File No: LTD/1613

July 2012

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

Betaines, coco alkyldimethyl(3-sulfopropyl)

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 Sustainability, Environment, Water, Population and Communities.

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

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

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Director
NICNAS

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SUMMARY

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

ASSESSMENT REFERENCE	APPLICANT(S)	CHEMICAL OR TRADE NAME	HAZARDOUS SUBSTANCE	INTRODUCTION VOLUME	USE
LTD/1613	Agfa-Gevaert Limited	Betaines, coco alkyldimethyl(3-sulfopropyl)	Yes	≤0.42 tonnes per annum	Component of products used in the printing and graphics industry

CONCLUSIONS AND REGULATORY OBLIGATIONS

Hazard classification
Based on studies conducted on a 50% aqueous solution of the notified chemical, the notified chemical is recommended for hazard classification according to the *Globally Harmonised System for the Classification and Labelling of Chemicals (GHS),* as adopted for industrial chemicals in Australia. The recommended hazard classification is presented in the table below.

Hazard classification	*Hazard statement*
Eye Damage (Category 1)	H318 Causes serious eye damage
Skin Irritation (Category 2)	H315 Causes skin irritation

Note: Given the severity of the effects is expected to increase at higher concentrations the neat notified chemical may be classified as:

Category 1: Causes severe burns and eye damage

Based on studies conducted on a 50% aqueous solution of the notified chemical, the notified chemical is recommended for hazard classification according to the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004) with the following risk phrase:

R38: Irritating to skin
R41: Risk of serious damage to eyes

Note: Given the severity of the effects is expected to increase at higher concentrations the neat notified chemical may be classified as:

R34: Causes burns

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

Hazard classification	*Hazard statement*
Acute Category 3	Harmful to aquatic life
Chronic Category 3	Harmful to aquatic life with long lasting effects

Human health risk assessment
Provided that adequate PPE are in place to mitigate exposure, under the conditions of the occupational settings described, the notified chemical is not considered to pose an unreasonable risk to the health of workers.

When used in the proposed manner, the notified chemical is not considered to pose an unreasonable risk to public health.

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

Recommendations

REGULATORY CONTROLS
Hazard Classification and Labelling

- Safe Work Australia should consider the following health hazard classification for the notified chemical:
  - H318 Causes serious eye damage
  - H315 Causes skin irritation

- Use the following risk phrases for products/mixtures containing the notified chemical:
  - ≥ 10%: Skin irritant 2: H315 Causes skin irritation;
  - ≥ 3%: Eye damage 1: H318 Causes serious eye damage

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 skin and eye contact

- Employers should ensure that the following personal protective equipment is used by workers to minimise occupational exposure to the notified chemical as introduced:
  - Coveralls
  - Safety goggles
  - Gloves

  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 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(1) of the Act; if
- the importation volume exceeds 0.42 tonnes per annum notified chemical;
- where the import volume is expected to exceed 0.42 tonnes, information regarding industrial sites involving the use frequencies of the notified chemical per annum, and supporting data for the removal of the notified chemical during sewage treatment will be required.

or

(2) Under Section 64(2) of the Act; if
- the function or use of the chemical has changed from component of products used in the printing and graphics industry, or is likely to change significantly;
- the amount of chemical being introduced has increased from 0.42 tonnes 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.

Material Safety Data Sheet
The MSDS of the notified chemical and a product containing the notified chemical provided by the notifier were reviewed by NICNAS. The accuracy of the information on the MSDS remains the responsibility of the applicant.

