio)*
Test Substance		
0 (vehicle control)	714.34	-
25	943.09	1.3
50	2,111.43	3.0
As supplied	2,555.00	3.6
Positive Control		
25	2,757.13	6.0

Remarks - Results	No mortalities and no signs of systemic toxicity or local irritation were noted in the test or control animals. A loss in bodyweight was noted for one female in the 25% group and another female in the 50% group. All remaining animals gained weight during the study period. The positive controls gave satisfactory responses confirming the validity of the test system.
CONCLUSION	There was evidence of induction of a lymphocyte proliferative response indicative of the skin sensitisation potential of the notified chemical. The EC3 value was determined to be 50% v/v.
TEST FACILITY	Huntingdon Life Sciences (2010d)

B.6. Skin sensitisation – human volunteers

TEST SUBSTANCE	Notified chemical
METHOD	Repeated insult patch test with challenge (Modified Shelanski-Shelanski method)
Study Design	Rest Period: 14 days
Study Group	99F, 14 M; age range 18-70years
Vehicle	2.5% w/w in alcohol SD39C: DEP (25:75)
Remarks - Method	Occluded. The notified chemical was applied to a 3.63 cm^2 patch and left to dry for 30 min. A panel of 113 healthy human subjects (devoid of any physical or dermatological conditions) was amassed. During the induction phase, the test article was placed onto an occlusive patch and applied to the back of each subject between the scapulae and waist. This application was repeated every Monday, Wednesday and Friday until 9 applications had been made. The subjected were instructed to remove the patches 24 hours after application on Tuesday and Thursday and after 48 hours on Saturday. After a rest period of 2 weeks, the challenge phase patch was applied to a virgin test site. The site was scored 24, 48 and 72 hours after application. Dermal responses were scored according to a 6-point scale (0, ±, 1 to 4).

RESULTS

Skin Reaction	*Reaction observed in Test subjects*	*Maximum Value **	*Maximum Duration of Any Effect **	*Maximum Value at End of Observation Period*
Erythema/Eschar	0/102**	0	0	0

*Calculated on the basis of the Challenge Scores at 24, 48 and 72 hours for test subjects.
** 11 subjects (11 females) discontinued for personal reasons unrelated to the conduct of the study. Data from these subjects up to the point of discontinuation was not used in the conclusions of the final report.

Remarks - Results: 102 subjects satisfactorily completed the test procedure. No clinical signs were noted for any test subject during the induction or challenge phases. The test material did not demonstrate a potential for eliciting dermal irritation or sensitisation under the test conditions.

CONCLUSION: The notified chemical was non-sensitising under the conditions of the test.

TEST FACILITY: Clinical Research Laboratories, Inc. N.J (2010)

B.7. Repeat dose toxicity

TEST SUBSTANCE: Analogue chemical (M3MC-Carboxylate)

METHOD: OECD TG 407 Repeated Dose 28-day Oral Toxicity Study in Rodents.
EC Directive 67/548/EC B.7 Repeated Dose (28 Days) Toxicity (Oral).

Species/Strain: Rat/CD (Crl:CD SD)
Route of Administration: Oral – gavage
Exposure Information: Total exposure days: 28 days
Dose regimen: 7 days per week
Post-exposure observation period: 14 days
Vehicle: Corn oil
Remarks - Method: No significant protocol deviations.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw/day*	*Mortality*
control	5 M, 5 F	0	0/10
low dose	5 M, 5 F	15	0/10
mid dose	5 M, 5 F	150	0/10
high dose	5 M, 5 F	1,000	0/10
control recovery	5 M, 5 F	0	0/10
high dose recovery	5 M, 5 F	1,000	0/10

Mortality and Time to Death
There were no unscheduled deaths during the study.

