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June 2010

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

FULL PUBLIC REPORT

D-Xylopyranoside, 2-octyldodecyl (INCI Name: Octyldodecyl xyloside)

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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1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)
Bronson and Jacobs (ABN 81 000 063 249)
70 Marple Avenue
Villawood, NSW, 2163

NOTIFICATION CATEGORY
Standard: Chemical other than polymer (more than 1 tonne 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

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
EU Reach

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
APX 20P

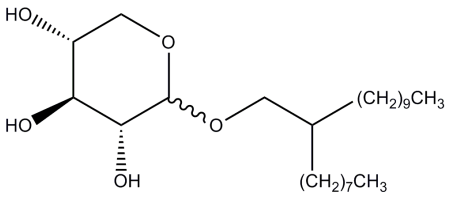
CAS NUMBER
423772-95-6

CHEMICAL NAME
D-Xylopyranoside, 2-octyldodecyl

OTHER NAME(S)
Octyldodecyl xyloside

MOLECULAR FORMULA
$C_{25}H_{50}O_5$

STRUCTURAL FORMULA



MOLECULAR WEIGHT 430.7

ANALYTICAL DATA
Reference NMR and UV spectra were provided. Analysis of monoxylosides and dixylosides was carried out by gas chromatography.

3. COMPOSITION

DEGREE OF PURITY 68%

NON HAZARDOUS IMPURITIES/RESIDUAL MONOMERS (>1% by weight)

Chemical Name	2-Octyldodecanol		
CAS No.	5333-42-6	*Weight %*	15
Chemical Name	2-Octyldodecyl dixyloside		
CAS No.	None assigned	*Weight %*	13.7
Chemical Name	2-Octyldodecyl trixyloside		
CAS No.	None assigned	*Weight %*	3.6

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20°C AND 101.3 kPa: Orange paste

Property	Value	Data Source/Justification
Melting Point/Freezing Point	25 °C	Measured
Boiling Point	Decomposes at> 98.5°C and 101.3 kPa	Measured
Density	0.983 kg/m^3 at 21°C	Measured
Vapour Pressure	<6.7 x 10^{-7} kPa at 25°C	Measured
Water Solubility	2.3 × 10^{-3} g/L at 20°C	Measured
Hydrolysis as a Function of pH	$t_{½}$ > 1 year at 25°C	Measured
Partition Coefficient (n-octanol/water)	log Pow = 7.69 at 20°C	Calculated
Adsorption/Desorption	log K_{oc} = < 1.32 at 25°C	Measured
Surface Tension	33.3 mN/m	Measured
Flash Point	224.5 °C at 101.3 kPa	Measured
Autoignition Temperature	Chars above 98°C. Expected to be > 225°C.	Estimated
Explosive Properties	Not expected to be explosive	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 room temperature in air and water.

Dangerous Goods classification
Based on the submitted physical-chemical data in the above table the notified chemical is not classified as hazardous according to the Australian Dangerous Goods Code (NTC, 2007). However the data above does 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/polymer.

5. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS
The notified chemical will be imported in premixes at up to 30% for formulation into cosmetic products.

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

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

PORT OF ENTRY
Sydney and Melbourne

IDENTITY OF MANUFACTURER/RECIPIENTS
Bronson and Jacobs Pty. Ltd. 70 Marple Avenue, Villawood NSW 2163
CRT Group. 221 Maidstone Street, Altona VIC 3018

TRANSPORTATION AND PACKAGING
The notified chemical will be imported in metal containers of various sizes up to 30 kg and transported from the wharf by road to the store and redistribution centre. From there it will be transported by road to local manufacturers for reformulation.

USE
The notified chemical is used as an ingredient of cosmetic products at up to 1.5%. It will be imported as a component in two premixes Easynov at up to 25% and Fluidanov 20X at up to 30%. Both Easynov and Fluidanov 20X are used at concentrations up to 5% in the manufacture of cosmetics including face and body care products, wipe impregnations, skin and cleansing masks, foundations, sunscreens, mascaras, baby lotions and hair treatment products.

OPERATION DESCRIPTION
Reformulation operations will involve weighing of an appropriate amount of the notified chemical into a weighing receptacle, and transferring to a mixing tank. Blending of these products will be conducted at the facilities of Bronson & Jacob's customers and typically involves manual weighing and blending equipment. During this process, the compounder would wear appropriate PPE such as safety glasses, protective clothing and gloves. When the compounding process is complete the final product would be transferred to smaller retail size containers and distributed to customers for sale to the public. Operators involved in the reformulation and testing of the finished products will follow the handling and storage guidelines as per the MSDS. This will entail wearing of protective clothing, eye protection and gloves.

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	8	4	12
Compounder	1	6	12
Chemist	1	2	12
Packers	2	8	12
Store person	2	4	12

EXPOSURE DETAILS
As the notified chemical will be sealed inside containers, transport and storage workers are unlikely to come into direct contact with the notified chemical unless there is a breakage or spillage. Compounders and chemists will not come into direct contact with the notified chemical as they will be protected by PPE.

Occupational exposure is also possible for workers in hair and beauty salons using products containing the notified chemical (<1.5%). Dermal exposure is expected to be extensive given that moisturiser products containing the notified chemical will be applied directly to the skin. Accidental ocular exposure could occur. There is also potential for accidental ingestion.

Although the level and route of exposure will vary depending on the method of application and work practices employed, extensive dermal exposure is expected in some occupational settings. This exposure is likely to be greater than that expected for the public

6.1.2. Public exposure

The public will be exposed to the notified chemical as a component of cosmetics at a maximum concentration of 1.5%.

Public exposure from transport, storage, reformulation or disposal is considered to be negligible. Public exposure to the notified chemical in Australia from use of skin cosmetics 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:

- The concentration of the notified chemical in rinse off and leave on cosmetic products = 1.5%;
- An individual uses all product types containing the notified chemical.

