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January 2010

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

FULL PUBLIC REPORT

4(3H)-Pyrimidinone, 2,6-diamino-

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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FULL PUBLIC REPORT

4(3H)-Pyrimidinone, 2,6-diamino-

1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)
Sun Ace Australia Pty Ltd (ABN 75 050 238 769)
32-38 Remington Drive
Dandenong South Victoria 3175

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: Flash point, Boiling Point, Adsorption/Desorption main test, Acute inhalation toxicity, Bioaccumulation

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
CEC/667, CER/17

NOTIFICATION IN OTHER COUNTRIES
None

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
DAHP
Astab COS

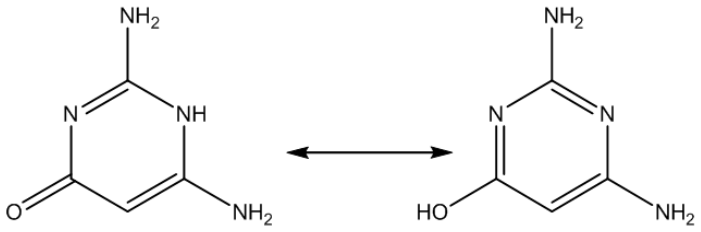
CAS NUMBER
56-06-4

CHEMICAL NAME
4(3H)-Pyrimidinone, 2,6-diamino-

OTHER NAMES
2,4-Diamino-6-hydroxypyrimidine

MOLECULAR FORMULA
$C_4H_6N_4O$

STRUCTURAL FORMULA



MOLECULAR WEIGHT
126.11 Da

ANALYTICAL DATA
Reference FTIR results were provided.
FTIR spectrometry
Major Peaks at: 3384, 3326, 3086, 1618, 1458, 1360, 1275, 1243, 1133, 979 cm^{-1}
[Sun Ace Kakoh Ltd. (2009a)]

3. COMPOSITION

DEGREE OF PURITY >99%

IMPURITIES/RESIDUAL MONOMERS The following chemicals have been noted as potential impurities, however concentrations have not been determined:
Methyl Cyanoacetate, DAHP Iso compounds, Guanidine Nitrate Sodium Sulfate, Sodium Chloride

ADDITIVES/ADJUVANTS None

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20°C AND 101.3 kPa: Whitish crystals

Property	Value	Data Source/Justification
Melting Point	276°C	Measured
Density	779 kg/m^3 at 20°C	Measured
Vapour Pressure	2.22 x 10^{-13} kPa at 25°C	Literature [Sun Ace Kakoh Ltd. (2009h)Zielenkiewicz X et al (1999)]
Water Solubility	5.28 g/L at 20°C	Measured
Hydrolysis as a Function of pH	$t_{½}$ > 1 year at 25°C for pH 4, 7 and 9	Measured
Partition Coefficient (n-octanol/water)	log P_{ow} = -0.94 at 20°C	Measured
Adsorption/Desorption	log K_{oc} = 1.59 at 20°C	Estimated
Dissociation Constant	$pKa_1 \approx 4.6$, $pKa_2 \approx 5.2$ at 25°C	Estimated
Particle Size	Inhalable fraction (<100 µm): 100%	Measured
Flash Point	Not determined	
Solid Flammability	Not Highly Flammable	Measured
Auto-ignition Temperature	>287°C	Measured
Explosive Properties	Not Explosive	Estimated (on basis of structure and reactivity) Sun Ace Kakoh Ltd. (2009i)

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

Reactivity
The notified chemical is expected to be stable under normal environmental conditions. There is no known incompatibility with other substances.

Dangerous Goods classification
Based on the data provided, the notified chemical is not classified under 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.

5. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS
The notified chemical will be imported as a powder at a concentration of 98% and will be reformulated in Australia.

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

Year	*1*	*2*	*3*	*4*	*5*
Tonnes	50	75	100	125	150

PORT OF ENTRY
Melbourne wharf.

IDENTITY OF MANUFACTURER/RECIPIENTS
Sun Ace Australia Pty Ltd

TRANSPORTATION AND PACKAGING
The notified chemical will be imported in 25 kg bags or 50 kg drums and transported by road from wharf to the notifier's site for reformulation. The reformulated product containing the notified chemical at 10-30% will be packed into 25 kg or 500 kg bulk bags and transported to the customer's premises by road and/or shipping.