Clinical Observations
Analysis of clinical appearance, functional observations, and food consumption did not reveal any toxicologically significant abnormalities between the treated and the control groups.
Effects considered to be treatment related were confined to animals receiving 1,000 mg/kg/day and consisted predominantly of underactivity, unsteady gait, hunched posture, reduced body tone and increased water consumption. Overall group mean bodyweight gains were lower for both sexes in the highest dose group during the treatment period.

Forelimb strength values were elevated for females receiving 1,000 mg/kg/day. Increased body tone and slightly elevated forelimb strength was noted in 4 and 2 female animals respectively in the 150 mg/kg/day group.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis
Increased triglyceride and ALT values and reduced chloride values compared to control were observed in females dosed at the highest level.

Hepatocyte hypertrophy was observed in the liver of some female animals treated at the highest dose. Statistically significantly reduced group mean total protein and albumin values and increased albumin/globulin ratio was also seen in this group, compared to the concurrent control. Higher than control ketone levels were noted for females receiving 1,000 mg/kg/day, which were comparable to control after the recovery period.

Animals receiving 1,000 mg/kg/day had a higher group mean total urinary volume and lower pH than observed in the concurrent controls. Males receiving 150 mg/kg/day were also considered to have lower pH values at the end of the treatment period. However, all parameters were considered to be similar to the respective control values during the period.

Effects in Organs

The macroscopic examination revealed enlargement of the liver and thymus in an increased incidence in females and increased adjusted kidney weights for both sexes, seen in the rats treated with 1,000 mg/kg/day compared to the control group. However these weight values were similar to control in the recovery group after two weeks.

An increased incidence in oedema and depressions on the epithelial aspect of the forestomach was noted in animals treated at the highest dose. The depression was also seen in one of the male animals treated with 150 mg/kg/day.

Remarks – Results

The changes in liver weights, histopathological stomach effects, increased water consumption and reduced body weight gain findings in the high dose group were considered to be adverse and hence the lower dose of 150 mg/kg bw/day was the dose where no adverse treatment related effects were observed.

Some treatment related disturbances in urinary pH in male animals and forelimb grip strength in female animals were noted in the 150mg/kg/day group, however these changes were considered by the study authors to be minor and not toxicologically significant.

CONCLUSION

The No Observed (Adverse) Effect Level (NO(A)EL) for systemic toxicity was established by the study authors as 150 mg/kg bw/day in this study, based on adverse effects at the highest dose tested.

TEST FACILITY Huntingdon Life Sciences (2009f)

B.8. Genotoxicity – bacteria

TEST SUBSTANCE Notified chemical

METHOD

	OECD TG 471 Bacterial Reverse Mutation Test. EEC Commission Regulation No. 440/2008 Method B.13/14 Mutagenicity – Reverse Mutation Test using Bacteria.
Species/Strain	*S. typhimurium*: TA1535, TA1537, TA98, TA100 *E. coli*: WP2uvrA (pKM101)
Metabolic Activation System	S9 fraction from phenobarbitone/β-naphthoflavone induced rat liver
Concentration Range in Main Test	a) With metabolic activation: 5–5,000 µg/plate b) Without metabolic activation: 2–5,000 µg/plate
Vehicle	Dimethyl sulfoxide
Remarks - Method	No significant protocol deviations. Test 1 was conducted as a plate incorporation assay. Test 2 was undertaken as a pre-incubation assay. Two positive control tests were conducted parallel to the main test using phenobarbital and 5,6-benzoflavone.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:*	
	Cytotoxicity in Main Test	*Genotoxic Effect*
Absent		

Test 1	>5,000	negative
Test 2	>5,000	negative
Present		
Test 1	>5,000	negative
Test 2	>5,000	negative

Remarks - Results

No significant increases in the frequency of revertant colonies were recorded for any of the bacterial strains, with any dose material, either with or without metabolic activation.

The positive controls produced satisfactory responses, thus confirming the activity of the S9-mix and the sensitivity of the bacterial strains.

The notified chemical at 5,000 µg/plate caused a visible reduction in the growth of the bacterial background lawn, with and without metabolic activation.

