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November 2009

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

FULL PUBLIC REPORT

D-Glucose, ether with glycerol (Glyceryl Glucoside)

This Assessment has been compiled in accordance with the provisions of the *Industrial Chemicals (Notification and Assessment) Act 1989* (Cwlth) (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 Ageing, 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, Water, Heritage and the Arts.

For the purposes of subsection 78(1) of the Act, this Full Public Report may be inspected at our NICNAS office by appointment only at 334-336 Illawarra Road, Marrickville NSW 2204.

This Full 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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D-Glucose, ether with glycerol (Glyceryl Glucoside)

1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)
Cognis Australia Pty Ltd (ABN 87 006 374 456)
4 Saligna Drive, TULLAMARINE VIC 3043

Beiersdorf Australia Ltd (ABN 98 000 025 623)
4 Khartoum Road, NORTH RYDE NSW 2113

NOTIFICATION CATEGORY
Standard: Chemical other than polymer (more than 1 tonne per year).

EXEMPT INFORMATION (SECTION 75 OF THE ACT)
Data items and details claimed exempt from publication:
Other Names, Structural Formula, Molecular Weight, Spectral and identification data and methods, Hazardous Impurities, Non-hazardous Impurities, Additives/Adjuvants, Import Volume, Purity

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
No variation to the schedule of data requirements is claimed.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
None

2. IDENTITY OF CHEMICAL

CHEMICAL NAME
D-Glucose, ether with glycerol

MARKETING NAME(S)
Hydagen GG (containing the notified chemical at ≤ 55%)

OTHER NAME(S)
Glyceryl Glucoside (INCI)

CAS NUMBER
100402-60-6

MOLECULAR FORMULA
$C_6H_{12}O_6.C_3H_8O_3$

MOLECULAR WEIGHT
NAMW < 500 Da

ANALYTICAL DATA
Reference IR and HPLC spectra were provided.

3. COMPOSITION

DEGREE OF PURITY 35-55%

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20°C AND 101.3 kPa: Brown solid

Property	Value	Data Source/Justification
Melting Point	> 240°C	Measured
Boiling Point	> 250°C at 101.3 kPa	Measured
Density	1454 kg/m^3 at 20°C	Measured
Vapour Pressure	1.2×10^{-5} kPa at 20°C	Measured
Water Solubility	> 20 g/L (temperature unknown)	Measured
Hydrolysis as a Function of pH	Not tested, expected to be stable.	Inferred from the structure.
Partition Coefficient (n-octanol/water)	log Pow = -4.1 at 20°C	Estimated
Adsorption/Desorption	log K_{oc} = 1	Estimated
Dissociation Constant	Not tested	No readily dissociable groups.
Particle Size	Not determined	Introduced in solution only.
Flash Point	> 100°C at 101.3 kPa	Hydagen GG (40-55% notified chemical in glycerin)
Flammability	Not considered to be flammable	Measured
Autoignition Temperature	> 400°C	Measured
Explosive Properties	Not expected to be explosive	The structural formula contains no explosophores.

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

Reactivity
The notified chemical is stable at normal conditions but could decompose at about 261°C.

5. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS
The notified chemical will not be manufactured in Australia. It will be imported as a component (at up to 7.5%) in finished skin care, hair care and cleansing products and other cosmetic products. The notified chemical may be imported as a raw material (at up to 55%) to be blended locally.

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

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

PORT OF ENTRY
Sydney and Melbourne

TRANSPORTATION AND PACKAGING
The notified chemical will be imported in 1000 L polyethylene containers or in 220 L polyethylene open top barrels.

The finished products containing the notified chemical will be imported in a variety of cosmetic containers suitable for sale. These containers will be packed in cartons and 12 cartons will be packed to a cardboard shipper. The shippers will be transported in a container from the wharf to the notifier's central warehouse. The cartons will be transported to retail warehouses and stores and to the general public.

USE
The notified chemical may be used as a humectant, skin conditioning agent, emulsion stabilising agent or film forming ingredient in cosmetic products at a level of up to 7.5%.

OPERATION DESCRIPTION

The notified chemical will be imported as a component in finished skin care, hair care and cleansing products and other cosmetic products.

The notified chemical may be used in the reformulation of cosmetic products. The process will involve a blending operation which will be mainly automated and occur in a fully enclosed environment, followed by automatic filling in containers of various sizes.

The final consumer products will be distributed to retail outlets, displayed and sold to the public.

