File No: LTD/1979

September 2017

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

Cyclotetrasiloxane, 2,4,6,8-tetramethyl-, reaction products with 1,1'-(methylethylidene)bis[4-(2-propen-1-yloxy)benzene]

This Assessment has been compiled in accordance with the provisions of the *Industrial Chemicals (Notification and Assessment) Act 1989* (the Act) and Regulations. This legislation is an Act of the Commonwealth of Australia. The National Industrial Chemicals Notification and Assessment Scheme (NICNAS) is administered by the Department of Health, and conducts the risk assessment for public health and occupational health and safety. The assessment of environmental risk is conducted by the Department of the Environment and Energy.

This Public Report is 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	CHEMICAL NAME	HAZARDOUS CHEMICAL	INTRODUCTION VOLUME	USE
LTD/1979	Admil Adhesives Pty Ltd	Cyclotetrasiloxane, 2,4,6,8-tetramethyl-, reaction products with 1,1'-(methylethylidene)bis[4-(2-propen-1-yloxy)benzene]	ND*	≤ 0.4 tonnes per annum	Component of liquid silicone rubber

*ND = not determined

CONCLUSIONS AND REGULATORY OBLIGATIONS

Hazard classification
Based on the available information, the notified chemical is not recommended for classification according to the *Globally Harmonised System of Classification and Labelling of Chemicals* (GHS), as adopted for industrial chemicals in Australia.

Human health risk assessment
Provided that the recommended controls are being adhered to, 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 its limited aquatic exposure and assessed use pattern, the notified chemical is not expected to pose an unreasonable risk to the environment.

Recommendations

CONTROL MEASURES

Occupational Health and Safety

- No specific engineering controls, work practices or personal protective equipment are required for the safe use of the notified chemical itself. However, these should be selected on the basis of all ingredients in the formulation.

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

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

- If products and mixtures containing the notified chemical are classified as hazardous to health in accordance with the *Globally Harmonised System of Classification and Labelling of Chemicals (GHS)* as adopted for industrial chemicals in Australia, workplace practices and control procedures consistent with provisions of State and Territory hazardous substances legislation should be in operation.

Disposal

- Where reuse or recycling are not appropriate, dispose of the notified chemical in an environmentally sound manner in accordance with relevant Commonwealth, state, territory and local government legislation.

Emergency procedures

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

Regulatory Obligations

Secondary Notification
This risk assessment is based on the information available at the time of notification. The Director may call for the reassessment of the chemical under secondary notification provisions based on changes in certain circumstances. Under Section 64 of the *Industrial Chemicals (Notification and Assessment) Act (1989)* the notifier, as well as any other importer or manufacturer of the notified chemical, have post-assessment regulatory obligations to notify NICNAS when any of these circumstances change. These obligations apply even when the notified chemical is listed on the Australian Inventory of Chemical Substances (AICS).

Therefore, the Director of NICNAS must be notified in writing within 28 days by the notifier, other importer or manufacturer:

(1) Under Section 64(1) of the Act; if
  - the importation volume exceeds one tonne per annum notified chemical;

or

(2) Under Section 64(2) of the Act; if
  - the function or use of the chemical has changed from component of liquid silicone rubber , or is likely to change significantly;
  - the amount of chemical being introduced has increased, 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.

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

ASSESSMENT DETAILS

1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT
Admil Adhesives Pty Ltd (ABN: 85 092 730 562)
5 Alimar Road
GLEN WAVERLEY VIC 3150

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

EXEMPT INFORMATION (SECTION 75 OF THE ACT)
Data items and details claimed exempt from publication: structural formula, molecular weight, analytical data and use details

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

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT
None

NOTIFICATION IN OTHER COUNTRIES
Japan (2004)

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
H4T-BP
H4T-BP-RM

CAS NUMBER
203874-34-4

CHEMICAL NAME
Cyclotetrasiloxane, 2,4,6,8-tetramethyl-, reaction products with 1,1'-(methylethylidene)bis[4-(2-propen-1-yloxy)benzene]

MOLECULAR FORMULA
Unspecified

MOLECULAR WEIGHT
> 500 Da

ANALYTICAL DATA
Reference IR, GPC, LCMS, NMR spectra were provided.