CONCLUSION

The notified chemical showed no evidence of mutagenic activity on bacteria under the conditions of the test.

TEST FACILITY

Huntingdon Life Sciences (2010e)

B.9. Genotoxicity – in vitro

TEST SUBSTANCE

Notified chemical

METHOD

OECD TG 476 In vitro Mammalian Cell Gene Mutation Test.
EEC Commission Regulation No. 440/2008 Method B.17 Mutagenicity - In vitro Mammalian Cell Gene Mutation Test.

Species/Strain	Mouse/
Cell Type/Cell Line	Lymphoma/(3.7.2c) cells/ L5178Y
Metabolic Activation System	S9 fraction from phenobarbital/β-naphthoflavone induced rat liver
Vehicle	Dimethyl sulfoxide

Remarks - Method

A preliminary cytogenetic assay were performed: tested both with and without the metabolic activation system (at 2% v/v) for concentrations up to 1,661 µg/mL with 3 hour exposure time and the absence of S9 mix only for 24 hour fixation time. Precipitation was noted in the culture mediums at 830.5 and 1,661 µg/mL in both the absence and presence of S9 mix following 3 hour exposure. Following continuous exposure for 24 hours, no precipitate was observed.

Vehicle and positive controls (MMS without metabolic activation and BaP with metabolic activation) were used in parallel with the test substance.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Expression Time*	*Selection Time*
Absent				
Test 1	5, 50, 100, 200, 250, 300, 350, 400, 450	3	2 d	10–14 d
Test 1a	50*, 200*, 225*, 250*, 275*, 300, 325, 350	3	2 d	10–14 d
Test 2	5*, 50*, 100, 150*, 200*, 250*, 300, 350, 400	24	2 d	10–14 d
Present				
Test 1	5*, 100*, 200*, 400*, 500*, 600, 700, 800, 850	3	2 d	10–14 d

* Cultures assessed for mutant phenotype

RESULTS

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

Test 1		N/A	>450	N/A
Test 1a	≥450	>350	>350	negative
Test 2	≥400	>250	>400	negative
Present				
Test 1	≥850	>500	>850	negative

Remarks - Results: There were no toxicologically (or statistically) significant increases or dose response relationships in mutant frequencies or number of small colony mutants, with or without metabolic activation.

The positive and vehicle controls gave satisfactory responses confirming the validity of the test system

CONCLUSION: The notified chemical did not demonstrate mutagenic potential to mouse lymphoma L5178Y cells treated in vitro under the conditions of the test.

TEST FACILITY: Huntingdon Life Sciences (2011)

B.10. Genotoxicity – in vitro

TEST SUBSTANCE: Notified chemical

METHOD: OECD TG 473 In vitro Mammalian Chromosome Aberration Test.
EC No. 440/2008 B.10 Mutagenicity – In Vitro

Species/Strain: Human
Cell Type/Cell Line: Human peripheral lymphocytes
Metabolic Activation System: S9 fraction from phenobarbital/β-naphthoflavone induced rat liver
Vehicle: Dimethyl sulfoxide
Remarks - Method: No significant protocol deviations.
Vehicle and positive controls (mitomycin C without metabolic activation and cyclophosphamide with metabolic activation) were used in parallel with the test substance.

Metabolic Activation	*Test Substance Concentration (μg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	16.70, 27.90, 46.50, 77.50, 129.20*, 215.30*, 358.80*, 598, 996.60, 1661	3 h	24 h
Test 2	20, 40, 80*, 120, 160*, 200, 240*, 280, 320, 360, 400	21 h	21 h
Present			
Test 1a	60, 120, 180, 240, 300, 360, 420, 480*, 495*, 540, 600	3 h	24 h
Test 1b	300, 400, 435, 450, 465, 480*, 495*, 510, 525*, 540	3 h	24 h
Test 2a	300, 400, 430, 445, 460, 475, 490, 505, 520, 535, 550*	3 h	24 h
Test 2b	500*, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1400, 1500, 1661	3 h	24 h
Test 2c	500*, 525, 550*, 575*, 625, 650, 675, 725, 750	3 h	24 h

* Cultures selected for metaphase analysis.