6. HUMAN HEALTH IMPLICATIONS

6.1 Exposure assessment

6.1.1 Occupational exposure

NUMBER AND CATEGORY OF WORKERS

Category of Worker	*Number*	*Exposure Duration (hours/day)*	*Exposure Frequency (days/year)*
Transport and Storage	10	4	12
Professional compounder	1	8	12
Chemist	1	3	12
Packers (Dispensing and Capping)	2	8	12
Store Persons	2	4	12
End Users	3×10^5	8	365

EXPOSURE DETAILS
Transport and distribution workers are not expected to be exposed to the notified chemical except in an unlikely event of an accident. In case of such accidental exposure, main routes of exposure would be dermal and ocular. However, the likelihood of such an accidental exposure is minimal.

In case of import of raw material for reformulation into consumer products, dermal and ocular exposure to the notified chemical (at up to 55%) may occur during manual transfer from the drums and pails in to the mixing vessel. However, this exposure could be minimised by the use of PPE for skin and eye protection by the workers.

Packers may also have dermal and ocular exposure to the notified chemical up to 7.5%. However exposure is likely to be minimised through the automation of the process and the use of safety glasses and gloves.

Overall, the exposure of workers to the notified chemical is expected to be low.

6.1.2. Public exposure

End-use products are designed to be sold to consumers. The general public will be repeatedly exposed to low-levels (< 7.5%) of the notified chemical via a number of different cosmetic products.

Chronic dermal exposure

Public exposure to the notified chemical in Australia has been estimated using the Scientific Committee on Consumer Products' (SCCP's) Notes of Guidance for the Testing of Cosmetic Ingredients and their Safety Evaluation and applying the following assumptions:

- Bodyweight of 60 kg for females (SCCP, 2006);
- The concentration of the notified chemical in all cosmetic and household products is 7.5%;
- 100% dermal absorption (SCCP, 2006);
- An individual uses all product types containing the notified chemical.

Product(s) used	*Use level for each product*	*Retention factor*	*Systemic Exposure (g/day)*
Shampoo	8.0 g per day	0.01	0.08
Hair conditioner	14.0 g × 0.28 applications/day	0.01	0.04
Hair styling products	5.0 g × 2 applications/day	0. 1	1.0
Shower gel	5.0 g × 2 applications/day	0.01	0.10
Face cream	0.8 g × 2 applications/day	1.0	1.6
Body lotion	8.0 g × 1 applications/day	1.0	8.0
Total product exposure =			10.82

Total exposure to the notified chemical at 7.5% in each of the products above = 180 mg/kg bw/day × 7.5%

Total	13.5 mg/kg bw/day

This exposure estimate was produced using highly conservative assumptions and is expected to reflect a worst-case scenario. In reality, the level of exposure is expected to be lower than 13.5 mg/kg bw/day as it is assumed that consumers would not use all these products to the extent shown above, and dermal absorption may be less than 100%.

Public exposure from transport, storage, reformulation or disposal is considered to be negligible.

6.2. Human health effects assessment

The results from toxicological investigations conducted on the notified chemical (40-55% in glycerin) are summarised in the table below. Details of these studies can be found in Appendix B.

Endpoint	*Result and Assessment Conclusion*
Rat, acute oral toxicity	LD50 > 2000 mg/kg bw, low toxicity
Rat, acute dermal toxicity	LD50 > 2000 mg/kg bw, low toxicity
Rabbit, skin irritation	non-irritating
Rabbit, eye irritation	slightly irritating
Mouse, skin sensitisation – local lymph node assay	no evidence of sensitisation
Rat, repeat dose oral toxicity – 90 days.	NOAEL = 1000 mg/kg bw/day
Mutagenicity – bacterial reverse mutation	non mutagenic
Genotoxicity – in vitro mammalian chromosome aberration test	non genotoxic

Toxicokinetics, metabolism and distribution

The notified chemical is highly water soluble and very hydrophilic (log P_{ow} of -4.1). Hence dermal absorption is unlikely as the notified chemical is not expected to be able to cross the lipid rich stratum corneum layer. However, absorption across the GI tract may occur by passive diffusion given the low molecular weight (NAMW~308 Da). Bioaccumulation is not likely given the high water solubility.

Acute toxicity

The notified chemical is expected to be of low acute toxicity *via* the oral and dermal routes.

Irritation
Based on the studies provided, the notified chemical is expected to be non-irritating to skin and slightly irritating to eyes. Slight conjunctival irritation was observed in the test animals within the first 24 hours. However, the effects were resolved at the 48 hour observation period.