USE
The notified chemical will be used as a stabiliser in PVC (polyvinyl chloride) products for the plastics industry, including pipe manufacture. Pipes may be used for distributing drinking water for human use.

OPERATION DESCRIPTION
The imported notified chemical in powder form will be reformulated with other ingredients and pelletized at the reformulation site. It will be manually weighed, transferred and loaded into the hopper. Mixing and blending of the notified chemical with other components is done automatically in a closed process. The blended product will then be transferred to a floveyor belt for pelletising. The pelletised material containing 10-30% of the notified chemical will then be packed into 25kg bags or 500 kg bulk bags for transfer to PVC manufacturing sites. Bags then would be sealed by the operator and transferred to a pallet and the pallet will be transferred to the warehouse using a forklift.

At the PVC manufacturing site, the granules will be added to the PVC resins, which will ultimately be extruded to produce PVC pipes. The percentage of the notified chemical in the final PVC pipes is between 0.1 and 0.30%.

6. HUMAN HEALTH IMPLICATIONS

6.1 Exposure assessment

6.1.1 Occupational exposure

Number and Category of Workers

Category of Worker	*Number*	*Exposure Duration (hr/day)*	*Exposure Frequency (days/year)*
Process operators	3	7.5	230
Warehouse	2	2	10
Quality control	1	1	230
Transport & storage	3	4	10

Exposure Details
Approximately 3 wharf & transport company workers will transport the notified chemical from the wharf to Sun Ace Australia sites in sealed bags. Two workers will be involved in receiving and storing the notified chemical at Sun Ace Australia sites with minimal exposure as the notified chemical will be in sealed bags. Worker exposure during transport and storage is not expected, except in the case of accidental breaching of the packaging.

At the formulation site where the notified chemical is incorporated into pellets, process workers will be involved in weighing, mixing, packing and loading the notified chemical into the mixers via the loading chute. The sealed bags would be cut open at the automatic lifting table. Workers may be exposed to the notified chemical via dermal, ocular or inhalation routes during these processes. Engineering controls such as local exhaust ventilation would be located at the weighing, mixing/blending and packing areas to minimise the potential exposure. Workers will be wearing gloves, full face masks or respirators and protective clothing as part of the process requirements.

During the automated packing operation of the pellets containing the notified chemical at 10-30%, dermal or ocular exposure of workers to the pellets may occur. As the pellets are expected to be non-dusting, inhalation exposure is not expected.

At the PVC manufacturing site, workers may have dermal or ocular contact with the pellets as they are mixed with the PVC resin. Once the PVC articles containing the notified chemical at 0.1%-0.3% are extruded, it is expected that the notified chemical will be incorporated in the polymer matrix, and will not be bioavailable.

6.1.2. Public exposure
The notified chemical will not be made available to the public.
The public may come into dermal contact with articles containing the notified chemical at 0.1-0.3%, however it will be incorporated in the polymer matrix and not expected to be available in significant quantities. Therefore, public exposure is not expected.
Potential low-level exposure of the public to the chemical could occur through drinking water, if leaching from the pipes occurred. As part of their scientific opinion on the safety of the notified chemical, EFSA (2009) reported that the specific migration of the chemical from rigid PVC into water was 75µg/kg. The conditions of this study were 10 days at 40ºC, the concentration of the chemicals in the PVC was nominally 0.18% (actual 0.13%), and the surface to volume ratio was 6 dm/L. The level of notified chemical tested for specific migration was approximately half the maximum level proposed for use in Australia (0.3%).

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 >5000 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
Rabbit, dermal irritation	slightly irritating
Guinea pig, skin sensitisation – adjuvant test	no evidence of sensitisation
Rat, oral toxicity repeated dose 90day	NOAEL 800mg/kg bw/day
Mutagenicity – bacterial reverse mutation	non mutagenic
Genotoxicity – in vitro (OECD TG 476 In vitro Mammalian Cell Gene Mutation Test)	non genotoxic
Genotoxicity – in vivo (OECD TG 474 Mammalian Erythrocyte Micronucleus Test)	non genotoxic

Toxicokinetics, metabolism and distribution
No pharmacokinetic data were provided. However the high water solubility is expected to limit dermal absorption to some extent. As the vapour pressure is very low, absorption via inhalation would only occur if dusts were inhaled. As the particle size is <100 μm and information is not available on the full particle size distribution, respirable particles may be present.