Product(s) used	*Use level for each product*	*Retention factor*	*Systemic Exposure* (g/day)*
Facial cleanser	0.8 g x 1 applications/day	0.01	0.01
Shampoo	10.46 g x 1 applications/ day	0.01	0.11
Conditioner	14.0 g x 0.28 applications/day	0.01	0.04
Shower gel	5.0 g x 2 applications/day	0.01	0.10
Makeup remover	2.5 g x 2 applications/day	0.10	0.50
Total rinse-off product exposure			**0.76**
Body Lotion	7.82g x 1 applications/day	1.0	7.82
Face Cream	1.54g x 1 applications/day	1.0	1.54
Total leave-on product exposure			**9.36**

* Using 100% dermal absorption (SCCP, 2006)

Total systemic exposure was calculated below for a female of 60 kg bw (SCCP, 2006) using rinse-off and leave-on cosmetic products containing 1.5% notified chemical.

Total systemic exposure =

[(Total exposure from rinse off use (g/day) x Concentration of notified chemical in products) + (Total exposure from leave on use (g/day) x Concentration of notified chemical in products)] x 1000 / Body Weight (kg)

Total systemic exposure = [(0.76 x 1.5%) + (9.36 x 1.5%) x 1000] /60 = 2.53 mg/kg bw/day

This exposure estimate was calculated using conservative use assumptions and is expected to reflect a worst case scenario. In reality, the level of exposure is expected to be lower than 2.48 mg/kg bw/day as it is assumed that consumers would not use all these products daily to the extent shown above, and dermal absorption should be lower than 100%.

$$\text{Systemic exposure for using all rinse-off products} = \frac{0.76 \times 1.5\% \times 1000}{60} = 0.19 \text{ mg/kg bw/day}$$

$$\text{Systemic exposure for using all leave-on products} = \frac{9.36 \times 1.5\% \times 1000}{60} = 2.34 \text{ mg/kg bw/day}$$

6.2. Human health effects assessment

The results from toxicological investigations conducted on the notified chemical 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 >2500 mg/kg bw; low toxicity
Rat, acute dermal toxicity	LD50>2000 mg/kg bw; low toxicity
Rabbit, skin irritation	slightly irritating
Rabbit, eye irritation	slightly irritating
Albino guinea pig, skin sensitisation <Buehler test>	No evidence of sensitisation
Guinea pig, skin sensitisation GPMT Maximisation test 1	Limited use (due to skin irritation observed)
Guinea pig, skin sensitisation – GPMT Maximisation test - 2	No evidence of sensitisation
Rat, repeat dose oral toxicity – 28 days.	NOEL=15 mg/kg bw; NOAEL=150 mg/kg bw
Mutagenicity – bacterial reverse mutation	non mutagenic
Genotoxicity – in vitro mammalian chromosome aberration test using Chinese Hamster Ovary cells	Not clastogenic (presence of metabolic activation) Weak clastogenic (absence of metabolic activation).
Cytogenetic-in vitro Assay Measuring Chromosomal Aberration Frequencies in Human Lymphocytes.	non genotoxic
Genotoxicity – in vivo Mammalian Erythrocyte Micronucleus	non genotoxic

Toxicokinetics, metabolism and distribution
Limited data is available to describe the likely toxicokinetic properties of the notified chemical. Given its relatively low water solubility 2.3 mg/L (measured), and molecular weight of <500 Da, absorption could occur but would be limited by the high calculated log P_{ow} of ~7.69.

Acute toxicity
The notified chemical is of low acute toxicity *via* the oral and dermal routes (LD50 >2500 mg/kg bw and LD50>2000 mg/kg bw respectively)

No acute inhalation toxicity study was conducted using the notified chemical. Inhalation toxicity is expected to be low as the notified chemical has a very low vapour pressure (<6.7 x 10^{-7} kPa).

Irritation
Slight erythema was noted at all treated skin sites at the 24 and 48-hour observations and at two treated skin sites at the 72-hour observation. Slight oedema was noted at one treated skin site at the 24, 48 and 72-hour observations.

Loss of skin elasticity was noted at one treated skin site at the 48-hour observation and at two treated skin sites at the 72-hour observation. One treated skin site appeared normal at the 72-hour observation and the remaining two treated skin sites appeared normal at the 7day observation.

Conjunctival irritation was noted in all treated eyes one hour after treatment and at the 24-hour observation. Minimal conjunctival irritation was noted in one treated eye at the 48-hour observation.

Overall, the notified chemical is expected to be slightly-irritating to skin and slightly irritating to eyes.

Sensitisation
Slight erythema followed dryness on the treated site was noted after the third application (Day 4) in the Buehler test. A scab was formed in 15 treated animals during the third week of the induction phase that was totally reversible during the rest phase. In all cases with animals showing skin reactions, only slight erythema was observed. Based on the similar reactions in the control group, this is likely to be due to irritation rather than sensitisation.

The results of the Magnusson & Kligman maximisation test (GPMT) were negative. A second maximisation test was inconclusive due to irritation effects.

Overall, there was no evidence of reactions indicative of skin sensitisation to the notified chemical under the conditions of the Buehler and GPMT tests.

Mutagenicity
The notified chemical was not mutagenic in a bacterial reverse mutation study, not genotoxic in an *in vitro* chromosome aberration study in human lymphocytes, and only weakly positive in an *in-vitro* mammalian chromosome aberration test with Chinese Hamster Ovary cells. It was not genotoxic in *vivo* in a Mammalian Erythrocyte Micronucleus test,

Repeat Dose/Chronic toxicity
The 28-day dermal toxicity study for the notified chemical showed no mortality at up to 750 mg/kg bw/day.
The test derives a NOEL of 15 mg/kg bw/day. The study author established a 'No Observed Adverse Effect Level' (NOAEL) of 750 mg/kg bw/day for animals of either sex.

Sporadic incidents of increased salivation were detected immediately after dosing in animals of either sex with 150 and 750 mg/kg bw/day and an elevation in absolute liver weight (up to 5.7% liver weight relative increase) and liver pathology changes were also noted in mid and high dose groups of both sexes.