Sensitisation
The sensitising potential of the notified chemical (40-55% in glycerin) was investigated in a modified local lymph node assay, which used a non-radioactive method of detection (lymph node cell counts). This method has not yet undergone formal validation, but has undergone inter- and intra-laboratory trials in Europe, which showed good reproducibility and sensitivity when compared to the established radioactive method (Ehling et al, 2005a and 2005b). There was no evidence of skin sensitisation to the test substance (40-55% notified chemical in glycerin) when tested under these conditions. Therefore, although this method has not yet been formally validated the notified chemical is considered to be unlikely to be a skin sensitiser given the clear negative response in this modified assay, and the absence of structural alerts in the notified chemical's structure.

Repeated dose oral toxicity
The No Observed Effect Level (NOEL) was established as 300 mg/kg bw/day for the notified chemical (40-55% in glycerin), based on systemic changes noted for the animals treated with 1000 mg/kg bw/day.

The No Observed Adverse Effect Level (NOAEL) was established as 1000 mg/kg bw/day because systemic changes noted for the animals treated with 1000 mg/kg bw/day were isolated and were not correlated to other parameters. The NOAEL of the notified chemical itself is therefore 400-550 mg/kg bw/day.

Genotoxicity
The notified chemical (40-55% in glycerin) was not mutagenic in a bacterial reverse mutation study and not genotoxic in an *in vitro* mammalian chromosome aberration test.

Health hazard classification
Based on the available data the notified chemical is not classified as hazardous under the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004).

6.3. Human health risk characterisation

6.3.1. Occupational health and safety
Dermal and possibly ocular exposure to the notified chemical could occur during the transfer of the raw material to the blending vessel. The level of exposure would vary from site to site depending on the level of automation of the formulation process. The estimated dermal exposure is 230 mg/day, based on EASE model using reasonable worst case defaults (without PPE) for the exposure scenario 'manual addition of liquids' (European Commission, 2003) and assuming the notified chemical is present at concentration of 55%. Therefore, for a 70 kg worker using a 100% dermal absorption factor, systemic exposure is estimated to be 3.3 mg/kg bw/day.

Based on a NOAEL of 400-550 mg/kg bw/day derived from a 90-day repeat dose oral toxicity study, the margin of exposure (MOE) for the transfer of the raw material to the blending vessel is 120-170. MOE greater than or equal to 100 accounting for intra- and inter-species differences are considered acceptable.

Overall, the notified chemical is not expected to pose an unacceptable risk to workers under the occupational conditions described.

6.3.2. Public health

The public may come into contact with the notified chemical (< 7.5%) through the use of a range of cosmetic products.

Systemic Toxicity

The worst -case long-term dermal exposure to the notified chemical is estimated as 13.5 mg/kg bw/day. A dermal NOAEL was not determined, however a NOAEL of 400-550 mg/kg bw/day was established in a 90-day oral study in the rat. The use of this NOAEL results in a margin of exposure (MOE) of 29-41. MOE greater than or equal to 100 are considered acceptable to account for intra- and inter-species differences. However, given that the exposure estimate is based on conservative assumptions (100% dermal absorption and consumer using all products containing maximum concentration of notified chemical) and that the NOAEL established was based on no adverse effects at the highest dose tested the risk to the public of systemic effects after the use of cosmetic products is not considered unacceptable.

Local Toxicity

The public will be exposed to the chemical at a maximum concentration of 7.5%. It is not a skin irritant or sensitiser but is slightly irritating to the eye. At this concentration the notified chemical is unlikely to be an eye irritant and therefore the risk of local irritancy effects is considered to be low.

Overall, the risk to public health from exposure to the notified chemical is not considered unacceptable under the conditions of use described.

7. ENVIRONMENTAL IMPLICATIONS

7.1. Environmental Exposure & Fate Assessment

7.1.1 Environmental Exposure

RELEASE OF CHEMICAL AT SITE

The notified chemical may be released as container residues (estimated 1%) and from equipment cleaning (estimated 3%).

RELEASE OF CHEMICAL FROM USE

The notified chemical is expected to be washed to sewer as a result of its use pattern (skin care, hair care and cleansing products and other cosmetic products).

RELEASE OF CHEMICAL FROM DISPOSAL

Spilt material is expected to be disposed of to landfill after containment and collection. Container residues will be disposed of to landfill with the containers or washed to sewer when containers are rinsed before recycling. Residues removed during equipment cleaning are likely to be flushed to sewer.

7.1.2 Environmental fate

The notified chemical is expected to be largely degraded during sewage treatment as it is readily biodegradable. A small proportion may be discharged to receiving waters in treated effluent as the notified chemical is water soluble, and would be expected to disperse and degrade. Bioaccumulation is not expected as the notified chemical is water soluble and readily biodegradable. For the details of the environmental fate studies please refer to Appendix C.

7.1.3 Predicted Environmental Concentration (PEC)

The PEC can be estimated as outlined below based on the hypothetical worst case assumptions of complete discharge to receiving waters via sewage treatment works nationwide.