RESULTS

Metabolic Activation	*Test Substance Concentration (μg/mL) Resulting in:*	
	Cytotoxicity in Main Test	*Genotoxic Effect*
Absent		
Test 1	> 358.80	negative
Test 2	≥ 80	positive
Present		
Test 1	> 525	negative
Test 2	> 575	negative

Remarks - Results: Each test was performed with different concentration ranges. In metaphase analysis, there were statistically significant increases in the frequency of

structural chromosome aberrations, following 21 hour continuous exposure only, without metabolic activation. Under all other experimental conditions, there was no evidence of causing an increase in the proportion of cells with aberrations.

No statistically significant increases in polyploidy metaphases were observed in the analysis of either test.

The positive and vehicle controls gave satisfactory responses confirming the validity of the test system.

CONCLUSION The notified chemical was clastogenic to human lymphocytes treated in vitro in the absence of S9 mix following 21 hour continuous exposure only.

TEST FACILITY Huntingdon Life Sciences (2010f)

B.11. Genotoxicity – in vitro

TEST SUBSTANCE Notified chemical

METHOD OECD TG 487 In Vitro Mammalian Cell Micronucleus Test
Species Human
Cell Type/Cell Line Human peripheral lymphocytes
Metabolic Activation System S9 fraction from phenobarbital/β-naphthoflavone induced rat liver
Vehicle Dimethyl sulfoxide
Remarks - Method

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	46.5*, 215.3*, 598*	3 h	20 h
Test 2	5, 20, 40	20 h	20 h
Present			
Test 1	300*, 580*, 582.5*	3h	20 h

* Cultures selected for metaphase analysis.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/mL) Resulting in:* *Cytotoxicity in Main Test*	*Genotoxic Effect*
Absent		
Test 1	≥598	negative
Test 2	≥40	negative
Present		
Test 1	≥582.5	negative

Remarks - Results Statistically significant increases in the number of binucleate cells containing micronuclei compared to the vehicle controls were obtained in both the absence and presence of S9 mix following 3 hour treatment. However the study authors state that the means values for all the test item treatment groups were within the historical control range. Therefore, they did not consider these increases to be biologically relevant.

The test substance did not induce any statistically significant increases in the number of binucleate cells containing micronuclei in the 20 hour treatment group.

No statistically significant increases in polyploidy metaphases were observed in the analysis of either test.

The positive and vehicle controls gave satisfactory responses confirming the validity of the test system.

CONCLUSION The notified polymer did not induce micronuclei in cultured human peripheral blood lymphocytes treated in vitro under the conditions of the test.

TEST FACILITY Huntingdon Life Sciences (2012)

B.12. Genotoxicity – in vivo

TEST SUBSTANCE Notified chemical

METHOD OECD TG 475 Mammalian Bone Marrow Chromosome Aberration Test.
EC Directive 2000/32/EC B.11 Mutagenicity - In vivo Mammalian Bone Marrow Chromosome Aberration Test.

Species/Strain	ICR mice (virus antibody-free)
Route of Administration	Dermal – intraperitoneal injection
Vehicle	Corn oil
Concentration in Preliminary test	62.5–2,000 mg/kg
Remarks - Method	The vehicle and positive controls were tested concurrently with the notified chemical.

Mortality was observed in the dose range-finding study, in 2/3 males and 2/3 females at 1,500 mg/kg and in 2/3 male and 3/3 females at 2,000 mg/kg.

During the dose range-finding study, clinical effects such as persistent piloerection, prostration, irregular breathing, hunched position and tremors were seen at doses 1,000 mg/kg and over. Lethargy and transient piloerection were seen in animals of both sexes at doses 62.5 mg/kg and over.