NICNAS considered 150 mg/kg bw/day as the No Observed (Adverse) Effect Level (NOAEL) based on treatment related clinical chemistry parameters and liver effects seen at this dose level and above in all animals.

Summary
The notified chemical is of low acute oral and dermal toxicity. It may be slightly irritating to eyes and skin, but not a skin sensitiser.

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

6.3. Human health risk characterisation

6.3.1. Occupational health and safety
Dermal and ocular exposure to transport and storage workers could only occur in the event of an accident of breakage or spillage of sealed containers containing the notified chemical up to 30%concetration.

Dermal and ocular exposure of the compounders and Chemists to the raw material premixes containing up to 30% concentration of the notified chemical could occur during formulation of cosmetics. The use of PPE such as protective clothings, gloves and safety glasses will minimise exposure.

Employees in hair and beauty salons will experience extensive dermal exposure during application of products containing the notified chemical (<1.5%) by hand. If these employees use products containing the notified chemical for personal use as well as in a work setting their level of exposure would be higher than that of consumers. However, exposure to the notified chemical at low concentrations (<1.5%) is not expected to cause skin or eye irritation. The risk of toxicity following repeated exposure is not anticipated to be unacceptable.

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 at up to 1.5% through the use of a range of cosmetic products. The irritation effects are not expected at this low concentration.

Conservative estimates of the margin of exposure (MOE) for the notified chemical could be estimated as follows using the exposures estimated in sec. 6.1.2:

$$\text{MOE (all rinse-off use)} = \frac{\text{Estimated NOAEL}}{\text{Estimated exposure}} = \frac{150 \text{ mg/kg bw/day}}{0.19 \text{ mg/kg bw/day}} = 789$$

$$\text{MOE (all leave-on use)} = \frac{\text{Estimated NOAEL}}{\text{Estimated exposure}} = \frac{150 \text{ mg/kg bw/day}}{2.34 \text{ mg/kg bw/day}} = 64$$

$$\text{MOE (combined use)} = \frac{\text{Estimated NOAEL}}{\text{Estimated combined daily exposure}} = \frac{150 \text{ mg/kg bw/day}}{2.53 \text{ mg/kg bw/day}} = 59$$

MOE greater than or equal to 100 are considered acceptable to account for intra- and inter-species differences. Based on a NOAEL of 150 mg/kg bw/day established in the 28-day repeat dose toxicity study, the MOE is calculated as 59 for a female using all types of products containing the notified chemical. This is an over estimation as it is highly unlikely that all products containing the notified chemical will be used together. The exposure calculations used 100% dermal absorption, but the dermal absorption is expected to be limited by the high log P_{ow} of ~7.69 calculated. The MOEs for leave-on use and combined use will be acceptable if dermal absorption is 50% or less. Therefore, the risk of repeated exposure is considered to be acceptable when using a few products containing the notified chemical at the same time.

Overall, based on the data provided, the notified chemical is not considered to pose an unacceptable risk to public health at concentrations up to 1.5% in cosmetic products.

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 constituent of two premixes which will be formulated into a variety of cosmetic products. Accidental spills and leaks during transport are expected to be physically contained and disposed of to landfill. It is estimated that 1% of the notified chemical will remain in drums as residues and be sent to landfill. Formulation of the notified chemical will occur in closed systems and should therefore experience minimal release to the sewerage system due to cleaning of equipment.

RELEASE OF CHEMICAL FROM USE

It is expected that the majority of the imported quantity of the notified chemical will be washed to the sewer as the chemical is intended for use in cosmetic products. This release will occur in a diffuse and widespread manner.

RELEASE OF CHEMICAL FROM DISPOSAL

Small amounts as residues in empty containers are expected to be disposed of to landfill with normal household rubbish.

7.1.2 Environmental fate

The notified chemical is not readily biodegradable. It is expected to have potential for bioaccumulation in the aquatic organisms given its low molecular weight and high estimated log Pow. For the details of the environmental fate studies refer to Appendix C. Most of the notified chemical is expected to be released to the sewage system. In the waste water treatment processes in the sewage treatment plant, most of the notified chemical is expected to partition to sludge or to suspended solids due to its low water solubility and its predicted hydrophobicity, where it will be removed for disposal to landfill. In landfill it is expected to slowly decompose by abiotic and biotic processes to form water and oxides of carbon. No significant amount of the notified chemical is expected to be released to the water environment. Therefore, the notified chemical is not expected to be bioavailable to the aquatic organisms despite its potential for bioaccumulation.

7.1.3 Predicted Environmental Concentration (PEC)

Most of the notified chemical is expected to be washed into the sewer. Therefore under a worst case scenario, with no removal of the notified chemical in the sewage treatment plant, the resultant Predicted Environmental Concentration (PEC) in sewage effluent on a nationwide basis, Predicted No-Effect Concentration (PNEC) and Risk Assessment (Q) are estimated as follows:

Predicted Environmental Concentration (PEC) for the Aquatic Compartment		
Total Annual Import/Manufactured Volume	2,000	kg/year
Proportion expected to be released to sewer	100%	
Annual quantity of chemical released to sewer	2,000	kg/year
Days per year where release occurs	365	days/year
Daily chemical release:	5.48	kg/day
Water use	200.0	L/person/day
Population of Australia (Millions)	21.161	million
Removal within STP	0%	
Daily effluent production:	4,232	ML
Dilution Factor - River	1.0	
Dilution Factor - Ocean	10.0	
PEC - River:	1.29	µg/L
PEC - Ocean:	0.13	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	LL50 > 100 mg/L	Not harmful up to the limit of its solubility in water
Daphnia Toxicity	EL50 > 100 mg/L	Not harmful up to the limit of its solubility in water
Algal Toxicity	E_rL50 > 100 mg/L	Not harmful up to the limit of its solubility in water

The results of the studies indicate that the notified chemical is expected to be not harmful to fish, daphnia, and algae up to its limit of solubility in water.