3. COMPOSITION

DEGREE OF PURITY
95.9%

IMPURITIES/RESIDUAL MONOMERS

Chemical Name	Benzene, 1,1'-(1-methylethylidene)bis[4-(2-propen-1-yloxy)-		
CAS No.	3739-67-1	*Weight %*	4.1
Hazardous Properties	ECHA CLP: H315 – Causes skin irritation H319 – Causes serious eye irritation H317 – May cause an allergic skin reaction		

ADDITIVES/ADJUVANTS
None

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20 °C AND 101.3 kPa: Clear liquid

Property	Value	Data Source/Justification
Melting Point/Freezing Point	< 0 °C	Liquid at ambient temperature
Boiling Point	Not determined	Forms a gel when heated
Density	1,000 kg/m^3 at 25 °C	SDS
Vapour Pressure	Not determined	Expected to be low based on structure and molecular weight
Water Solubility	Not determined	The notified chemical is not expected to be water soluble based on the predominantly hydrophobic chemical structure
Hydrolysis as a Function of pH	Not determined	The notified chemical is not expected to significantly hydrolyse under environmental conditions (pH 4-9)
Partition Coefficient (n-octanol/water)	Not determined	The notified chemical is expected to partition from water to n-octanol on the basis of its expected low water solubility
Adsorption/Desorption	Not determined	The notified chemical is expected to sorb strongly to soil and sediment based on its predominantly hydrophobic structure
Dissociation Constant	Not determined	The notified chemical does not contain functionality that is expected to dissociate under environmental conditions
Flash Point	Not determined	Forms a gel when heated
Flammability	Not determined	Not expected to be highly flammable
Autoignition Temperature	Not determined	Not expected to autoignite
Explosive Properties	Not determined	Contains no functional groups that would imply explosive properties
Oxidising Properties	Not determined	Contains no functional groups that would imply oxidative properties

DISCUSSION OF PROPERTIES

Reactivity
The notified chemical is expected to be stable under normal conditions of use. However, flammable hydrogen gas may be emitted when the notified chemical comes into contact with acidic, basic or oxidising materials or water. The notified chemical is also noted to be reactive with alcohols, catalytic metals and metallic compounds.

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 of 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 not be manufactured in Australia. It will be imported as a component of liquid silicone rubber at ≤ 1% concentration.

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

Year	*1*	*2*	*3*	*4*	*5*
Tonnes	0.3	0.3 – 0.4	0.3 – 0.4	0.3 – 0.4	0.3 – 0.4

PORT OF ENTRY
Melbourne and Sydney

TRANSPORTATION AND PACKAGING
The liquid silicone containing the notified chemical at ≤ 1% concentration will be imported into Australia by sea in plastic bags contained within 200 kg drums.

USE
The notified chemical will be used as a component of liquid silicone rubber.

OPERATION DESCRIPTION
The liquid silicone rubber containing the notified chemical at ≤ 1% concentration will be contained in a plastic bag within a container. The plastic bag will be opened manually and then the container will be attached to the injection moulding machine. Once attached to the injection moulding machine all following steps will proceed automatically from mixing to moulding. When the container becomes empty, the container will be changed and the plastic bag will be closed for disposal. When it is necessary to change the working material for injection moulding, the new working material will be pumped into the moulding to push out the remaining liquid silicone rubber, which will be collected in the used inner plastic bag.

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)*
Reformulation workers	2.5	30 - 40

EXPOSURE DETAILS
Transport and warehouse workers
Transport and storage workers are not expected to be exposed to the notified chemical except in the unlikely event of an accident.

Reformulation
During reformulation, workers may be exposed to the notified chemical at ≤ 1% concentration via the dermal and ocular routes when opening the bag containing the notified chemical, and connecting and disconnecting the packaging to and from the injection moulding machine.