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Sacrifice Time hours*
I (vehicle control)	5 M	-	18
II (low dose)	5 M	312	18
III (mid dose)	5 M	625	18
IV (high dose)	5 M	1,250	18
V (positive control, CP)	5 M	50	18
Vehicle recovery	5 M	-	48
High dose recovery	10 M	1,250	48

CP=cyclophosphamide.

RESULTS

Doses Producing Toxicity	≥1,250 mg/kg bw.
Genotoxic Effects	None
Remarks - Results	In the definitive study, no mortality was observed in any of the treatment groups. Clinical signs noted at all doses were piloerection and lethargy. Prostration and irregular breathing were noted at 1,250 mg/kg. These observations were not considered by the study authors to be of toxicological significance.

No appreciable reductions in the mean Mitotic Index (MI) compared to the vehicle control were observed in any of the treatment groups.

No statistically significant increase in the number of cells with structural

	aberrations compared to the vehicle control were noted in any of the test substance treatment groups. No numerical aberrations were observed in the test substance or control groups. The positive and vehicle controls gave satisfactory responses confirming the validity of the test system.
CONCLUSION	The notified chemical was not clastogenic under the conditions of the test.
TEST FACILITY	BioReliance (2013)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready biodegradability

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 310 Ready Biodegradability: CO_2 in Sealed Vessel Test.
Inoculum	Activated sewage sludge from a predominantly domestic sewage treatment plant.
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	Total Inorganic Carbon (TIC)
Remarks - Method	Conducted in accordance with the test guidelines above, and in compliance with GLP standards and principles.

RESULTS

Notified chemical		*Sodium benzoate*	
Day	*% Degradation*	*Day*	*% Degradation*
7	1.4	7	75.9
14	0	14	88.8
21	0	21	87.8
28	0.2	28	85.7

Remarks - Results	On two occasions during the test, the maximum temperature of water exceeded that stated in the study protocol. However, as all the validity criteria were fulfilled, this deviation was not considered to be significant. The toxicity control containing sodium benzoate and the test substance attained > 77% degradation by day 7 of the study thereby confirming that the test substance was not toxic to the sewage treatment micro-organisms used in the study.
CONCLUSION	The notified chemical is not readily biodegradable.
TEST FACILITY	Huntingdon (2010g)

C.1.2. Ready biodegradability

TEST SUBSTANCE	Analogue chemical (M3MC-Carboxylate)
METHOD	OECD TG 301 F Ready Biodegradability: Manometric Respirometry Test.
Inoculum	Activated sewage sludge from a predominantly domestic sewage treatment plant
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	Biological Oxygen Demand (BOD)
Remarks – Method	Conducted in accordance with the test guidelines above, and in compliance with GLP standards and principles.

RESULTS

Notified chemical		*Sodium benzoate*	
Day	*% Degradation*	*Day*	*% Degradation*
7	10	3	60
17	41	17	87
28	74	28	92

Remarks – Results	The validity criteria for the test were met. The toxicity control containing sodium benzoate and the test substance attained 61% degradation by day 3 of the study thereby confirming that the test substance was not toxic to the sewage treatment micro-organisms used in the study.

	The Analogue M3MC-Carboxylate was not considered readily biodegradable as the value obtained at the end of 10 day window was 41%. However, as biodegradation of exceeded 60% by day 28, the test substance was considered to be biodegradable under the test conditions.
CONCLUSION	The test substance and, by inference, the notified chemical are not readily biodegradable.
TEST FACILITY	Huntingdon (2009e)

C.2. Ecotoxicological Investigations

C.2.1. Acute toxicity to fish

TEST SUBSTANCE	Analogue chemical (M3MC-Carboxylate)
METHOD	OECD TG 203 Fish, Acute Toxicity Test - Semi-Static.
Species	*Brachydanio rerio* (Zebra fish)
Exposure Period	96 hour
Auxiliary Solvent	None
Water Hardness	126 mg $CaCO_3$/L
Analytical Monitoring	GC/FID
Remarks – Method	After a range finding test, a definitive test was conducted in accordance with the guidelines above and in compliance with GLP standards and principles. No significant deviations to the test protocol were reported. The test was performed with renewal of the test solution every 24 hours.