7.2.1 Predicted No-Effect Concentration

Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment		
Fish	> 100	mg/L
Assessment Factor	100	
PNEC:	> 1000	µg/L

An assessment factor of 100 has been used since full study reports for endpoints of three trophic levels are available for the environmental risk assessment.

7.3. Environmental risk assessment

Risk Assessment	PEC µg/L	PNEC µg/L	Q
Q - River	1.29	1000	0.0013
Q - Ocean	0.13	1000	0.00013

The Risk Quotients (Q=PEC/PNEC) for the worst case scenario consideration have been calculated to be << 1 for both river and ocean compartments. This indicates the notified chemical is not expected to pose an unacceptable risk to the aquatic environment based on its reported use pattern.

8. CONCLUSIONS AND REGULATORY OBLIGATIONS

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

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 calculated and the reported use pattern, the notified chemical is not expected to pose a risk to the environment.

Recommendations

CONTROL MEASURES
Occupational Health and Safety

- Employers should ensure that the following safety directions are used by workers to minimise occupational exposure to the notified chemical during formulation:
  - Avoid contact with eyes and skin

- 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.

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

Disposal

- The notified chemical should be disposed of to landfill.

Emergency procedures

- Spills or accidental release of the notified chemical should be handled by physical 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 amount of chemical being introduced has increased from two tonnes per year, 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 notified chemical provided by the notifier was 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/Freezing Point 25 °C

Method OECD TG 102 Melting Point/Melting Range.
EC Directive 92/69/EEC A.1 Melting/Freezing Temperature.

Remarks	Measured as solidification point
Test Facility	Defitraces (2003a)

Boiling Point >98.5 ºC with decomposition

Method	OECD TG 103 Boiling Point. EC Directive 92/69/EEC A.2 Boiling Temperature.
Remarks	Changed colour to yellow above 82 ºC, then produced black particles floating in the liquid at 98.5 ºC as it decomposed.
Test Facility	Defitraces (2003b)

Density 0.983 kg/m^3 at 21ºC

Method	OECD TG 109 Density of Liquids and Solids. EC Directive 92/69/EEC A.3 Relative Density.
Remarks	Stereopycnometer method.
Test Facility	Defitraces (2003c)

Vapour Pressure < 6.7 x 10^{-7} kPa at 25ºC

Method	OECD TG 104 Vapour Pressure. EC Directive 92/69/EEC A.4 Vapour Pressure.
Remarks	Vapour Pressure balance.
Test Facility	Safepharm Laboratories (2007a)

Water Solubility 2.3 × 10^{-3} g/L at 20ºC

Method	OECD TG 105 Water Solubility. EC Directive 92/69/EEC A.6 Water Solubility.
Remarks	Flask Method. Mean of 5 results, RSD = 10.4%.
Test Facility	Defitraces (2003d)

Hydrolysis as a Function of pH

Method	EC Directive 92/69/EEC C.7 Degradation: Abiotic Degradation: Hydrolysis as a Function of pH.

pH	*T (℃)*	*$t_{½}$ years*
4	49.7 – 50.0	> 1
7	49.7 – 50.0	> 1
9	49.7 – 50.0	> 1

Remarks	There was practically no hydrolysis of the test substance over 5 days taking into account a 20% standard deviation at the concentration analysed.
Test Facility	Defitraces (2009)

Partition Coefficient (n-octanol/water) log Pow = 7.69

Method	KOWWIN (v1.66)
Test Facility	US EPA

Adsorption/Desorption – screening test log K_{oc} < 1.32

Method	OECD TG 121 Estimation of the Adsorption Coefficient (Koc) on Soil and Sewage Sludge using High Performance Liquid Chromatography (HPLC).
Remarks	The mixture of the reference items was dissolved in CH_3OH/H_2O 65/35% v/v rather than the guideline ratio of 55/45%. This deviation would not have affected the outcome of the test.

Test Facility	LAUS GmbH (2008a)

Flash Point 224.5 ºC at 101.3 kPa

Method	EC Directive 92/69/EEC A.9 Flash Point.
Remarks	Sticky paste test item.
Test Facility	Defitraces (2003e)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral

TEST SUBSTANCE: 100% APX 20P

METHOD: OECD TG 423 Acute Oral Toxicity – Acute Toxic Class Method.
Method.
Species/Strain: Sprague-Dawley Rats
Vehicle: Arachis oil BP
Remarks - Method: Only one dose level was used (2000 mg/kg bw). No other significant protocol deviations.

RESULTS: No deaths were noted in either group.

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
1	3 Female	2000	none
2	3 Female	2000	none

LD50: >2500 mg/kg bw (estimated)
Signs of Toxicity: None
Effects in Organs: No effects noted in bodyweight, clinical signs or upon individual necropsy.
Remarks - Results

CONCLUSION: The notified chemical is of low toxicity via the oral route.

TEST FACILITY: Safepharm Laboratories (2003b)

B.2. Acute toxicity – dermal

TEST SUBSTANCE: 100% APX 20P

METHOD: OECD TG 402 Acute Dermal Toxicity.
Species/Strain: 5 F and 5 M Sprague-Dawley Rats
Vehicle: Distilled water
Type of dressing: Occlusive/Semi-occlusive.
Remarks - Method: No significant protocol deviations.

RESULTS: No deaths were noted at 2000 mg/kg bw. Neither cutaneous reactions nor systemic clinical signs related to the administration of the test item were observed.

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
1	5 F and 5 M	0	None
2	5 F and 5 M	2000	None

LD50: >2000 mg/kg bw
Signs of Toxicity - Local: No cutaneous reaction was noted aside from a slight brown staining around the site of application which cleared following Day 3.
Signs of Toxicity - Systemic: No signs of systemic toxicity was noted.
Effects in Organs: Gross pathological examination showed no sign of any toxicity.

CONCLUSION: The notified chemical is of low toxicity via the dermal route.