Acute toxicity, Irritation and Sensitisation
The notified chemical is of low acute toxicity via oral and dermal routes. Acute inhalation toxicity data are not provided.

In animal studies the notified chemical was slightly irritating to the skin and eyes, and it did not elicit skin sensitisation in a guinea pig maximisation test.

Repeated dose toxicity
No mortality was observed during a 90 day rat study with doses up to 1600mg/kg bw/d. Although most animal at high and mid dose group had mild to severe salivation from day 1 onward, no pathological lesions of salivary glands were observed.

Ophthalmological examinations did not show any abnormalities.

Neurobehavioural and clinical observations did not reveal any test substance related abnormality in treatment groups.

The No Observed Adverse Effect Level (NOAEL) was established at 800 mg/kg bw/day based on hind leg effects seen at this dose level.

Mutagenicity
The notified chemical was not mutagenic to bacteria and was not genotoxic under in vitro and in vivo tests conducted.

No data are provided on carcinogenicity or toxicity for reproduction effects of the notified chemical.

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

The notified chemical is expected to have slight skin and eye irritation potential. No data are provided on inhalation toxicity of the notified chemical. The highest potential exposure to workers is during the blending/mixing stage, when handling of the notified chemical in powder form occurs.

The potential risk of irritation and inhalation of dust to workers will be minimised by the use of safe work practices and engineering controls such as local exhaust ventilation and the use of PPE such as protective clothing, gloves and full face mask or respirators.

Systemic effects from repeated exposure are not expected due to the relatively high NOAEL of 800 mg/kg bw/day established in the 90 day study and the workplace controls that will limit exposure.. Therefore the risk to workers is not considered to be unacceptable.

6.3.2. Public health

The notified chemical has slight skin and eye irritation potential and the NOAEL from a 90-day repeated dose oral study in rats is 800 mg/kg bw/day. Exposure of the public to the notified chemical through dermal contact with articles is considered to be very low.

Consumption of drinking water is also a potential source of exposure, if the notified chemical is leached from pipes containing it. EFSA (2009) concluded that the notified chemical is safe for use in rigid PVC in contact with non-acidic and non-alcoholic aqueous food if the migration of the chemical is up to 5 mg/kg food. The specific migration data for the chemical reported in the EFSA evaluation was 75 µg/kg water, considerably lower than this limit. Based on the toxicological profile of the chemical and the expected low specific migration value determined experimentally, the risk to the public is not considered to be unacceptable.

7. ENVIRONMENTAL IMPLICATIONS

7.1. Environmental Exposure & Fate Assessment

7.1.1 Environmental Exposure

RELEASE OF CHEMICAL AT SITE

The notified chemical will not be manufactured in Australia. Release to the environment during shipping, transport and warehousing will only occur in the unlikely event of accidental spills from the import containers.

RELEASE OF CHEMICAL FROM USE

Imported chemical will be used in the production of pelletised stabiliser formulations. Potential release sources during this activity are:

- Spills
- Residue from packaging
- Dust generation
- Equipment cleaning

Most of the spilled material will be swept or vacuumed and will be re-used in the formulations. Material unable to be re-used in production will be placed in suitable containers ready for disposal by licensed waste collectors.

Generated dust is recycled back into the production by automated systems. Therefore, release to air is considered minimal. Residue from packaging is expected to be insignificant due to the nature of the product. All packaging materials are disposed as prescribed waste. Equipment cleaning is only carried out when a product change occurs. Total quantity released from these activities is expected to be less than 0.5% of the annual introduction volume (<750 kg).

RELEASE OF CHEMICAL FROM DISPOSAL

The notified chemical will be combined in the matrix of extruded PVC articles. The ultimate fate of the notified chemical is therefore linked to the disposal of used PVC articles. This is expected to be used in landfill where degradation of the polymer matrix may release the notified chemical slowly over time.