ASSESSMENT DETAILS

1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)
Agfa-Gevaert Ltd (ABN 12 000 404 722)
15 Dalmore Drive
SCORESBY VIC 3179

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

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

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
Variation to the schedule of data requirements is claimed for all physico-chemical properties.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT
None

NOTIFICATION IN OTHER COUNTRIES
None

2. IDENTITY OF CHEMICAL

MARKETING NAME
Ralufon DCH (contains ~50% notified chemical)

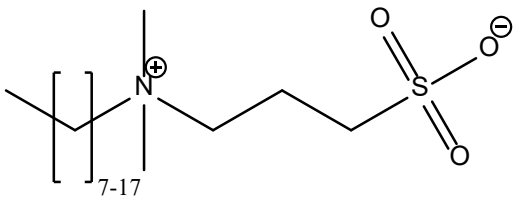
CAS NUMBER
68201-55-8

CHEMICAL NAME
Betaines, coco alkyldimethyl(3-sulfopropyl)

OTHER NAME(S)
Sulfobetaines, coco alkyldimethyl(3-sulfopropyl)
Dimethylcocobetaine
3-[dodecyl(dimethyl)ammonio]propane-1-sulfonate
N,N-Dimethyl-N-coco-N-(3-sulfopropyl) ammonium betaine
Coconut dimethylammonium-3-sulfopropyl betaine
Dodecaniminium-N,N-dimethyl-N-(3-sulfopropyl)-hyrdoxide, inner salt

MOLECULAR FORMULA
Unspecified

STRUCTURAL FORMULA



MOLECULAR WEIGHT
The notified chemical contains various species with molecular weights ranging from 279-419 Da.
Mn 335.54 Da (average). Based on the average molecular weight, the average alkyl chain length is 12.58.

ANALYTICAL DATA
Reference IR spectrum was provided.

3. COMPOSITION

DEGREE OF PURITY 100%

HAZARDOUS IMPURITIES/RESIDUAL MONOMERS None

NON HAZARDOUS IMPURITIES/RESIDUAL MONOMERS (>1% by weight) None

ADDITIVES/ADJUVANTS None

LOSS OF MONOMERS, OTHER REACTANTS, ADDITIVES, IMPURITIES
Not expected under normal conditions of use.

DEGRADATION PRODUCTS
Not expected under normal conditions of use.

4. PHYSICAL AND CHEMICAL PROPERTIES

The following information relates to the notified chemical as an aqueous solution (~50% concentration) in the product Ralufon DCH:

APPEARANCE OAT 20 °C AND 101.3 kPa: Yellow liquid

Property	Value	Data Source/Justification
Melting Point/Freezing Point	0- -4°C	MSDS
Boiling Point	100°C	MSDS
Density	1020-1030 kg/m^3 at 20°C	MSDS
Vapour Pressure	8.59×10^{-17} kPa at 20°C	Estimated using MPBPVP v 1.43 (modified Grain method).
Water Solubility	Not determined	Fully miscible according to the notifier. The notified chemical is considered to be at least dispersible in water given the chemical structure has a surfactant characteristics.
Hydrolysis as a Function of pH	Not determined	The notified chemical does not contain functional groups that are likely to hydrolyse under the environmental conditions.
Partition Coefficient (n-octanol/water)	log P_{OW} = -0.47 – 1.65	Reported by Davies *et al* (2004) for components of the notified chemical having C8 – C12 alkyl chains. A low value is expected based on the structural information of the notified chemical.
Adsorption/Desorption	Not determined	A chemical having cationic amine groups is expected to absorb to soil or sediment.
Dissociation Constant	pKa = 1.3	MSDS. The notified chemical is expected to be ionised in the environmental pH range of 4 – 9.
Particle Size	Not determined	Not introduced in solid form.
Flash Point	> 100°C	MSDS
Flammability	Not determined	Not expected to be highly flammable
Autoignition Temperature	Not expected to self-ignite	Statement provided on MSDS
Explosive Properties	Not expected to be explosive	Based on structural information and the lack of structural alerts.
Oxidising Properties	Not expected to have oxidative properties	Based on structural information and the lack of structural alerts.

DISCUSSION OF PROPERTIES

Reactivity
Stable under normal conditions of use.

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

5. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS
The notified chemical will be imported at up to 5% concentration as a component of products used in the printing and graphics industry.