Dermal and ocular exposure of the notified chemical to workers should be mitigated through the stated use by the notifier of personal protective equipment (PPE) including coveralls, impervious gloves and safety glasses. Inhalation exposure to the notified chemical during liquid silicone rubber curing above 150 °C may also occur. The stated use of local ventilation should minimise exposure.

Workers may come into contact with the finished silicone rubber products containing the notified chemical at ≤ 0.5% concentration. Once the silicone rubber is cured the notified chemical will be reacted into an inert polymer matrix and will not be bioavailable.

6.1.2. Public Exposure
The finished silicone-based rubber containing the notified chemical at ≤ 0.5% concentration will be used in masks for medical oxygen breathing apparatus. Once the silicone rubber is cured the notified chemical will be reacted into an inert polymer matrix and will not be bioavailable. Therefore public exposure to the notified chemical is not expected.

6.2. Human Health Effects Assessment
The results from toxicological investigations conducted on the notified chemical are summarised in the following table. For full details of the studies performed on the notified chemical, refer to Appendix A.

Endpoint	*Substance*	*Result and Assessment Conclusion*
Rat, repeat dose oral toxicity – 28 days.	Notified chemical	NOAEL = 1000 mg/kg bw/day
Mutagenicity – bacterial reverse mutation	Notified chemical	non mutagenic
Genotoxicity – in vitro chromosomal aberration test	Notified chemical	non genotoxic

Toxicokinetics
Based on the relatively high molecular weight (> 500 Da) and expected low water solubility, absorption of the notified chemical across biological membranes is expected to be limited.

Irritation and sensitisation
The notified chemical does not contain structural alerts for irritation and sensitisation.

Repeated dose toxicity
In a 28-day repeated dose oral toxicity with a 14-day recovery study in rats with the notified chemical at exposure doses of 0, 40, 200 or 1000 mg/kg bw/day, the NO(A)EL was established as 1000 mg/kg bw/day based on the absence of adverse effects at all doses tested.

Mutagenicity/Genotoxicity
The notified chemical contains allyl ether groups which may present a concern for genotoxicity. The notified chemical tested negative in both a bacterial reverse mutation assay and a chromosomal aberration test using Chinese hamster lung fibroblast cells. The notified chemical is therefore not expected to present as a genotoxicant.

Health hazard classification
Based on the available information, the notified chemical is not recommended for classification according to the *Globally Harmonised System of Classification and Labelling of Chemicals (GHS),* as adopted for industrial chemicals in Australia.

6.3. Human Health Risk Characterisation

6.3.1. Occupational Health and Safety
Based on available toxicity studies, the notified chemical is of low systemic toxicity from repeated exposure and is not expected to be genotoxic. The notified chemical does not contain structural alerts for irritation and sensitisation and it is not expected to cross biological membranes. Therefore, based on the limited information available, the notified chemical is expected to be of low hazard.

Workers handling the notified chemical during the various rubber production processes may be exposed to the notified chemical at up to 1% concentration via the dermal, ocular and inhalation routes. Exposure would be reduced by the use of engineering controls for some processing steps, including local exhaust ventilation. The proposed use of PPE including coveralls, impervious gloves, and safety glasses will also reduce exposure to the notified chemical during processing. Once bound in the rubber, the notified chemical is not expected to be significantly bioavailable. Overall, the risk to the health of workers is not considered to be unreasonable.

6.3.2. Public Health
The public may come into contact with the finished silicone-based rubber products containing the notified chemical at ≤ 0.5% concentration. Once incorporated into the rubber, the notified chemical will be reacted into an inert polymer matrix and will not be available for exposure, hence the risk to the public 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 as a component of liquid silicone rubber. There is unlikely to be any significant release to the environment from transport and storage, except in the case of accidental spills and

leaks. In the event of spills, the product containing the notified chemical is expected to be collected with inert material for disposal through waste management contractors by thermal decomposition.