7.1.2 Environmental fate

A ready biodegradability study was provided for the notified chemical which indicated that the notified chemical is not readily biodegradable and not toxic to the inoculum microorganisms For the details of the environmental fate studies, refer to Appendix C. The notified chemical is not expected to be discharged to the aquatic environment in significant quantities based on the intended use and probable disposal pathway. The most likely pathway for release of the notified chemical is in landfill as PVC articles slowly degrade. The notified chemical may be mobile in soil-water systems, but is unlikely to bioaccumulate based on its high water solubility.

7.1.3 Predicted Environmental Concentration (PEC)

No significant concentrations of the notified chemical are expected in the aquatic environment based on the limited possibility for release of the notified chemical. The PEC for the notified chemical has therefore not been calculated.

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	96 h LC_{50} >100 mg/L	Not harmful to fish
Daphnia Toxicity	48 h EC_{50} = 31.23 mg/L	Harmful to aquatic invertebrates
Algal Toxicity	72 h EC_{50} = 0.0465 mg/L	Very toxic to algae

The ecotoxicology test results indicate that primary producers such as algae are likely to be the most sensitive organisms to the notified chemical.

7.2.1 Predicted No-Effect Concentration

No significant aquatic exposure is anticipated based on the intended use and probable disposal pathway of the notified chemical. Hence, a Predicted No Effect Concentration (PNEC) was not calculated.

7.3. Environmental risk assessment

The notified chemical will not be released in significant quantities to the aquatic environment as it will be compounded into PVC articles. The possibility of significant exposure of aquatic organisms to the notified chemical is therefore low. On this basis, the environmental risk of the notified chemical is considered to be acceptable.

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 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 implement the following engineering controls to minimise occupational exposure to the notified chemical as introduced :
  - Local exhaust ventilation where dust is present
  - Automated system for loading

- Employers should implement the following safe work practices to minimise occupational exposure during handling of the notified chemical as introduced:
  - Avoid contact with skin and eyes
  - Do not inhale dust

- 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 via licensed waste collectors.

Emergency procedures

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

Regulatory Obligations
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 concentration of the notified chemical in PVC pipes exceeds 0.3%
  - significant migration rate occurs from PVC pipes into water (e.g. exceeding EFSA limit)

or

(2) Under Section 64(2) of the Act; if
  - the function or use of the chemical has changed from stabiliser for PVC articles, or is likely to change significantly;
  - the amount of chemical being introduced has increased from 150 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.

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 276ºC

Method Inhouse measurement method.
Remarks Opti-Melt tester was used
Test Facility Sun Ace Kakoh Ltd. (2009b)

Density 779 kg/m^3 at 20ºC

Method OECD TG 109 Density of Liquids and Solids.
EC Directive 92/69/EEC A.3 Relative Density.
Remarks The pycnometer method was used.
Test Facility JAI Research Foundation (2009a)

Water Solubility 5.284 g/L at 20ºC

Method OECD TG 105 Water Solubility.
Remarks Flask Method with validated HPLC analysis.
Test Facility Sun Ace Kakoh Ltd. (2009c)

Hydrolysis as a Function of pH

Method OECD TG 111 Hydrolysis as a Function of pH

pH	*T (ºC)*	*$t_{½}$*
4	50	> 5 days
7	50	> 5 days
9	50	> 5 days

Remarks Analysis with validated HPLC method
Test Facility Sun Ace Kakoh Ltd. (2009d)

Partition Coefficient (n-octanol/water) log P_{ow} = -0.94 ± 0.01 at 20 ± 1ºC

Method OECD TG 107 Partition Coefficient (n-octanol/water): Shake Flask Method
OPPTS 830.7550 Partition Coefficient (n-octanol/water), Shake Flask Method
Remarks Analysis with validated HPLC method
Test Facility JAI Research Foundation (2009b)

Adsorption/Desorption – screening test log K_{oc} = 1.59 at 20°C

Method Estimated using regression equation relating log K_{oc} to water solubility as taken from Handbook of Chemical Property Estimation Methods (1990)
Remarks Regression Equation: Log K_{oc} = -0.55 log S + 3.64, where S = water solubility (mg/L).
Test Facility Sun Ace Kakoh Ltd. (2009e)

Dissociation Constant $pKa_1 \approx 4.6$, $pKa_2 \approx 5.2$ at 25°C

Method Estimated using the principles of Taft and Hammett Correlations
Remarks Based on the pK_as of aniline and pyridine
Test Facility Sun Ace Kakoh Ltd. (2009f)

Particle Size <100 µm

Method OECD TG 110 Particle Size Distribution/Fibre Length and Diameter Distributions.