Predicted Environmental Concentration (PEC) for the Aquatic Compartment		
Total Annual Import/Manufactured Volume	5000	kg/year
Proportion expected to be released to sewer	100%	
Annual quantity of chemical released to sewer	5000	kg/year
Days per year where release occurs	365	days/year
Daily chemical release:	13.7	kg/day
Water use	200.0	L/person/day
Population of Australia (Millions)	21.374	million
Removal within STP	0%	
Daily effluent production:	4,275	ML
Dilution Factor - River	1.0	
Dilution Factor - Ocean	10.0	
PEC - River:	3.2	µg/L
PEC - Ocean:	0.32	µg/L

7.2. Environmental effects assessment

The results from ecotoxicological investigations conducted on the notified chemical are summarised in the table below. Details of these studies can be found in Appendix C.

Endpoint	*Result*	*Assessment Conclusion*
Fish Toxicity	EC50 > 660 mg/L	Not harmful
Daphnia Toxicity	EC50 > 1000 mg/L	Not harmful
Algal Toxicity	ErC50 > 800 mg/L	Not harmful

The notified chemical is not harmful to aquatic life, consistent with its water solubility.

7.2.1 Predicted No-Effect Concentration

The PNEC can be determined as outlined below by application of a hundredfold assessment factor to the most sensitive aquatic toxicity test result, as data are available for three trophic levels.

Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment		
Fish toxicity (LC50)	> 660	mg/L
Assessment Factor	100	
PNEC:	> 6600	µg/L

7.3. Environmental risk assessment

The risk quotients (Q = PEC/PNEC) are tabulated below.

Risk Assessment	PEC µg/L	PNEC µg/L	Q
Q – River	3.2	> 6600	< 0.0005
Q - Ocean	0.32	> 6600	< 0.00005

The notified chemical is not considered to pose a risk to the environment as risk quotients are much less than one.

8. CONCLUSIONS AND REGULATORY OBLIGATIONS

Hazard classification
Based on the available data the notified chemical is not classified as hazardous under the *Approved Criteria for Classifying Hazardous Substances* [NOHSC:1008(2004)].

and

As a comparison only, the classification of the notified chemical using the Globally Harmonised System for the Classification and Labelling of Chemicals (GHS) (United Nations 2003) is not required, as testing indicates that it is not harmful to aquatic life.

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

When used in the proposed manner, the notified chemical is not considered to pose an unacceptable 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 a risk to the environment.

Recommendations

CONTROL MEASURES
Occupational Health and Safety

- Employers should implement the following safe work practices to minimise occupational exposure during handling of the notified chemical as introduced:
  - Avoid contact with eyes

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

- A copy of the MSDS should be easily accessible to employees.

- If products and mixtures containing the notified chemical are classified as hazardous to health in accordance with the *Approved Criteria for Classifying Hazardous Substances* [NOHSC:1008(2004)] workplace practices and control procedures consistent with provisions of State and Territory hazardous substances legislation must be in operation.

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, 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(2) of the Act; if
- the function or use of the chemical has changed from an ingredient in cosmetic products at a level of up to 7.5%, or is likely to change significantly;
- the amount of chemical being introduced has increased from 5 tonne per annum, or is likely to increase, significantly;
- the chemical has begun to be manufactured in Australia;
- additional information has become available to the person as to an adverse effect of the chemical on occupational health and safety, public health, or the environment.

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

No additional secondary notification conditions are stipulated.

Material Safety Data Sheet
The MSDS of the products containing the notified polymer provided by the notifier were reviewed by NICNAS. The accuracy of the information on the MSDS remains the responsibility of the applicant.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Melting Point > 240ºC

Method EC Directive 92/69/EEC A.1 Melting/Freezing Temperature.
Remarks No melting was shown below 240ºC. From room temperature to 220ºC an evaporation of 3.0±0.4wt% was visible.
The study report was not signed.
Test Facility Henkel (2007a)

Boiling Point > 250ºC at 101.3 kPa

Method EC Directive 92/69/EEC A.2 Boiling Temperature.
Remarks No boiling of main component(s) was shown below 250ºC. From room temperature to 250ºC an evaporation of 3.4±0.2wt% was visible.
The study report was not signed.
Test Facility Henkel (2007b)

Density 1454 kg/m^3 at 20ºC

Method EC Directive 92/69/EEC A.3 Relative Density.
Remarks Pycnometer was used.
Test Facility Henkel (2007c)