RESULTS

Concentration mg/L		*Number of Fish*	*Mortality*			
Nominal	*Measured*		*24 h*	*48 h*	*72 h*	*96 h*
Control	-	7	0	0	0	0
1.0	1.11	7	0	0	0	0
1.8	2.05	7	0	0	0	0
3.2	3.21	7	0	0	0	0
5.6	5.82	7	0	0	1	2
10	10.45	7	0	1	4	7

EC50	6.73 mg/L at 96 hours. The 95% confidence limit is 5.587–8.12 mg/L.
NOEC	3.21 mg/L.
Remarks – Results	The validity criteria for the test were met. The LC50 and 95% confidence limit values were calculated using the geometric mean measured concentrations values.
CONCLUSION	The test substance and, by inference, the notified chemical are toxic to fish.
TEST FACILITY	Safety Evaluation Centre (2010g)

C.2.2. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE	Analogue chemical (M3MC-Carboxylate)
METHOD	OECD TG 202 *Daphnia* sp. Acute Immobilisation Test – Static.
Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	None
Water Hardness	50–220 mg $CaCO_3$/L
Analytical Monitoring	GC-MS
Remarks - Method	After a range finding test, a definitive test was conducted in accordance with the guidelines above and in compliance with GLP standards and principles. No significant deviations to the test protocol were reported.

RESULTS

Concentration mg/L		*Number of D. magna*	*Number Immobilised*	
Nominal	*Measured*		*24 h*	*48 h*
Control	nd	20	0	0

3.41	2.67	20	0	0
7.51	6.79	20	0	0
16.5	14.3	20	0	0
36.4	31.6	20	19	20
80	70.9	20	19	20

LC50	21.3 mg/L at 48 hours. The 95% confidence limit is 14.3–31.6 mg/L.
NOEC	14.3 mg/L
Remarks - Results	The LC50 and 95% confidence limit values were calculated using the geometric mean measured concentrations values.
CONCLUSION	The test substance and, by inference, the notified chemical are harmful to aquatic invertebrates.
TEST FACILITY	Huntingdon (2010h)

C.2.3. Algal growth inhibition test

TEST SUBSTANCE	Analogue chemical (M3MC-Carboxylate)
METHOD	OECD TG 201 Alga, Growth Inhibition Test.
Species	*Pseudokirchneriella subcapitata*
Exposure Period	72 hours
Concentration Range	Nominal: 0.441, 0.97, 2.13, 4.7, 10.3, 22.7 and 50 mg/L Measured: 0.196, 0.444, 1.19, 2.51, 6.86, 16.3 and 37.4 mg/L
Auxiliary Solvent	None
Water Hardness	0.4 mM Ca^{2+} and Mg^{2+}
Analytical Monitoring	GC-MS
Remarks - Method	After a range finding test, a definitive test was conducted in accordance with the guidelines above and in compliance with GLP standards and principles. No significant deviations to the test protocol were reported.