Range (μm)	*Mass (%)*
< 100	100
< 75	76
< 45	25

Remarks Sieve method
Test Facility Sun Ace Kakoh Ltd. (2009g)

Solid Flammability Not Highly flammable

Method EC Directive 92/69/EEC A.10 Flammability (Solids).
Remarks Result based on Preliminary Screening test.
Test Facility JAI Research Foundation (2009c)

Autoignition Temperature >287°C

Method EC Directive 92/69/EEC A.16 Relative Self-Ignition Temperature for Solids.
Remarks Tested up to the melting point temperature
Test Facility JAI Research Foundation (2009d)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral

TEST SUBSTANCE: Notified chemical

METHOD: OECD TG 425 Acute Oral Toxicity: Up-and-Down Procedure.

Species/Strain	Rat /Wistar
Vehicle	Peanut oil
Remarks - Method	No significant protocol variations

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
1	1F	175	None
2	1F	550	None
3	1F	1750	None
4	3F	5000	None

LD50	>5000 mg/kg bw
Signs of Toxicity	None
Effects in Organs	None
Remarks - Results	No mortality, signs of toxicity, or adverse effects in organs were observed at the dose level of 5000 mg/kg bw.

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

TEST FACILITY: JAI Research Foundation (2008)

B.2. Acute toxicity – dermal

TEST SUBSTANCE: Notified chemical

METHOD: OECD TG 402 Acute Dermal Toxicity – Limit Test.

Species/Strain	Rat (Rattus norvegicus)/Wistar
Vehicle	Pulverised powder moistened with distilled water
Type of dressing	Semi-occlusive.
Remarks - Method	No significant protocol variations. The notified chemical was applied to approximately 10% of the body surface.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
I	5M, 5F	0 (control)	None
II	5M, 5F	2000	None

LD50	>2000 mg/kg bw
Signs of Toxicity - Local	None
Signs of Toxicity - Systemic	None
Effects in Organs	Observation of uterus distension in 2 rats (females). (1 out of 10 from control group and 1out of 10 from treatment group) was not considered to be treatment related.
Remarks - Results	No other effects or abnormalities were detected.

CONCLUSION	The notified chemical is of low toxicity via the dermal route.
TEST FACILITY	JAI Research Foundation (2009e)

B.3. Irritation - skin

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 404 Acute Dermal Irritation/Corrosion.
Species/Strain	Rabbit/New Zealand White
Number of Animals	3M
Vehicle	Pulverised powder moistened with distilled water
Observation Period	72 hours
Type of Dressing	Semi-occlusive.
Remarks - Method	Initially one rabbit was tested with a single patch for a period of 4 hours. Based on the observation at 24 hour post patch removal, the irritation response was confirmed by testing two additional rabbits simultaneously to confirm the irritation response.

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	0.33	0.00	0.00	1	<48 h	0
Oedema	0.00	0.00	0.00	0	0	0

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

Remarks - Results	Erythema was seen in 1/3 animals at 24h, but had resolved at 48h
CONCLUSION	The notified chemical is slightly irritating to the skin.
TEST FACILITY	JAI Research Foundation (2009f)

B.4. Irritation – eye

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 405 Acute Eye Irritation/Corrosion.
Species/Strain	Rabbit/New Zealand White
Number of Animals	3F
Vehicle	Distilled water
Observation Period	72 hours
Remarks - Method	One rabbit was tested initially. Based on observation at 24 hours two additional rabbits were tested simultaneously to confirm irritation response. Fluorescein testing was carried out at 24h.