Vapour Pressure 1.2×10^{-5} kPa at 20ºC

Method EC Directive 92/69/EEC A.4 Vapour Pressure.
Remarks For generating the vapour pressure curve, pressure-dependent Differential Scanning Calorimetry (DSC) boiling peaks were used.
An unequivocal indication of boiling is the shift of an endothermic peak towards lower temperatures when reducing the pressure (normally the pressure is reduced from 101.3 kPa down to 0.1 kPa in defined steps).
The practical evaluation of the vapour pressure, with the boiling process being superimposed by decomposition, would result in a vapour pressure of 2.7×10^{-17} kPa at 20ºC.
The DSC measurements showed that there was no unequivocal indication of boiling, but instead a mixture of decomposition/boiling and vaporisation of the decomposition products. This implied that the vapour pressure value, which could be determined by means of DSC, was adulterated by superimposed thermodynamic effects.
For this reason, an estimation method was used to determine the vapour pressure.
The study report was not signed.
Test Facility Henkel (2007d)

Water Solubility > 20 g/L (temperature unknown)

Method EC Directive 92/69/EEC A.6 Water Solubility.
Remarks While suspending and stirring approximately 550 mg of the test substance in 25 mL water, the test substance was completed dissolved.
When adding more test substance to find a point of saturation, first the test substance turned out to be miscible with water in any proportion. Then, the solution became highly viscous and syrupy. At the end, the solution could not even be stirred by a magnetic stir bar.
No point of saturation of the test substance with water could be determined. Moreover, the test substance turned out to be very hygroscopic.
Test Facility Henkel (2007e)

Hydrolysis as a Function of pH Not tested

Remarks The notified chemical is expected to be stable to hydrolysis, based on its structure and shelf-life in aqueous-based cosmetic formulations.

Partition Coefficient (n-octanol/water) log Pow = -3.9 to -6.6 at 20ºC

Remarks Estimated (EPI Suite). The notified chemical contains a number of water soluble components that are expected to have very low partition coefficients.

Adsorption/Desorption – screening test log K_{oc} = 1

Remarks Estimated (EPI Suite). The notified chemical contains a number of water soluble components that are expected to have low soil organic carbon partition coefficients.

Flammability Not considered to be flammable.

Method EC Directive 92/69/EEC A.10 Flammability (Solids).
Remarks The study report was not signed.
Test Facility Henkel (2007f)

Autoignition Temperature > 400ºC

Method EC Directive 92/69/EEC A.16 Relative Self-Ignition Temperature for Solids.
Remarks The study report was not signed.
Test Facility Henkel (2007g)

Explosive Properties Not explosive

Remarks There are no chemical groups that would imply explosive properties, therefore the result has been predicted negative.

Stability Testing Stable to 261ºC

Method In house-Determination of Exotheric Reactions by Differential Scanning Calorimetry
Remarks The temperature was raised at the rate of 2ºC/min from 5 to 300ºC. Sample was 25 mg. Purge gas was nitrogen at 3L/h.
Results showed an exothermic reaction, probably decomposition, at about 261ºC with a reaction enthropy of 534J/g.
Test Facility Henkel (2009)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral

TEST SUBSTANCE: 40-55% notified chemical in glycerin

METHOD: OECD TG 423 Acute Oral Toxicity – Acute Toxic Class Method.
- Species/Strain: Rat/SPF Wistar
- Vehicle: Water
- Remarks - Method: No deviations from the protocol.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
1	3 F	2000	0
2	3 F	2000	0

- LD50: > 2000 mg/kg bw
- Signs of Toxicity: All animals showed piloerection 30 min and 2 hours after the application of the test substance. No abnormalities were observed at other times.
- Effects in Organs: The gross necropsy revealed no pathological abnormalities.
- Remarks - Results: There were no mortalities and no severe signs of toxicity.

CONCLUSION: The test substance is of low toxicity via the oral route.

TEST FACILITY: Frey-Tox (2007a)

B.2. Acute toxicity – dermal

TEST SUBSTANCE: 40-55% notified chemical in glycerin

METHOD: OECD TG 402 Acute Dermal Toxicity – Limit Test.
EC Directive 92/69/EEC B.3 Acute Toxicity (Dermal) – Limit Test.
- Species/Strain: Rat/SPF Wistar
- Vehicle: None
- Type of dressing: Semi-occlusive.
- Remarks - Method: Prior to the main study a pilot study was performed using one female rat given a dose of 2000 mg/kg bw. Slight signs of toxicosis (i.e. apathetic within the first day) were observed in this rat.