TEST FACILITY: Phycher (2007a)

B.3. Irritation – skin

TEST SUBSTANCE	100% APX 20P
METHOD	OECD TG 404 Acute Dermal Irritation/Corrosion and EC Directive 92/69/EEC B.4 Acute Toxicity (Skin Irritation).
Species/Strain	Rabbit/New Zealand White
Number of Animals	3M
Vehicle	None
Observation Period	7 days
Type of Dressing	Semi-occlusive.
Remarks - Method	Four hours after application the corset and patches were removed from each animal and any residual test material removed by gentle swabbing with cotton wool soaked in 74% Industrial Methylated Spirits.

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	0.67	1.0	1.0	1	72	1
Oedema	0.0	0.0	1.0	1	72	1

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

Remarks - Results	Slight erythema was noted at all treated skin sites at the 24 and 48-hour observations and at two treated skin sites at the 72-hour observation. Slight oedema was noted at one treated skin site at the 24, 48 and 72-hour observations. Loss of skin elasticity was noted at one treated skin site at the 48-hour observation and at two treated skin sites at the 72-hour observation. One treated skin site appeared normal at the 72-hour observation and the remaining two treated skin sites appeared normal at the 7day observation.
CONCLUSION	The notified chemical is slightly irritating to the skin.
TEST FACILITY	Safepharm Laboratories (2003c)

B.4. Irritation – eye

TEST SUBSTANCE	100% APX 20P
METHOD	OECD TG 405 Acute Eye Irritation/Corrosion. EC Directive 92/69/EEC B.5 Acute Toxicity (Eye Irritation).
Species/Strain	Rabbit/New Zealand White
Number of Animals	3M
Observation Period	72 hours

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	0.3	1.0	0.3	2	24-48 hours	0
Conjunctiva: chemosis	0.3	0.7	0.0	1	24-48 hours	0
Conjunctiva: discharge	0.3	0.7	0.3	2	24-48 hours	0
Corneal opacity	0.0	0.0	0.0	0	0	0
Iridial inflammation	0.0	0.0	0.0	0		0

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

Remarks - Results	Minimal to moderate conjunctival irritation was noted in all treated eyes one hour after treatment and at the 24-hour observation. Minimal conjunctival irritation was noted in one treated eye at the 48-hour observation.
CONCLUSION	The notified chemical is slightly irritating to the eye.
TEST FACILITY	Safepharm Laboratories (2003d)

B.5. Skin sensitisation

TEST SUBSTANCE	100% APX 20P
METHOD	OECD TG 406 Skin Sensitisation – non-adjuvant <Buehler test>. EC Directive 96/54/EC B.6 Skin Sensitisation - < Buehler test >.
Species/Strain	Guinea pig/Dunkin-Hartley
PRELIMINARY STUDY	Maximum Non-irritating Concentration: topical: 25%
MAIN STUDY	
Number of Animals	Test Group: 20 males Control Group: 10 males
INDUCTION PHASE	Induction Concentration: topical: 100%
Signs of Irritation	Slight erythema followed dryness on the treated site was noted after the third application (Day 4). A scab was formed in 15 treated animals during the third week of the induction phase that was totally reversible during the rest phase.
CHALLENGE PHASE	
1st challenge	topical: 12.5 and 25% in paraffin oil.
2nd challenge	Not performed
Remarks - Method	The induction phase was performed by topical application at Day 0, 2, 4, 7, 9, 11, 14, 16 and 18 with the test substance at 100%. There was a 17-day rest phase before the challenge test.

RESULTS

Animal	*Challenge Concentration*	*Number of Animals Showing Skin Reactions after: 1st challenge*		
		24 h	*48 h*	*72 h*
Test Group	12.5%	2/18	2/18	0/18
	25%	1/18	1/18	0/18
Positive Control Group	12.5%	4/10	2/10	0/10
	25%	4/10	2/10	0/10

Remarks - Results	Two treated animals died during the test (on the 8th and 17th day). The macroscopical examinations revealed a deep content on the intestinal transit in one animal and an adherence in the rib cage on the other. The study authors report that these mortalities were not attributable to the test material. In all cases with animals showing skin reactions, only slight erythema was observed. Based on the similar reactions in the control group, this is likely to be due to irritation rather sensitisation. The results of the positive control (22% positive), benzocaine (CAS No. 94-09-7), demonstrated the sensitivity of the test.
CONCLUSION	There was no evidence of reactions indicative of skin sensitisation to the notified chemical under the conditions of the test.
TEST FACILITY	Phycher (2004a)

B.6. Skin sensitisation

TEST SUBSTANCE	100% APX 20P

METHOD OECD TG 406 Skin Sensitisation – adjuvant test. (GPMT Maximisation test 1)
EC Directive 96/54/EC B.6 Skin Sensitisation – adjuvant test. (Magnusson & Kligman maximisation assay)

Species/Strain Guinea pig/Dunkin-Hartley

PRELIMINARY STUDY Maximum Non-irritating Concentration:
topical: 6.25%

MAIN STUDY

Number of Animals Test Group: 10 Control Group: 5

INDUCTION PHASE Induction Concentration:
intradermal: 1.562% in olive oil
topical: 100%

Signs of Irritation Not reported

CHALLENGE PHASE

1st challenge topical: 3.125% and 6.25% in olive oil

2nd challenge topical: 3.125% and 1.56% in olive oil

Remarks - Method 10% Sodium lauryl sulphate was applied 24 hours prior to the second induction.
Rest phase before the 1st challenge was 17 days.
Rest phase before 2nd challenge was 6 days.

RESULTS

Animal	*Challenge Concentration 1st challenge/2nd challenge*	*Number of Animals Showing Skin Reactions after*: 1st challenge*		*2nd challenge*	
		24 h	*48 h*	*24 h*	*48 h*
Test Group	6.25% / 3.125%	4/9	2/9	4/9	2/9
	3.125% / 1.56%	4/9	1/9	3/9	0/9
Positive Control Group	6.25% / 3.125%	1/4	1/4	0/4	0/4
	3.125% / 1.56%	1/4	1/4	0/4	0/4

* Skin reactions have only been counted where animals have a skin reaction with a grading of 2 or more given the control animals also showed signs of irritation.