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

Year	*1*	*2*	*3*	*4*	*5*
Tonnes	0.19	0.22	0.26	0.32	0.42

PORT OF ENTRY
Various

IDENTITY OF MANUFACTURER
Raschig GmbH, Germany

TRANSPORTATION AND PACKAGING
Finished products will be imported in 20 L plastic containers and transported to the notifier's warehouse for storage before distribution to end users by road.

USE
Finished products containing the notified chemical at ≤ 5% will be used as developer, finisher and cleaning solution in the printing and graphics industry.

OPERATION DESCRIPTION
Finished products containing the notified chemical at ≤ 5% will be added to printing equipment using hoses or manually and either automatically or manually cleaned and rinsed out.

6. HUMAN HEALTH IMPLICATIONS

6.1. Exposure Assessment

6.1.1. Occupational Exposure

CATEGORY OF WORKERS

Category of Worker	*Exposure Duration (hours/day)*	*Exposure Frequency (days/year)*
Transport	4	5
Distribution	2	20
Storage	1	240
End users	1-2	30-60
Product disposal	2	10

EXPOSURE DETAILS
Workers involved in the transport, distribution and storage of imported products containing the notified chemical at ≤ 5% will only be exposed in the event of an accident involving the breach of product containers.

Workers handling products containing the notified chemical at ≤ 5% during end use and disposal may experience dermal and accidental ocular exposure during addition of product to printing equipment, cleaning, maintenance and subsequent disposal of product after use. The level of exposure experienced by workers may vary between sites depending on the level of automation in the cleaning and maintenance processes. However, exposure is expected to be minimised during these activities by the use of personal protective equipment (PPE), which include gloves, goggles and coveralls.

Inhalation exposure is not expected given the low vapour pressure and the use of local exhaust ventilation in areas surrounding the printing equipment.

6.1.2. Public Exposure
Products containing the notified chemical will not be made available to the general public and hence exposure to the public is not expected during use.

6.2. Human Health Effects Assessment

The results from toxicological investigations conducted on the notified chemical at 50% concentration are summarised in the table below. Details of these studies can be found in Appendix A.

Endpoint	*Result and Assessment Conclusion*
Rabbit, skin irritation	moderately irritating
Rabbit, eye irritation	severely irritating
Mutagenicity – bacterial reverse mutation	non mutagenic

Toxicokinetics

The notified chemical has a molecular weight < 500 Da, is expected to be soluble in water and have a low partition coefficient (log Pow = -0.47 – 1.65) and is therefore expected to be absorbed following dermal, oral and inhalation exposure.

Irritation and Sensitisation

Studies conducted in rabbits on the notified chemical at ~50% in water showed it to be irritating to skin and severely irritating to the eye. The severity of these effects is expected to increase at higher concentrations.

The notified chemical contains a quaternary ammonium functional group which is a structural alert for skin sensitisation (Barratt, et al., 1994).

Mutagenicity

The notified chemical at ~50% showed no signs of mutagenicity in a reverse mutation test.

Health hazard classification

Based on studies conducted on a 50% aqueous solution of the notified chemical, the notified chemical is recommended for hazard classification according to the *Globally Harmonised System for the Classification and Labelling of Chemicals (GHS),* as adopted for industrial chemicals in Australia. The recommended hazard classification is presented in the table below.

Hazard classification	***Hazard statement***
Eye Damage (Category 1)	H318 Causes serious eye damage
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Note: Given the severity of the effects is expected to increase at higher concentrations the neat notified chemical may be classified as:

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R38: Irritating to skin
R41: Risk of serious damage to eyes

Note: Given the severity of the effects is expected to increase at higher concentrations the neat notified chemical may be classified as:

R34: Causes burns

6.3. Human Health Risk Characterisation

6.3.1. Occupational Health and Safety

Based on the data provided, the notified chemical is a skin irritant and may cause serious damage to the eye at ~50% concentration. Based on the presence of a structural alert, the notified chemical may also present as a skin sensitiser.