RESULTS

Biomass		*Growth*	
EyC50 mg/L at 72 h	*NOEC mg/L*	*ErC50 mg/L at 72 h*	*NOEC mg/L*
14.3	1.19	27.7	1.19

Remarks - Results	The validity criteria for the test were met.
CONCLUSION	The test substance and, by inference, the notified chemical are harmful to algae.
TEST FACILITY	Huntingdon (2010i)

C.2.4. Inhibition of microbial activity

TEST SUBSTANCE	Analogue chemical (M3MC-Carboxylate)
METHOD	OECD TG 209 Activated Sludge, Respiration Inhibition Test.
Inoculum	Activated sewage sludge from domestic sewage treatment plant
Exposure Period	3 hours
Concentration Range	Nominal: 10, 30, 100, 300 and 1000 mg/L Actual: Not measured
Remarks – Method	The test was conducted in accordance with the test guideline without significant deviations. Good Laboratory Practice (GLP) protocol was followed.
RESULTS	
IC50	210 mg/L. The 95% confidence limit is 176–248 mg/L.
Remarks – Results	During the test, the temperature of the incubator ranged between 20.8 and 24.0ºC. This was outside the range of stated in the protocol. However, as all the validity criteria were fulfilled, this deviation was not considered to be significant.
CONCLUSION	The test substance and, by inference, the notified chemical are not expected to be inhibitory to micro-organisms at concentrations < 210 mg/L.
TEST FACILITY	Huntingdon (2009f)

BIBLIOGRAPHY

BioReliance (2013) Bone Marrow Chromosome Aberration Test in Mice following Intraperitoneal Injection (IP) of TM 09-218c (Study number AD39TT.108M.BTL, 14 November 2013) Maryland, US, BioReliance (Unpublished report submitted by notifier).

CRL (2010) Repeated Insult Patch Test: Test Item 10-204-10 (CRL study number 52910-1, 19 August 2010) New Jersey, USA, Clinical Research Laboratories Inc. (Unpublished report submitted by notifier).

Huntingdon (2008a) M3MC-Carboxylate Physicochemical Properties (HLS Study No. IFF0391, 3 September 2008) Suffolk, UK, Huntingdon Life Sciences Ltd (Unpublished report submitted by notifier).

Huntingdon (2008b) M3MC-Carboxylate: Acute Oral Toxicity to the Rat (Acute Toxic Class Method) (HLS Study No. IFF0392, 29 July 2008) Suffolk, UK, Huntingdon Life Sciences Ltd (Unpublished report submitted by notifier).

Huntingdon (2009a) M3MC-Carboxylate Physicochemical Properties (HLS Study No. IFF0400, 10 March 2009) Suffolk, UK, Huntingdon Life Sciences Ltd (Unpublished report submitted by notifier).

Huntingdon (2009b) M3MC-Carboxylate: Acute Dermal Toxicity to the Rat (HLS Study No. IFF0402, 30 March 2009) Suffolk, UK, Huntingdon Life Sciences Ltd (Unpublished report submitted by notifier).

Huntingdon (2009c) M3MC-Carboxylate: Toxicity Study by Oral Administration to CD rats for 4 weeks followed by 2 week Recovery Period (HLS Study No. IFF0408, 21 October 2009) Suffolk, UK, Huntingdon Life Sciences Ltd (Unpublished report submitted by notifier).

Huntingdon (2009d) M3MC-Carboxylate Abiotic Degradation: Hydrolysis as a Function of pH (HLS Study No. IFF0401, 25 March 2009) Suffolk, UK, Huntingdon Life Sciences Ltd (Unpublished report submitted by notifier).

Huntingdon (2009e) M3MC-Carboxylate Assessment of Ready Biodegradability (HLS Study No. IFF0416, 25 March 2009) Suffolk, UK, Huntingdon Life Sciences Ltd (Unpublished report submitted by notifier).

Huntingdon (2009f) M3MC-Carboxylate Activated Sludge: Respiration Inhibition Test (HLS Study No. IFF0415, 1 April 2009) Suffolk, UK, Huntingdon Life Sciences Ltd (Unpublished report submitted by notifier).

Huntingdon (2010a) TM 09-218 Physicochemical Properties (HLS Study No. JMN0003, 26 May 2010) Suffolk, UK, Huntingdon Life Sciences Ltd (Unpublished report submitted by notifier).