Remarks - Results Moderate erythema 24 hours following the removal of the occlusive dressing was observed in 44% (4/9) of treated animals, on the area treated at 6.25% and 3.125%, and in 25% (1/4) of control animals, on the area treated at 6.25% and 3.125%, and in 25% (1/4) of control animals on the area treated at 6.25% and 3.125%. These reactions were also recorded at the 48 hour reading, in 22% (2/9) and 11% (1/9) of treated animals on the area treated at 6.25% and 3.125% respectively and in 25% (1/4) of control animals on the area treated at 6.25% and 3.125%.

In order to confirm this reaction, a second challenge test has been conducted with the test product diluted at 3.125% and 1.56% in paraffin oil, after a 6-days rest phase. After 24 hours following the removal of the occlusive dressing, moderate erythema was observed in 44% (4/9) and 33% (3/9) of treated animals, on the area treated at 3.125% and 1.56% respectively. These reactions were also recorded at the reading 48 hours in 22% (2/9) of treated animals, on the area treated at 3.125%.

Given these results are inconclusive the test was repeated using a challenge concentration of 3.125% and 1.56% (see below).

Number of Animals Test Group: 11 Control Group: 6

INDUCTION PHASE Induction Concentration:
intradermal: 1.562% in olive oil
topical: 100%

Signs of Irritation Not reported

CHALLENGE PHASE

1st challenge	topical: 3.125% and 1.56% in olive oil
2nd challenge	Not conducted
Remarks - Method	10% Sodium lauryl sulphate was applied 24 hours prior to the second induction. Rest phase before the 1st challenge was 18days.

RESULTS

Animal	*Challenge Concentration*	*Number of Animals Showing Skin Reactions after*:*			
		1st challenge		*2nd challenge*	
		24 h	*48 h*	*24 h*	*48 h*
Test Group	3.125%	0/9	0/9	-	-
	1.56%	0/9	0/9	-	-
Positive Control Group	3.125%	0/5	0/5	-	-
	1.56%	0/5	0/5	-	-

* Skin reactions have only been counted where animals have a skin reaction with a grading of 2 or more given the control animals also showed signs of irritation.

Remarks - Results	Slight erythema (grade 1) was observed at the 24 hour observation period in an animal treated at 3.125% and 1.56%. No other signs of irritation were noted in the other animals at both the 24 and 48 hour observation periods.
CONCLUSION	Difficulty in interpreting the results was noted due to irritation in both treated and control animals. Limited of the study due to inconclusive results.
TEST FACILITY	Phycher (2004b)

B.7. Skin sensitisation

TEST SUBSTANCE	100% APX 20P
METHOD	OECD TG 406 Skin Sensitisation – (GPMT Maximisation test – 2)
Species/Strain	Guinea pig/Hartley albino
PRELIMINARY STUDY	Maximum Non-irritating Concentration: intradermal: Moderate erythema at lowest dose = 0.375% topical: 100%
MAIN STUDY	
Number of Animals	Test Group: 10 Vehicle Control Group: 5
INDUCTION PHASE	Induction Concentration: intradermal: 0.375% in olive oil topical: 100%
Signs of Irritation	No irritation was observed in either controls or treated group.
CHALLENGE PHASE	
Challenge	topical: 100%
Remarks - Method	-<u>Vehicle Control group</u> Three pairs of intradermal injections of 0.1 ml volume were given in the shoulder region cleared of hair on each side of the midline. 1-FCA diluted at 50 % with distilled water (v/v) 2-Olive oil (vehicle) 3-mixture 50/50 of the solutions 1- and 2- (v/v) - <u>Treated group</u> Three pairs of intradermal injections of 0.1 ml volume were given in the same sites and order as in the control animals. 1-FCA diluted at 50 % with distilled water (v/v) 2-test item with olive oil at the dose previously defined (MIC_{ID}). 3-test item at the same dose as in 2- in a 50/50 mixture (v/v) of FCA and

distilled water (v/v).

RESULTS

Animal	*Challenge Concentration*	*Percentage of Animals Showing Skin Reactions after:*	
		24 h	*48 h*
Treated Group	100%	0	0
Vehicle Control Group	100%	0	0

Remarks - Results: Positive control: Preparation with 2-mercaptobenzothiazole which is known for its skin sensitization potential.
The percentage of reactive animals during the challenge exposure was equal to 40 %.

CONCLUSION: There was no evidence of reactions indicative of skin sensitisation to the notified chemical under the conditions of the test.

TEST FACILITY: Evic France (2004)

B.8. Repeat dose toxicity

TEST SUBSTANCE: 100% APX 20P

METHOD: OECD TG 407 Repeated Dose 28-day Oral Toxicity Study in Rodents.
Species/Strain: 20F, 20M/ Sprague-Dawley
Route of Administration: Oral – gavage/diet/drinking water
Exposure Information: Total exposure days: 28 days
Vehicle: Arachis oil BP
Physical Form: liquid (as a suspension)
Remarks - Method: The test material was administered by gavage to three groups, each of five male and five female Sprague-Dawley Crl:CD® (SD) IGS BR strain rats, for twenty-eight consecutive days, at dose levels of 15, 150 and 750 mg/kg bw/day. A control group of five males and five females was dosed with vehicle alone (Arachis oil BP).

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw/day*	*Mortality*
control	5F, 5M	0	0
low dose	5F, 5M	15	0
mid dose	5F, 5M	150	0
high dose	5F, 5M,	750	0

Mortality and Time to Death
No mortality from 0 to 750 mg/kg bw

Clinical Observations
Sporadic incidents of increased salivation were detected immediately after dosing in animals of either sex at with 150 or 750 mg/kg bw/day. This was coupled with sporadic episodes of generalised red/brown staining and isolated incidents of wet fur in animals of either sex treated with 750 mg/kg bw/day. An increase in water consumption was detected for animals of either sex treated with 750 mg/kg bw/day during the final two weeks of the treatment period for males and during the final week of treatment for the females.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis
No toxicologically significant effects were detected. Animals of either sex treated with 750 mg/kg bw/day showed elevations in albumin, albumin/globulin ratio, aspartate aminotransferase and alanine aminotransferase.