RESULTS

Number and Sex of Animals	*Dose (mg/kg bw)*	*Mortality*
5 per sex	2000	0

- LD50: > 2000 mg/kg bw
- Signs of Toxicity - Local: All animals tested appeared apathetical on day 0 after 1 hour, 3 hours and 6 hours. From day 1 to end of the observation period on day 14 no abnormalities were observed.
- Effects in Organs: The post mortem inspection of the female animals revealed no pathological abnormalities. Two of the male rats had marbled kidneys that was not evident in the remaining animals.
- Remarks - Results: There were no mortalities and no severe signs of toxicity.

CONCLUSION: The test substance is of low toxicity via the dermal route.

TEST FACILITY: Frey-Tox (2007b)

B.3. Irritation – skin

TEST SUBSTANCE 40-55% notified chemical in glycerin

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

Species/Strain	Rabbit/New Zealand White
Number of Animals	3 F
Vehicle	None
Observation Period	72 hours
Type of Dressing	Semi-occlusive.
Remarks - Method	No deviations from the protocol.

RESULTS

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

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

Remarks - Results No skin reactions were observed in all treated animals.

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

TEST FACILITY Frey-Tox (2007c)

B.4. Irritation – eye

TEST SUBSTANCE 40-55% notified chemical in glycerin

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

Species/Strain	Rabbit/New Zealand White
Number of Animals	3 F
Observation Period	72 hours
Remarks - Method	No deviations from the protocol. Conjunctiva: discharge was not recorded.

RESULTS

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

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

Remarks - Results One hour after the application of the test substance all tested animals showed some hyperaemic conjunctival blood vessels.
24 h after the application of the test substance some hyperaemic conjunctival blood vessels were still observed at two animals, whereas one animal did not show any ocular reactions.
48 h and 72 h after the application of the test substance all animals were free of any signs of eye irritation.

CONCLUSION	The test substance is slightly irritating to the eye.
TEST FACILITY	Frey-Tox (2007d)

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

TEST SUBSTANCE	40-55% notified chemical in glycerin
METHOD	Modified Local Lymph Node Assay (Ehling et al, 2005a and 2005b)
Species/Strain	Female mice/Hsd Win:NMRI
Vehicle	Dimethylformamide (DMF)
Remarks - Method	Cell proliferation was measured by cell counting instead of radioactive labelling. This non-radioactive method has not yet formally validated, but has undergone inter- and intra-laboratory trials in Europe which showed good reproducibility and sensitivity when compared to the established radioactive method (Ehling et al, 2005a and 2005b).

RESULTS

Concentration (% w/w)	*Weight index (Test/Control Ratio)*	*Cell count index (Test/Control Ratio)*
Test Substance		
0 (vehicle control)	1.00 ± 0.19	1.00 ± 0.26
2	1.06 ± 0.20	0.94 ± 0.26
10	1.02 ± 0.19	1.04 ± 0.21
50	1.07 ± 0.18	0.99 ± 0.29

Remarks - Results	The NMRI mice did not show any increase in the stimulation indices for cell counts or for weights of the draining lymph nodes after application of the test substance. The "positive level" which is 1.4 for the cell count index for this breed of mice was never reached or exceeded in any dose group. The "positive level" of ear swelling which is 2×10^{-2} mm increase, i.e. about 10% of the control value, has not been reached or exceeded in any dose group. No increases of the ear weights could be determined compared to control animals either. The body weights of the animals were not affected by any treatment. This study did neither point to non-specific (irritant) nor to a specific immunostimulating (sensitising) potential of the test substance.
CONCLUSION	There was no evidence of induction of a lymphocyte proliferative response indicative of skin sensitisation to the test substance.
TEST FACILITY	Bayer HealthCare (2007)

B.6. Repeat dose toxicity

TEST SUBSTANCE	40-55% notified chemical in glycerin
METHOD	OECD TG 408 Repeated Dose 90-Day Oral Toxicity Study in Rodents. EC Directive 88/302/EEC B.26 Sub-Chronic Oral Toxicity Test: 90-Day Repeated Oral Dose Study using Rodent Species.
Species/Strain	Rat/CD/Crl:CD (SD)
Route of Administration	Oral – gavage
Exposure Information	Total exposure days: 90 days Dose regimen: 7 days per week
Vehicle	Tap water
Remarks - Method	No deviations from the protocol.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw/day*	*Mortality*
control	10 per sex	0	0
low dose	10 per sex	100	0
mid dose	10 per sex	300	0
high dose	10 per sex	1000	0

Mortality and Time to Death
No mortalities occurred.