Workers may experience dermal and accidental ocular exposure to products containing the notified chemical at ≤ 5% concentration during end-use (i.e. addition to printing equipment, cleaning and maintenance and disposal). At this concentration there is potential for skin sensitisation and irritant effects to the skin and eye. However, the use of PPE (gloves, goggles and coveralls) should minimise the potential for exposure.Therefore, provided that adequate PPE are in place, the risk to workers is not considered unreasonable.

6.3.2. Public Health

Exposure to the notified chemical by the public is not expected except in the unlikely event of a transport accident or spill. Hence the risk to the public from the notified chemical when used in the proposed manner is not considered unreasonable.

7. ENVIRONMENTAL IMPLICATIONS

7.1. Environmental Exposure & Fate Assessment

7.1.1. Environmental Exposure

RELEASE OF CHEMICAL AT SITE

The notified chemical will be imported into Australia as a component of a final product. Therefore, no releases from manufacturing and reformulation processes, and no significant releases from transportation are expected.

RELEASE OF CHEMICAL FROM USE

The imported formulated products are water based and will be used in the cleaning of printing plates nationwide. This may involve brushing, wiping or immersion techniques. The amount of the product used in each application is estimated to be up to 20 L, which is equivalent to 1 kg of the notified chemical.

No detailed information regarding waste treatment and release of the notified chemical from use has been provided. The notifier indicates that the washing waste will be contained in wash tanks, collected by a licensed waste contractor, and most likely delivered to a waste water treatment plant for treatment. The after-treatment effluent can then be discharged into the environment through the sewer or drainage system. Based on the proposed use pattern, 100% of the imported notified chemical is assumed for the worst case scenario to be disposed of directly to sewer after use.

RELEASE OF CHEMICAL FROM DISPOSAL

Any residues in the empty containers are expected to be sent to landfill with the container, which is not expected to be of significant amount.

7.1.2. Environmental Fate

The notified chemical is not readily biodegradable. For the details of the environmental fate studies please refer to Appendix B. The notified chemical is not expected to have high potential for bioaccumulation based on its expected low log P_{OW}, despite of its low molecular weight of < 1000. It is assumed that 100% of the notified chemical will be released to sewer after use. In a sewage treatment plant, the notified chemical may be partially absorbed on the sludge that will eventually be sent to landfill. The notified chemical may also partially remain in effluent and be released into surface water. In landfill or surface water, the notified chemical is expected to degrade slowly via biotic and abiotic pathways, forming water and oxides of carbon, nitrogen and sulphur.

7.1.3. Predicted Environmental Concentration (PEC)

The Predicted Environmental Concentration (PEC) has been calculated assuming for the worst case scenario, whereby 100% release of the notified chemical to sewage system nationwide, one application per season, and no removal from a sewage treatment plant (STP).

Predicted Environmental Concentration (PEC) for the Aquatic Compartment		
Total Annual Import/Manufactured Volume	420	kg/year
Proportion expected to be released to sewer	100%	
Annual quantity of chemical released to sewer	420	kg/year
Days per year where release occurs	4	days/year
Daily chemical release:	105	kg/day
Water use	200	L/person/day
Population of Australia (Millions)	22.613	million
Removal within STP	0%	
Daily effluent production:	4,523	ML
Dilution Factor - River	1.0	
Dilution Factor - Ocean	10.0	
PEC - River:	23.22	µg/L
PEC - Ocean:	2.32	µg/L

STP effluent re-use for irrigation occurs throughout Australia. The agricultural irrigation application rate is assumed to be 1000 L/m^2/year (10 ML/ha/year). The notified chemical in this volume is assumed to infiltrate and accumulate in the top 10 cm of soil (density 1500 kg/m^3). Using these assumptions, irrigation with a concentration of 23.2 µg/L may potentially result in a soil concentration of approximately 0.155 mg/kg. Assuming accumulation of the notified chemical in soil for 5 and 10 years under repeated irrigation, the concentration of notified chemical in the applied soil in 5 and 10 years may be approximately 0.774 mg/kg and 1.55 mg/kg, respectively. However, these values are expected to be maximum concentrations due to the likely sorption of the notified chemical to STP sludge, and its potential for degradation.