Effects in Organs
An elevation in absolute liver weight and liver weight relative to terminal bodyweight was detected for animals of either sex treated with 150 or 750 mg/kg bw/day (up to 5.7% liver weight relative increase).
Liver pathology changes were also noted in mid and high dose groups of both sexes. These changes consisted of centrilobular hepatocyte enlargement in males and generalised hepatocyte enlargement in females. These changes were considered to be adaptive in nature.

Remarks – Results
The 'No Observed Effect Level' for animals of either sex was considered to be 15 mg/kg/day. Considering the effects detected at 150 or 750 mg/kg bw/day as adaptive, the study author established a 'No Observed Adverse Effect Level' (NOAEL) of 750 mg/kg bw/day for animals of either sex.

CONCLUSION
NICNAS considered 150 mg/kg bw/day as the No Observed (Adverse) Effect Level (NOAEL) based on treatment related but non adverse clinical chemistry parameters and liver effects seen at this dose level in all animals.

TEST FACILITY Safepharm Laboratories (2007b)

B.9. Genotoxicity – bacteria reverse mutation test

TEST SUBSTANCE	100% APX 20P
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
Species/Strain	*S. typhimurium*: TA1535, TA1537, TA98, TA100, *E. coli*: WP2uvrA,
Metabolic Activation System	Phenobarbitone/β-naphthoflavone
Concentration Range in Main Test	a) With metabolic activation: 50 to 5000 μg/plate b) Without metabolic activation: 50 to 5000 μg/plate
Vehicle	dimethyl sulphoxide
Physical Form	Gas/vapour
Remarks - Method	OECD TG 471 Bacterial Reverse Mutation Test.
RESULTS	The vehicle (dimethyl sulphoxide) control plates gave counts of revertant colonies within the normal range. All of the positive control chemicals used in the test induced marked increases in the frequency of revertant colonies, both with and without metabolic activation. At 5000 μg/plate, only oily precipitate occurred but did not prevent the scoring of revertant colonies. No significant increases in the frequency of revertant colonies were recorded for any of the bacterial strains, with any dose of the test material, either with or without metabolic activation.
CONCLUSION	The notified chemical was not mutagenic to bacteria under the conditions of the test.
TEST FACILITY	Safepharm Laboratories (2003e)

B.10. Genotoxicity – in vitro 1

TEST SUBSTANCE	100% APX 20P
METHOD	OECD TG 473 In vitro Mammalian Chromosome Aberration Test. EC Directive 2000/32/EC B.10 Mutagenicity - In vitro Mammalian Chromosome Aberration Test.

Species/Strain	Chinese Hamster Ovary cells (CHO)
Cell Type/Cell Line	(CHO-K1 [ATCC n° CCL 61], cell cycle 12 – 14 hours, chromosome number 21 ± 2).
Metabolic Activation System	Culture medium : Mc Coy 5A
RESULTS	In the absence of metabolic activation, the test substance, induced a weak clastogenic effect: [in short treatment (4 h) : 15.4 % for 50 µg/mL ($P < 0.01$) and in long term treatment (20 h) for all concentrations studied from 12.5 µg/mL (13 %, $P < 0.001$) to 50 µg/mL (13.3 %, $P < 0.001$)]. In the presence of metabolic activation, the test substance is not clastogenic at the concentrations studied (12.5 to 50 µg/mL) suggesting complete metabolization of the test substance by S9-mix.
CONCLUSION	In the presence of metabolic activation, the notified chemical was not clastogenic to <CHO cells> treated in vitro and in the absence of metabolic activation S9, the notified chemical was weak clastogenic under the conditions of the test.
TEST FACILITY	Lemi (2007)

B.11. Genotoxicity – in vitro 2

TEST SUBSTANCE	100% APX 20P
METHOD	OECD TG 473 *In vitro* Mammalian Chromosome Aberration Test.
Cell Type/Cell Line	Human peripheral lymphocytes
Metabolic Activation System	S9 mix from Aroclor 1254-treated male Wilstar rat liver
Vehicle	Dimethyl sulfoxide
Remarks - Method	The notified chemical was cytotoxic ≥ 300 µg/mL and caused haemolysis ≥ 150 µg/mL

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	9.38*, 18.75*, 37.5*, 75* and 150*	4 hr	32 hr
Test 2	4.69*, 9.38*, 18.75*, 37.5*, 75 and 150	32 hr	32 hr
Present			
Test 1	9.38*, 18.75*, 37.5*, 75* and 150*	4 hr	32 hr

*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	≥ 300 µg/mL			
Test 1		≥ 150 µg/mL	> 150 µg/mL	Negative
Test 2		≥ 150 µg/mL	> 150 µg/mL	Negative
Present	≥ 300 µg/mL			
Test 1		≥ 150 µg/mL	> 150 µg/mL	Negative

Remarks - Results	A slight increase in the number of structural aberrations at the high concentration of 150 µg/mL was observed compared to the solvent control but this was not statistically significant.
CONCLUSION	The notified chemical was not clastogenic to human peripheral lymphocytes treated in vitro under the conditions of the test.
TEST FACILITY	IIBAT (2007)

B.12. Genotoxicity – in vivo

TEST SUBSTANCE	100% APX 20P
METHOD	OECD TG 474 Mammalian Erythrocyte Micronucleus Test. EC Directive 2000/32/EC B.12 Mutagenicity - Mammalian Erythrocyte Micronucleus Test.
Species/Strain	Rats/SD
Route of Administration	Oral – gavage
Vehicle	Distilled water and olive oil
Remarks - Method	No significant protocol deviations. A single dose level of 2000 mg/kg bw was used on the basis of a preliminary study in which no toxicity was noted at any dose tested.