Clinical Observations
Rats treated did not reveal any influence on the behaviour or external appearance as well as the parameters of functional observations.
No influence was noted on the body weight, body weight gain, the body weight at autopsy and food and drinking water consumption.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis
No influence was noted on haematological parameters as well as for the biochemical parameters in female animals. A slightly reduced bilirubin plasma level was noted for the male rats treated with 1000 mg/kg bw/day. This is regarded as an isolated finding without any correlation to other haematological parameters.
No test substance-related influence was noted on the urinary parameters, the eyes or optic region and the auditory acuity at any of the tested dose levels.

Effects in Organs
Relative and absolute uterus weights were slightly (statistically not relevant) increased for the females treated with 1000 mg/kg bw/day. No histopathological correlate was noted for the increased uterus weights.

The histopathological examination revealed an increased severity in the degree of swollen gastric chief cells in the pars glandularis of the stomach of the male animals treated with 1000 mg/kg bw/day. However, a minimal to mild degree of swollen gastric chief cells was also noted in 8/10 male animals and 7/10 female animals of the control. The severity of the findings was distinctly increased for the male animals treated with 1000 mg/kg bw/day compared to the control group. Therefore, this finding is regarded to be test substance-related.

CONCLUSION
The No Observed Effect Level (NOEL) for the test substance was established as 300 mg/kg bw/day in this study, based on systemic changes noted for the animals treated with 1000 mg/kg bw/day.

The No Observed Adverse Effect Level (NOAEL) for the test substance was established as 1000 mg/kg bw/day in this study because systemic changes noted for the animals treated with 1000 mg/kg bw/day were isolated and were not correlated to other parameters.

TEST FACILITY LPT (2008)

B.7. Genotoxicity – bacteria

TEST SUBSTANCE 40-55% notified chemical in glycerin

METHOD OECD TG 471 Bacterial Reverse Mutation Test.
EC Directive 2000/32/EC B.13/14 Mutagenicity – Reverse Mutation Test using Bacteria.
Plate incorporation procedure (Test 1)/Pre incubation procedure (Test 2)

Species/Strain *S. typhimurium*: TA1535, TA1537, TA98, TA100, TA102

Metabolic Activation System S9 was prepared from the livers of male Wistar rats that had each orally received three consecutive daily doses of phenobarbital/β-naphthoflavone (80/100 mg/kg bw) prior to S9 preparation on Day 4.

Concentration Range in Main Test a) With metabolic activation: 0, 31.6, 100, 316, 1000, 2500 and 5000 µg/plate

	b) Without metabolic activation: 0, 31.6, 100, 316, 1000, 2500 and 5000 µg/plate
Vehicle	DMSO
Remarks - Method	E. coli was not used. The deviation from the protocol did not influence the quality and integrity of the study.

RESULTS

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

Remarks - Results	No biologically relevant increases in revertant colony numbers of any of the five tester strains were observed following treatment with the test substance at any concentration level, neither in the presence nor absence of metabolic activation by both procedures. The reduction in the number of revertants down to a mutation factor of 0.5 found in tester strain TA 102 at a dose of 31.6 µg/plate (with metabolic activation) was regarded as not biologically relevant due to the lacking dose-response relationship. The reference mutagens induced a distinct increase of revertant colonies indicating the validity of the experiments.
CONCLUSION	The test substance was not mutagenic to bacteria under the conditions of the test.
TEST FACILITY	BSL BIOSERVICE (2007a)

B.8. Genotoxicity – in vitro

TEST SUBSTANCE	40-55% notified chemical in glycerin
METHOD	OECD TG 473 In vitro Mammalian Chromosome Aberration Test. EC Directive 2000/32/EC B.10 Mutagenicity - In vitro Mammalian Chromosome Aberration Test.
Cell Type/Cell Line	Chinese hamster/ V79 Cells
Metabolic Activation System	The S9 liver microsomal fraction was prepared from male Wistar rats induced with phenobarbital and β-naphthoflavone.
Vehicle	None
Remarks - Method	No deviations from the protocol.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	0*, 125, 250, 500, 1000*, 2500*, 5000*	4 h	20 h
Test 2	0*, 31.25, 62.5, 125, 250, 500, 1000*, 2500*, 5000*	20 h	20 h
Present			
Test 1	0*, 125, 250, 500, 1000*, 2500*, 5000*	4 h	20 h
Test 2	0*, 500, 1000, 2000, 3000*, 4000*, 5000*	4 h	20 h

*Cultures selected for metaphase analysis.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/mL) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent	> 5000			
Test 1		> 5000	> 5000	negative
Test 2		> 5000	> 5000	negative
Present	> 5000			
Test 1		> 5000	> 5000	negative
Test 2		> 5000	> 5000	negative

Remarks - Results

In both experiments no biologically relevant increase of the aberration rates was noted after treatment with the test substance with and without metabolic activation. The aberration rates of all dose groups treated with the test item were within the historical control data of the negative control.
In addition no biologically relevant increase in the frequencies of polyploid cells was found after treatment with the test substance as compared to the controls with and without metabolic activation.
Ethylmethanesulfonate (600 and 900 µg/mL) and cyclophosphamide (0.83 µg/mL) were used as positive controls and induced distinct and biologically relevant increases in cells with structural chromosomal aberration.