The reformulation process will involve transfer of the notified chemical into blending vessels, followed by blending operations that will be highly automated and expected to occur within a fully enclosed environment. Therefore, significant release of the notified chemical from this process to the environment is not expected. Wastes containing the notified chemical generated during reformulation include spilt materials and empty plastic bags, and are expected to be collected and disposed of to landfill in accordance with local government regulations.

RELEASE OF CHEMICAL FROM USE
Upon application as an additive in liquid silicone rubber, the notified chemical will react rapidly and become part of a cured inert matrix. No significant release of the notified chemical to the aquatic compartment is expected from use. Accidental spills and leaks are expected to be collected for disposal to landfill in accordance with local government regulations.

RELEASE OF CHEMICAL FROM DISPOSAL
Residues of the notified chemical in empty plastic bags are expected to be collected by waste management contractors for disposal by recycling. The notified chemical will share the fate of the articles to which it is bound. At the end of their useful lives, articles containing the notified chemical are expected to be disposed of to landfill. Therefore, no significant aquatic release of the notified chemical is expected from disposal.

7.1.2. Environmental Fate
Based on the result of the biodegradability study, the notified chemical is not considered readily biodegradable (1% in 28 days). Although the notified chemical is not readily biodegradable, it is not expected to be bioaccumulative based on the results of a bioaccumulation study (BCF $\leq$ 55). For details of the environmental fate studies, please refer to Appendix B. Based on its expected low water solubility and adsorption coefficient, the notified chemical is expected to bind strongly to soil and sediment, and is therefore not expected to be mobile.

The majority of the notified chemical will be bound within an inert matrix, and will not be mobile or bioavailable once cured. The notified chemical will share the fate of the articles to which it is adhered, and will most likely entail disposal to landfill. Therefore, in landfill and in recycling, the notified chemical is expected to eventually degrade through biotic and abiotic processes to form water and oxides of carbon and silica.

7.1.3. Predicted Environmental Concentration (PEC)
The predicted environmental concentration (PEC) has not been calculated for the notified chemical, as no significant aquatic release is expected from the proposed use pattern.

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 B.

Endpoint	*Result*	*Assessment Conclusion*
Fish Toxicity	96 h LC50 > 100 mg/L	Not harmful to fish

Based on the above ecotoxicological endpoints for the notified chemical, it is not expected to be harmful to aquatic life. Therefore, the notified chemical is not formally classified under the Globally Harmonised System of Classification and Labelling of Chemicals (GHS) (United Nations, 2009) for acute and chronic toxicities.

7.2.1. Predicted No-Effect Concentration
The predicted no-effects concentration (PNEC) has not been calculated, as the notified chemical is not expected to be harmful to aquatic life, and no significant release is expected from the proposed use pattern.

7.3. Environmental Risk Assessment
A Risk Quotient (RQ = PEC/PNEC) has not been calculated, as no significant release of the notified chemical to the environment is expected from the proposed use pattern. The notified chemical is not considered readily biodegradable and has low potential for bioaccumulation. On the basis of the assessed use pattern as an industrial additive for liquid silicone rubber and the expected limited aquatic release, the notified chemical is not expected to pose an unreasonable risk to the environment.

APPENDIX A: TOXICOLOGICAL INVESTIGATIONS

A.1. Repeat dose toxicity

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 407 Repeated Dose 28-day Oral Toxicity Study in Rodents.
Species/Strain	Rat/ Crj:CD(SD)
Route of Administration	Oral
Exposure Information	Total exposure days: 28 days Dose regimen: 7 days per week Post-exposure observation period: 14 days
Vehicle	Olive oil
Remarks - Method	No significant deviations from the OECD guideline.

RESULTS

Group	*Number and Sex of Animals*	*Dose (mg/kg bw/day)*	*Mortality*
control	12(6 F / 6 M)	0	0/12
low dose	12 (6 F / 6 M)	40	0/12
mid dose	12 (6 F / 6 M)	200	0/12
high dose	12 (6 F / 6 M)	1000	0/12
control recovery	12 (6 F / 6 M)	0	0/12
high dose recovery	12 (6 F / 6 M)	1000	0/12

Mortality and Time to Death

No deaths reported

Clinical Observations

Salivations just after administration that were transient were observed in all groups including control animals. No abnormalities were observed in any animals.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

No abnormalities were observed in haematology examinations in males in any group. In females treated at 1000 mg/kg bw/day increased segmental neutrophils was observed at termination of dosing period.