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Sacrifice Time hours*
1 – Distilled Water	5M , 5F	0	24
2 – Distilled Water	5M , 5F	0	48
3 - Positive control, cyclophosphamide	5M , 5F	50	24
4 - Test group	5M , 5F	2000	24
5 - Test group	5M , 5F	2000	48

RESULTS

Doses Producing Toxicity	There were no signs of toxicity at the tested dose of 2000 mg/kg bw.
Genotoxic Effects	The test substance showed a slight increase in the frequency of micronucleated PCE over the levels observed in the vehicle control group but did not induce a statistically significant increase. There was a statistically significant increase in the number of micronucleated cells in the positive control group, as compared to the vehicle control group, thus validating the conduct of assay.
Remarks - Results	As there were no clinical signs in the test animals, and no change in the ratio of polychromatic cells, compared to the controls, and cannot be confirmed that the test material reached the bone marrow. There was no statistically significant increase in the number of micronucleated cells in the test substance group at all time points, as compared to the concurrent vehicle control groups.
CONCLUSION	The test substance was not clastogenic under the conditions of this in vivo mouse micronucleus test.
TEST FACILITY	Phycher (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 D Ready Biodegradability: Closed Bottle Test.

Inoculum	Aerobic activated sludge from a domestic wastewater treatment plant
Exposure Period	28 days
Auxiliary Solvent	None reported
Analytical Monitoring	Yellow Springs oxygen meter and BOD probe
Remarks - Method	The test substance was prepared with the aid of high shear mixing to form a homogeneous dispersion. Standard protocol was followed in this study.

RESULTS

Test substance		*Sodium Benzoate*	
Day	*% Degradation*	*Day*	*% Degradation*
3	13	3	54
6	14	6	65
9	24	9	65
12	34	12	65
15	35	15	67
18	33	18	69
21	33	21	73
24	33	24	74
28	36	28	78

Remarks - Results: The reference substance was degraded >60% by day 14, indicating a valid test. All validation criteria for the test were satisfied. The test substance did not reach 60% biodegradation by the 28th day and hence did not reach the pass level for this test.

CONCLUSION: The notified chemical is not readily biodegradable

TEST FACILITY: Safepharm Laboratories (2003f)

C.2. Ecotoxicological Investigations

C.2.1. Acute toxicity to fish

TEST SUBSTANCE: Notified chemical

METHOD: OECD TG 203 Fish, Acute Toxicity Test – Semi Static

Species	Zebra fish (*Danio rerio*)
Exposure Period	96 hours
Auxiliary Solvent	None reported
Water Hardness	1.08 mmol/L (Ca^{2+} and Mg^{2+})
Analytical Monitoring	None
Remarks – Method	The water accommodated fraction (WAF) was prepared by adding the nominal weight to dilution water and shaken vigorously for 24 hours. The resulting solution was passed through a 0.45 µm membrane filter. Standard protocol was followed except that the temperature at 24 h was 0.2ºC below the minimum value recommended in the guidelines. This slight deviation would not have affected the results of the test.

RESULTS

Concentration mg/L		*Number of Fish*	*Mortality*			
Nominal	*Actual*		*24 h*	*48 h*	*72 h*	*96 h*
0	0	7	0	0	0	0
100	Not measured (WAF)	7	0	0	0	0

LL50	>100 mg/L at 96 hours (based on loading rates).
NOEL	100 mg/L at 96 hours (based on loading rates).
Remarks – Results	All validation criteria for the study were satisfied. No abnormal behaviour was observed in the fish.
CONCLUSION	The notified chemical is not harmful to fish up to the limit of its solubility in water
TEST FACILITY	LAUS GmbH (2007a)

C.2.2. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 202 Daphnia sp. Acute Immobilisation Test - Static
Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	None reported
Water Hardness	250 mg $CaCO_3$/L
Analytical Monitoring	None
Remarks - Method	The water accommodated fraction (WAF) was prepared by adding the nominal weight to dilution water and shaken vigorously for 24 hours. The resulting solution was passed through a 0.45 µm membrane filter. Standard protocol guidelines were followed with no significant deviations reported.

RESULTS

Concentration mg/L		*Number of D. magna*	*Number Immobilised*	
Nominal	*Actual*		*24 h*	*48 h*
0	0	20	1	1
1	Not measured (WAF)	20	0	0
10	Not measured (WAF)	20	0	0
100	Not measured (WAF)	20	0	0

EL50	>100 mg/L at 48 hours. Based on loading rates.
NOEL	100 mg/L at 48 hours. Based on loading rates.
Remarks - Results	The 24 h-EC50 of potassium dichromate was tested in a current reference test. The value was determined as 1.4 mg/L, which is within the required range of 0.6 – 2.1 mg/L. One daphnia in the control group died. No other daphnia showed signs of abnormal behaviour throughout the test. Since <10% of the control daphnids were immobilised the test is considered to be valid. All validation criteria for the study were satisfied.
CONCLUSION	The notified chemical is not harmful to aquatic invertebrates up to the limit of its solubility in water
TEST FACILITY	LAUS GmbH (2007b)

C.2.3. Algal growth inhibition test

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 201 Alga, Growth Inhibition Test.
Species	*Desmodesmus subspicatus*
Exposure Period	72 hours
Concentration Range	Nominal: 1, 10 and 100 mg/L (WAF) Actual: Not measured
Auxiliary Solvent	None reported
Water Hardness	Not reported
Analytical Monitoring	None
Remarks - Method	Standard protocols were followed except that the temperature range in the main study (23-25ºC) was higher than recommended by the test guidelines (21-24ºC). This slight deviation would not have affected the results of the test.

RESULTS

Biomass		*Growth*	
E_bL_{50}	*NOEL*	*E_rL_{50}*	*NOEL*
mg/L at 72 h	*mg/L*	*mg/L at 72 h*	*mg/L*
>100	100	>100	100

Remarks - Results	All validity criteria for the study were satisfied. The EC50s of potassium dichromate were tested in a current reference test. The values determined were in the normal range of the laboratory.
CONCLUSION	The notified chemical is not harmful to algae up to the limit of its solubility in water
TEST FACILITY	LAUS GmbH (2007c)

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