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	1	1	2	48 h	0
Conjunctiva: chemosis	0.00	0.33	0.33	1	24 h	0
*Conjunctiva:discharge***	-	-	-	-	-	-
Corneal opacity	0.00	0.00	0.00	0	0	0
Iridial inflammation	0.00	0.00	0.00	0	0	0

*Calculated on the basis of the scores at 24, 48, and 72 hours for EACH animal.
**Results were not noted for conjunctival discharge

CONCLUSION The notified chemical is slightly irritating to the eye.

TEST FACILITY JAI Research Foundation (2009g)

B.5. Skin sensitisation

TEST SUBSTANCE Notified chemical

METHOD OECD TG 406 Skin Sensitisation - Guinea-pig Maximisation Test Method.

Species/Strain Guinea pig/Hartley

PRELIMINARY STUDY Maximum Non-irritating Concentration:
intradermal: 5%
Topical: 100% moistened with 80% ethanol.

MAIN STUDY

Number of Animals Test Group: 20 (10M, 10F) Control Group: 10 (5M, 5F)

INDUCTION PHASE Induction Concentration: 5%, 2.5%, 1.0% and 0.5%
Intradermal injection: 2.5%
Topical:100%

Signs of Irritation Very slight to well-defined erythema and very slight to slight oedema were observed

CHALLENGE PHASE

1st challenge Topical application: 2.5, 50, 75 and 100 mg of notified chemical moistened with acetone

Remarks-Method For the topical induction phase, the skin was treated with sodium lauryl sulfate.

RESULTS

Animal	*Challenge Concentration*	*Number of Animals Showing Skin Reactions after: 1st challenge*		*2nd challenge*
		24 h	*48 h*	
Test Group	100 mg	0/20	0/20	Not performed
Control Group	100 mg	0/10	0/10	Not performed

Remarks - Results No positive skin response was seen after challenge. The reliability and sensitivity of the system was confirmed through testing of the positive control α-Hexylcinnamaldehyde

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

TEST FACILITY JAI Research Foundation (2009h)

B.6. Repeat dose toxicity

TEST SUBSTANCE Notified chemical

METHOD OECD TG 408 Repeated Dose 90-Day Oral Toxicity Study in Rodents.

Species/Strain Rat/Wistar
Route of Administration Oral – gavage

Exposure Information Total exposure days: 90 days

Vehicle Peanut oil

Remarks - Method No significant protocol variations

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw/day*	*Mortality*
I (control)	10 per sex	0	0/10
II (low dose)	10 per sex	200	0/10
III (mid dose)	10 per sex	800	0/10
IV (high dose)	10 per sex	1600	0/10
V (control recovery)	10 per sex	0	0/10
VI (high dose recovery)	10 per sex	0	0/10

Mortality and Time to Death
No mortality occurred.

Clinical Observations
Clinical sign of mild to severe salivation was observed in the test substance treated groups during the course of study. Significant reduction in motor capacity was observed in group IV and hind leg foot splay in group III male. There were no significant effect on body weight of treated rats.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis
Evaluation of clinical chemistry and haematological parameters revealed alteration of MCV and cholesterol level in group IV in both sexes.

Effects in Organs
Microscopic examination revealed varying degrees of pathological changes in different organs belonging to various treatment groups (II, III and IV) which were compared well with the control group (I) suggesting that the test substance did not adversely affect any organ and clinical changes were at par with historical control data and considered spontaneous / incidental & physiological in nature.

Remarks – Results

CONCLUSION
The No Observed Adverse Effect Level (NOAEL) of the notified chemical in Wistar rats treated over a period of 90 days was established as 800 mg/kg bw/day in this study, based on clinical observations observed in 1 male at this dose level.