CONCLUSION

The test substance was not clastogenic to the V79 Chinese hamster cell line treated in vitro under the conditions of the test.

TEST FACILITY

BSL BIOSERVICE (2007b)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready biodegradability

TEST SUBSTANCE: Notified chemical

METHOD: OECD TG 301 B Ready Biodegradability: CO_2 Evolution Test.
- Inoculum: Activated sludge
- Exposure Period: 28 days
- Auxiliary Solvent: None
- Analytical Monitoring: Inorganic carbon in absorber flasks
- Remarks - Method:

RESULTS

Test substance		*Sodium benzoate*	
Day	*% Degradation*	*Day*	*% Degradation*
11	73.1	10	72.4
14	76.8	14	79.2
28	99.2	28	105.4

Remarks - Results: The 10 day window for the test substance was 1-11 days.

CONCLUSION: The notified chemical is readily biodegradable

TEST FACILITY: Hydrotox (2007)

C.1.2. Bioaccumulation

No bioaccumulation testing was conducted, as no bioaccumulation of the water soluble and readily biodegradable notified chemical is to be expected.

C.2. Ecotoxicological Investigations

C.2.1. Acute toxicity to fish

TEST SUBSTANCE: Notified chemical

METHOD: OECD TG 203 Fish, Acute Toxicity Test - static.
EC Directive 92/69/EEC C.1 Acute Toxicity for Fish - static.
- Species: Zebra fish (*Danio rerio*)
- Exposure Period: 96 hours
- Auxiliary Solvent: None
- Water Hardness: 10-250 mg $CaCO_3$/L
- Analytical Monitoring: GC-MS
- Remarks – Method: A limit test only was conducted

RESULTS

Concentration mg/L		*Number of Fish*	*Mortality*				
Nominal	*Actual*		*1 h*	*24 h*	*48 h*	*72 h*	*96 h*
0		7	0	0	0	0	0
660	615-708	7	0	0	0	0	0

LC50: > 660 mg/L at 96 hours.

NOEC	660 mg/L at 96 hours.
Remarks – Results	No sublethal effects were observed.

CONCLUSION The notified chemical is not harmful to fish.

TEST FACILITY Noack (2008a)

C.2.2. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE Notified chemical

METHOD OECD TG 202 Daphnia sp. Acute Immobilisation Test and Reproduction Test - static.
EC Directive 92/69/EEC C.2 Acute Toxicity for Daphnia - static.

Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	None
Water Hardness	160-180 mg $CaCO_3$/L
Analytical Monitoring	GC-MS
Remarks - Method	

RESULTS

Concentration mg/L		*Number of D. magna*	*Number Immobilised*	
Nominal	*Actual*		*24 h*	*48 h*
0		20	0	0
62.5	60.1	20	0	0
125	114	20	0	0
250	215	20	0	0
500	321	20	0	0
1000	918	20	0	0

LC50	> 1000 mg/L at 48 hours
NOEC	1000 mg/L at 48 hours
Remarks - Results	The variable analytical recoveries may be caused by a precipitate that formed after derivatisation. This only occurred in the daphnia medium.

CONCLUSION The notified chemical is not harmful to daphnids.

TEST FACILITY Noack (2008b)

C.2.3. Algal growth inhibition test

TEST SUBSTANCE Notified chemical

METHOD OECD TG 201 Alga, Growth Inhibition Test.
EC Directive 92/69/EEC C.3 Algal Inhibition Test.

Species	*Desmodesmus subspicatus*
Exposure Period	72 hours
Concentration Range	Nominal: 50, 100, 200, 400, 800 mg/L Actual: 31.6, 66.7, 147, 289, 603 mg/L
Auxiliary Solvent	None
Water Hardness	Typical algal culture medium (soft water)
Analytical Monitoring	GC-MS
Remarks - Method	

RESULTS

Biomass		*Growth*	
E_bC50 mg/L at 72 h	*NOEC mg/L*	*E_rC50 mg/L at 72 h*	*NOEC mg/L*
659	400	> 800	400

Remarks - Results: Aggregation of algal cells was observed at 200-800 mg/L. The inhibitory effects seen at 800 mg/L were reversible.

CONCLUSION: The notified chemical is not harmful to green algae.

TEST FACILITY: Noack (2008c)

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