In females treated at 1000 mg/kg bw/day increased haemoglobin concentration and haematocrit value were observed at termination of recovery period.

No abnormalities were observed in blood chemical examinations in all male groups at termination of dosing period. In females decreased albumin concentration and A/G ratio was observed at 1000 mg/kg bw/day and decreased GOT was observed at 40 and 200 mg/kg bw/day at termination of dosing period. At termination of recovery period increased potassium and decreased γ–GTP were observed in males treated at 1000 mg/kg bw/day and decreased γ–GTP was observed in females treated at 1000 mg/kg bw/day.

No abnormalities were observed in urinalyses in any treatment groups.

Effects in Organs

No abnormalities were observed in organ weights in any treatment groups at termination of dosing period. Only a decrease in relative kidney weight in males treated at 1000 mg/kg bw/day at termination of recovery period was observed.

No abnormalities were observed in the gross necropsy in any treatment and recovery groups except blackish change of pupillary process of liver was observed in one female treated at 1000 mg/kg bw/day and loss of hair in one female treated at 200 mg/kg bw/day.

No abnormalities were observed in the histopathological examinations at the termination of dosing period except in one female treated at 1000 mg/kg bw/day (capsulation for blood clots on capsule of liver and solitary cyst in medulla of kidney) were observed. Solitary cyst in medulla of kidney was also noted in one of the control group.

At termination of recovery period basophilic tubules of kidney in one male treated at 1000 mg/kg bw/day was observed. Histopathological examinations for female animals at the termination of recovery period were not performed.

Remarks – Results
The study authors consider the observed changes to be either within the range of historical control data or incidental since they were sporadic and showed unclear dose-relationship.

CONCLUSION
The No Observed (Adverse) Effect Level (NO(A)EL) was established as 1000 mg/kg bw/day in this study by the study authors based on the absence of adverse effects at all doses tested.

TEST FACILITY Ceri (2003)

A.2. Genotoxicity – bacteria

TEST SUBSTANCE Notified chemical

METHOD OECD TG 471 Bacterial Reverse Mutation Test.
Pre incubation procedure

Species/Strain *S. typhimurium*: TA1535, TA1537, TA98, TA100
E. coli: WP2uvrA

Metabolic Activation System S9 fraction from phenobarbital /5,6-benzoflavone induced rat liver

Concentration Range in Main Test
a) With metabolic activation: 0-5000 µg/plate
b) Without metabolic activation: 0-5000 µg/plate

Vehicle Acetone

Remarks - Method The test substance was judged positive for mutagenic activity when clear dose-related increase in the number of the revertant colonies and two-fold or more increase in the number of the revertant colonies compared with the negative control observed with reappearance.

Positive controls:
With S9 mix:
2-Aminoanthracene (2AA) used for strains TA1535 & WP2uvrA and benzo(a)pyrene (BP) used for strains TA100, TA98 & TA1537

Without S9 mix:
9-Aminoacridine (9AA) used for strain TA1537, sodium azide (NaN3) used for strain TA1535 and 2-(2-furyl)-3-(5-nitro-2furyl)acrylamide (AF-2) used for strains TA100, TA98 and WP2uvrA

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	> 5000	-	> 5000	Negative
Test 2	-	> 5000	> 5000	Negative
Present				
Test 1	> 5000	-	≥ 5000	Negative
Test 2	-	> 5000	≥ 2500	Negative

Remarks - Results The positive controls produced satisfactory results, validating the S9 fraction and the test system.

The test substance did not increase the number of the revertant colonies in any bacterial strains both in the presence or absence of metabolic activation.