TEST FACILITY JAI Research Foundation (2009i)

B.7. Genotoxicity – bacteria

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 471 Bacterial Reverse Mutation Test. Plate incorporation procedure
Species/Strain	*S. typhimurium*: TA1535, TA1537, TA98, TA100, *E. coli*: WP2uvrA.
Metabolic Activation System	Extract of rat liver (treated with Aroclor 1254) called S9 fraction was used at 5% in test 1 and 10% in test 2. The S9 fraction was buffered and supplemented with the essential co-factors β NADP and glucose-6-phosphate to form S9 mix.
Concentration Range in Main Test	a) With metabolic activation: 0 – 2500 µg/plate. b) Without metabolic activation: 0 - 2500 µg/plate.
Vehicle	Dimethyl sulfoxide
Remarks - Method	No significant protocol variations.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	>2500		-	Negative
Test 2			-	Negative
Present				
Test 1	>2500	>2500	-	Negative
Test 2		>2500	-	Negative

Remarks - Results	No mutagenic effects were observed with or without metabolic activation. Results with positive controls demonstrated the validity of the test system.
CONCLUSION	The notified chemical was not mutagenic to bacteria under the conditions of the test.
TEST FACILITY	JAI Research Foundation (2009j)

B.8. Genotoxicity – in vitro

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 476 In vitro Mammalian Cell Gene Mutation Test.
Species/Strain	Chinese hamster ovary
Cell Type/Cell Line	Cell line CHO-K1
Metabolic Activation System	Extract of rat liver (treated with Aroclor 1254) called S9 fraction was used. The S9 fraction was buffered and supplemented with the essential co-factors β NADP, KCl and glucose-6-phosphate to form S9 mix.
Vehicle	Dimethyl sulfoxide
Remarks - Method	No significant protocol variations. Concentrations for testing were selected on the basis of a preliminary cytotoxicity test. S9 was used at 2% in Test 2 with metabolic activation, and at 1% in the remaining tests.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Expression Time*	*Selection Time*
Absent				
Test 1	31.25, 62.5*, 125*, 250*, 500*	4 h	24 h	48 h
Test 2	31.25, 62.5*, 125*, 250*, 500*	23 h	24 h	48 h
Present				
Test 1	31.25, 62.5*, 125*, 250*, 500*	4 h	24 h	48 h
Test 2	31.25, 62.5*, 125*, 250*, 500*	4 h	24 h	48 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	>500			
Test 1		>500	>500	Negative
Test 2		>500	>500	Negative
Present	>500			
Test 1		>500	>500	Negative
Test 2		>500	>500	Negative

Remarks - Results: The increase in mutation frequency with the positive controls ethyl methanesulfonate and benzo(a)pyrene (in the absence and presence of metabolic activation respectively) confirmed the validity of the test system. A very slight increase only in mutation frequency was seen at the highest concentration only (500 µg/mL) of the notified chemical.

CONCLUSION: The notified chemical was not clastogenic to CHO-K1 treated in vitro under the conditions of the test.

TEST FACILITY: JAI Research Foundation (2009k)

B.9. Genotoxicity – in vivo

TEST SUBSTANCE: Notified chemical

METHOD: OECD TG 474 Mammalian Erythrocyte Micronucleus Test.

Species/Strain: Swiss albino mice
Route of Administration: Oral – gavage

Vehicle: Corn oil

Remarks - Method: Use of 2 animals per sex per group. The dose ranges in a preliminary study were 250, 500, 1000, 2000 mg/kg bodyweight. As no mortality was observed at any dose and toxicity was limited to clinical signs in one animal at the highest dose, the main study was carried out at 2000 mg/kg/bw and animals were given this dose twice, at a 24 h interval.

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Sacrifice Time hours*
I (vehicle control)	5 per sex	0	24 h
II (limit dose)	5 per sex	2000	24 h after second dosing
III (positive control, M)	5 per sex	1	24 h

M=mitomycin C. 1mg/kg/bw

RESULTS

Doses Producing Toxicity	No mortality was observed in both control and treated groups. Two animals from the treated group showed lethargy on Day 2 after dosing. Toxicity to bone marrow, as measured by alteration to the PCE/NCE ratio, was not observed, however the clinical signs shown by two of the test animals indicate that the notified chemical was systemically available...
Genotoxic Effects	The average number of micronucleated polychromatic erythrocytes was slightly increased in the female treated group compared to the control but this small increase was not statistically significant. The number was significantly increased in the positive control group, verifying the sensitivity of the system.
CONCLUSION	The notified chemical was not clastogenic under the conditions of this in vivo mice micronucleus test.
TEST FACILITY	JAI Research Foundation (2009l)

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	River water (0.1 mL/L) from Daman Ganga River, Vapi.
Exposure Period	28
Auxiliary Solvent	None
Analytical Monitoring	BOD
Remarks - Method	No significant protocol deviations were reported.