7.2. Environmental Effects Assessment

No reliable ecotoxicity data were submitted. Ecotoxicity data in the published literature (Davies *et.al.,* 2004) reports a 48 h EC50 for *Daphnia magna* of 34.8 mg/L the C12 alkyl chain homologue of the notified chemical. The notified chemical is a mixture of chemicals with alkyl chains ranging C8 – C18, and the weighted average alkyl chain length is 12.58. Thus the above reported endpoint provides an indication of the likely toxicity of the notified chemical to daphnids.

The environmental hazard classification of the notified chemical is conducted in accordance with the guidance provided in the global harmonised system (GHS) (GHS, 2009).

Under the GHS the notified chemical is considered to be harmful to aquatic invertebrates. Based on its predicted acute toxicity to aquatic invertebrates the notified chemical is formally classified under the GHS as “Acute Category 3; Harmful to aquatic life”.

Since no chronic endpoints to aquatic life are available for the notified chemical, based on its predicted acute toxicity to daphnids, and given the notified chemical is not considered rapidly biodegradable, it is formally classified for long-term hazard under the GHS as “Chronic category 3; Harmful to aquatic life with long lasting effects”.

7.2.1. Predicted No-Effect Concentration

Predicted No-Effect Concentration (PNEC) for the aquatic compartment has been calculated based on the above reported EC50 of 34.8 mg/L for daphnids. An assessment factor of 1000 has been used since only one endpoint is available.

EC50 (Invertebrates)	34.8	mg/L
Assessment Factor	1,000	
PNEC:	34.8	µg/L

7.3. Environmental Risk Assessment

The Risk Quotient (PEC/PNEC) is calculated based on the estimated PEC and PNEC.

Risk Assessment	*PEC µg/L*	*PNEC µg/L*	*Q*
Q - River:	23.22	34.8	0.67
Q - Ocean:	2.32	34.8	0.067

The RQ value of < 1 indicates an acceptable risk to the aquatic life from the use of the notified chemical. Therefore, the notified chemical is not expected to pose an unreasonable risk to the aquatic life from its assessed use pattern. The notified chemical is not considered rapidly biodegradable. However, it is not expected to have high potential for bioaccumulation based on its expected low log P_{OW}, despite of its low molecular weight of < 1000.

APPENDIX A: TOXICOLOGICAL INVESTIGATIONS

B.1. Irritation – skin

TEST SUBSTANCE: Notified chemical at ~50%

METHOD
- Species/Strain: Rabbit/New Zealand White
- Number of Animals: 3 (2 males, 1 female)
- Vehicle: None
- Observation Period: 14 days
- Type of Dressing: Occlusive.
- Remarks - Method: No significant protocol deviations.

RESULTS

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3			
Erythema/Eschar	2	2	2	3	> 7 days	0
Oedema	1	1	1	1	> 7 days	0

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

Remarks - Results: Well defined erythema and very slight oedema was reported in all 3 animals at each of the 24, 48 and 72 hour observations. At the 7-day observation, moderate to severe erythema was reported in 1 animal and well defined erythema persisted in the other 2 animals. Very slight oedema was also reported in all 3 animals at the 7-day observation. No signs of irritation were observed at the 14-day observation.

CONCLUSION: The notified chemical at ~50% concentration is moderately irritating to the skin.

TEST FACILITY: RCC Ltd (1983a)

B.2. Irritation – eye

TEST SUBSTANCE: Notified chemical at ~50%

METHOD
- Species/Strain: Rabbit/New Zealand White
- Number of Animals: 3 (2 males, 1 female)
- Observation Period: 14 days
- Remarks - Method: No significant protocol deviation.