Huntingdon (2010b) M3MC-Carboxylate: Skin Irritation to the Rabbit (HLS Study No. IFF0403, 23 February 2010) Suffolk, UK, Huntingdon Life Sciences Ltd (Unpublished report submitted by notifier).

Huntingdon (2010c) M3MC-Carboxylate: Eye Irritation to the Rabbit (HLS Study No. IFF0405, 23 February 2010) Suffolk, UK, Huntingdon Life Sciences Ltd (Unpublished report submitted by notifier).

Huntingdon (2010d) TM 09-218: Assessment of Skin Sensitization Potential using the Local Lymph Node Assay in the Mouse (HLS Study No. JMN0007, 18 June 2010) Suffolk, UK, Huntingdon Life Sciences Ltd (Unpublished report submitted by notifier).

Huntingdon (2010e) TM 09-218: Bacterial Reverse Mutation Test (HLS Study No. JMN0005, 19 August 2010) Suffolk, UK, Huntingdon Life Sciences Ltd (Unpublished report submitted by notifier).

Huntingdon (2010f) TM 09-218: In Vitro Mammalian Chromosome Aberration Test in Human Lymphocytes (HLS Study No. JMN0006, 26 November 2010) Suffolk, UK, Huntingdon Life Sciences Ltd (Unpublished report submitted by notifier).

Huntingdon (2010g) TM 09-218 Assessment of Ready Biodegradability Sealed Vessel CO_2 Evolution Test (HLS Study No. JMN004, 7 October 2010) Suffolk, UK, Huntingdon Life Sciences Ltd (Unpublished report submitted by notifier).

Huntingdon (2010h) M3MC-Carboxylate Acute Toxicity to *Daphnia magna* (HLS Study No. IFF0412, 24 February 2010) Suffolk, UK, Huntingdon Life Sciences Ltd (Unpublished report submitted by notifier).

Huntingdon (2010i) M3MC-Carboxylate Algal Growth Inhibition Test (HLS Study No. IFF0411, 26 February 2010) Suffolk, UK, Huntingdon Life Sciences Ltd (Unpublished report submitted by notifier).

Huntingdon (2011) TM 09-218: In Vitro Mutation Test Using Mouse Lymphoma L5178Y Cells HLS Study No. JMN0030, 02 December 2011) Suffolk, UK, Huntingdon Life Sciences Ltd (Unpublished report submitted by notifier).

Huntingdon (2012) TM 09-218: In Vitro Micronucleus Test in Cultured Human Lymphocytes (HLS Study No. JMN0031, 14 February 2012) Suffolk, UK, Huntingdon Life Sciences Ltd (Unpublished report submitted by notifier).

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

OECD SIDS 2002. SIDS Initial Assessment Report on 5-ethylidene-2-norbornene. Accessed April 2014 at http://webnet.oecd.org/hpv/ui/SIDS_Details.aspx?id=e9c155ab-2bec-438e-a869-19c98446fa4b.

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

Safety Evaluation Center (2010) Acute Toxicity of M3MC-Carboxylate to Zebra fish (*Brachidanio rario*) (Study No. G0923J0159 translated version, 5 May 2010) Shenyang, China, Supervision and Test Center for Pesticide Safety Evaluation and Quality Control (Unpublished report submitted by notifier).

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

Steinmann SN, Vogel, P, Mo, Y, Corminboeuf, C 2011, 'The norbornene mystery revealed', *Chemical Communications*, vol. 47, pp. 227-229.

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 (2009) Estimations Programs Interface Suite™ for Microsoft® Windows, v 4.00. United States Environmental Protection Agency. Washington, DC, USA. Accessed on 19 March 2014.

US EPA (2012) Estimating Toxicity of Industrial Chemicals to Aquatic Organisms using ECOSAR (Ecological Structure Activity Relationship) for MS-Windows Version 1.11. United States Environmental Protection Agency. Washington, DC, USA, http://www.epa.gov/oppt/newchems/tools/21ecosar.htm. Accessed on 19 March 2014.