RESULTS

Test substance		*Potassium hydrogen phthalate*	
Day	*% Degradation*	*Day*	*% Degradation*
7	2.33	7	62.98
14	2.52	14	74.89
21	2.88	21	80.43
28	3.63	28	80.85

Remarks - Results	The test substance did not inhibit degradation of the reference substance by more than 25% after 14 days, indicating that the test substance did not adversely affect the inoculum microorganisms.
CONCLUSION	The test substance is not readily biodegradable and not toxic to the inoculum microorganisms.
TEST FACILITY	JAI Research Foundation (2009m)

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	Common Carp (*Cyprinus carpio*)
Exposure Period	96 h
Auxiliary Solvent	DMSO (Dimethyl sulfoxide)
Water Hardness	204.0 – 216.0 mg $CaCO_3$/L
Analytical Monitoring	HPLC
Remarks – Method	No significant protocol deviations were reported.

RESULTS

Concentration mg/L		*Number of Fish*	*Mortality*					
Nominal	*Actual*		*3hr*	*6h*	*24h*	*48h*	*72h*	*96h*
Control		10	0	0	0	0	0	0
Vehicle control		10	0	0	0	0	0	0
0.1		10	0	0	0	0	0	0
1.0		10	0	0	0	0	0	0
10.0		10	0	0	0	0	0	0
50.0		10	0	0	0	0	0	0
100.0		10	0	0	0	0	0	0

LC50	>100 mg/L at 96 hours.
NOEC	100 mg/L at 96 hours.
Remarks – Results	Based on the results of the analytical testing (99.19% recovery of the test substance from the 100 mg/L test concentration), the results are based on the nominal concentration only.
CONCLUSION	The test substance is not harmful to fish
TEST FACILITY	JAI Research Foundation (2009n)

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
Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	DMSO (Dimethyl sulfoxide)
Water Hardness	208.0 mg $CaCO_3$/L
Analytical Monitoring	HPLC
Remarks - Method	Reference substance = Potassium dichromate. No significant protocol deviations were reported.

RESULTS

Concentration mg/L		*Number of D. magna*	*Number Immobilised*	
Nominal	*Actual*		*24 h*	*48 h*
	0	20	0	0
	10	20	0	0
	17.7	20	2	5
	31.6	20	4	9
	56.2	20	11	16
	100	20	16	20

EC50	52.86 mg/L at 24 hours 31.23 mg/L at 48 hours
LOEC	17.7 mg/L at 48 hours
NOEC	10.0 mg/L at 48 hours
Remarks – Results	All test parameters were found to be within guideline limits. The results obtained with the positive and negative controls established the suitability, validity and reliability of the test system and the methods followed.
CONCLUSION	The notified chemical is harmful to aquatic invertebrates
TEST FACILITY	JAI Research Foundation (2009o)

C.2.3. Algal growth inhibition test

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 201 Alga, Growth Inhibition Test.
Species	*Pseudokirchneriella subcapitata*
Exposure Period	72 hours
Concentration Range	Nominal: 0, 0.0040, 0.0074, 0.0138, 0.0257, 0.0479 mg/L
Auxiliary Solvent	DMSO (Dimethyl sulfoxide)
Water Hardness	Not reported.
Analytical Monitoring	HPLC
Remarks - Method	Reference substance = Potassium dichromate. No significant protocol deviations were reported.

RESULTS

Biomass		*Growth*	
E_bC_{50} *mg/L at 72 h*	*NOE_bC* *mg/L at 72 h*	*E_rC_{50}* *mg/L at 72 h*	*NOE_rC* *mg/L at 72 h*
0.0167 95% C.I. 0.01495 – 0.01865		0.0465 95% C.I. 0.03386 – 0.04879	0.0040

Remarks - Results	All test parameters were found to be within guideline limits. The results obtained with the positive and negative controls established the suitability, validity and reliability of the test system and the methods followed.
CONCLUSION	The test substance is very toxic to algae
TEST FACILITY	JAI Research Foundation (2009p)

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