RESULTS

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3			
Conjunctiva: redness	1.67	2	2	3	> 14 days	1
Conjunctiva: chemosis	1.67	1	0.67	2	> 72 hrs	0
Conjunctiva: discharge	2.33	2	1.33	3	> 72 hrs	0
Corneal opacity	1.67	1.67	1	3	> 14 days	1
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: Redness (up to grade 3), chemosis (up to grade 2) and discharge (up to grade 3) was reported in the conjunctiva of each animal at each

observation point until Day 7, where grade 1 redness was reported in 1 animal which persisted until the end of the 14 day observation period.

Corneal opacity (up to grade 3) was reported in all 3 animals at each observation point until Day 14 where grade 1 corneal opacity persisted in 1 animal until the end of the 14 day observation period.

CONCLUSION The notified chemical at ~50% concentration is severely irritating to the eye.

TEST FACILITY RCC Ltd (1983b)

B.3. Genotoxicity – bacteria

TEST SUBSTANCE Notified chemical at ~50%

METHOD EEC Directive 79/831, Annex V, Method No. 431. Similar to OECD TG 471

Plate incorporation procedure

Species/Strain	*S. typhimurium:* TA1535, TA1537, TA1538, TA98, TA100
Metabolic Activation System	S9 fraction from Aroclor 1254 induced rat liver.
Concentration Range in Main Test	a) With metabolic activation: 0-5000 µg/plate b) Without metabolic activation: 0-500 µg/plate
Vehicle	Distilled water
Remarks - Method	No significant protocol deviations

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	≥ 500	≥ 500	None	Negative
Test 2		≥ 500	None	Negative
Present				
Test 1	5000	5000	None	Negative
Test 2		5000	None	Negative

Remarks - Results Cytotoxicity was observed at doses of 500 µg/plate and above without metabolic activation which prevented testing at higher doses. Cytotoxicity was observed only at 5000 µg/plate with metabolic activation.

No significant increases in the frequency of revertant colonies were recorded for any of the bacterial strains up to 500 µg/plate without metabolic activation and up to 5000 µg/plate with metabolic activation.

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

CONCLUSION The notified chemical at ~50% concentration was not mutagenic to bacteria under the conditions of the test.

TEST FACILITY RCC Ltd (1983c)

APPENDIX B: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready biodegradability

TEST SUBSTANCE	Notified chemical (50% aqueous solution)
METHOD	OECD TG 301 B Ready Biodegradability: CO_2 Evolution Test.
Inoculum	Activated sludge
Exposure Period	28 days
Auxiliary Solvent	No
Analytical Monitoring	The last CO_2-measurement was conducted on day 29 for determination of the degree of biodegradation.
Remarks - Method	The test substance was tested in duplicate at 39.5 mg/L, corresponding to 12 mg TOC/L at pH 7.6 – 7.7 and 20.1 – 21.9°C. Two blank control vessels without test substance, one positive control vessel using sodium acetate (40 mg/L or 12 mg TOC/L), and one toxicity control vessel using test substance plus sodium acetate (12 mg TOC/L for each) were also established.

RESULTS

Test substance		*Sodium acetate*	
Day	*% Degradation*[*]	*Day*	*% Degradation*
7	3	7	54
14	6	9	62
29	26	14	71

[*] Average values of duplicate results

Remarks - Results	All the test guideline validity criteria were met. A biodegradation degree of 35% was reached by Day 14 for the toxicity control. The test substance is therefore not considered to be toxic to the sludge micro-organisms. Based on the test results, the notified chemical is not considered to be readily biodegradable. However, it is considered to have potential to biodegrade in the aquatic environment.
CONCLUSION	The notified chemical is not readily biodegradable.
TEST FACILITY	NOTOX B.V. (2011)

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