CONCLUSION	The notified chemical was not mutagenic to bacteria under the conditions of the test.
TEST FACILITY	Genetic Laboratory (2003)

A.3. Genotoxicity – in vitro

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 473 In vitro Mammalian Chromosome Aberration Test.
Species/Strain	Chinese Hamster (lung fibroblasts)
Cell Type/Cell Line	CHL/IU
Metabolic Activation System	S9 fraction from phenobarbital /5,6-benzoflavone induced rat liver
Vehicle	Acetone
Remarks - Method	The positive controls used in this test study are: Mitomycin C (MMC) without S9-Mix Cyclophosphamide, monohydrate (CPA) with S9-Mix The amount of mitotic metaphases in each treatment group was qualitatively examined.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	0, 19.5, 39.1, 78.1, 156, 313, 625, 1250*, 2500*, 5000*	6 h	24 h
Test 2	0, 19.5, 39.1, 78.1, 156, 313, 625, 1250*, 2500*, 5000*	24 h	24 h
Present			
Test 1	0, 19.5, 39.1, 78.1, 156, 313, 625, 1250*, 2500*, 5000*	6 h	24 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				
Test 1	> 5000	> 5000	≥ 39.1	negative
Test 2	≥ 5000	> 5000	≥ 39.1	negative
Present				
Test 1	≥ 2500	≥ 2500	≥ 39.1	negative

Remarks - Results	In both main tests, no statistically significant increases in the frequency of cells with structural or numerical chromosome aberrations were observed in the presence or absence of metabolic activation. All of the positive control chemicals used in the test induced statistically significant increases in the frequency of cells with aberrations indicating that the sensitivity of the assay and the efficacy of the S9-mix were validated either with or without metabolic activation.
CONCLUSION	The notified chemical was not clastogenic to CHL/IU cells treated in vitro under the conditions of the test.
TEST FACILITY	Hita Laboratory (2003)

APPENDIX B: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

B.1. Environmental Fate

B.1.1. Ready biodegradability

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 301 C Ready Biodegradability: Modified MITI Test (I).
Inoculum	Activated sludge
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	Biochemical oxygen demand (BOD) and HPLC
Remarks - Method	Conducted in accordance with the test guidelines above, and in compliance with GLP standards and principles.

RESULTS

Test substance		*Aniline*	
Day	*% Degradation*	*Day*	*% Degradation*
7	0	7	66
14	0	14	82
21	1	21	84
28	1	28	83

Remarks - Results	All validity criteria for the test were satisfied. The reference compound, aniline, reached the 60% pass level by day 7 indicating the suitability of the inoculum. The degree of degradation of the test substance after 28 days was 1%. The test substance cannot be classified as readily biodegradable according to the OECD (301) guideline.
CONCLUSION	The notified chemical is not readily biodegradable.
TEST FACILITY	Kurume (2002)

B.1.2. Bioaccumulation

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 305 Bioconcentration: Flow-through Fish Test.
Species	*Cyprinus carpio* (Common carp)
Exposure Period	Exposure: 28 days Depuration: None
Auxiliary Solvent	None
Concentration Range	Nominal: 0.1 and 1 mg/L Actual: 0.0946 and 0.879 mg/L (average)
Analytical Monitoring	Atomic absorption spectrometry
Remarks - Method	The test was conducted under continuous flow-through conditions using groundwater. The test item and dispersant (HCO-20) (50 times the amount of test item) were kneaded together. Ion-exchanged water was added to the mixture to prepare 1,000 mg/L stock solution. The test was conducted in accordance with the test guideline above, with no significant deviation in protocol reported.

RESULTS

Bioconcentration Factor	BCF = 5.9 – 55
CT50	Not determined
Lipid content in test fish	The average lipid content in the test fish was as follows: Before test: 2.68% After test: 2.58%

Remarks - Results	All validity criteria for the test were satisfied. No abnormality in behaviour or appearance were observed during the uptake test period. The test item concentration was maintained at ≥83% of the nominated concentrations and the variations were within ±20 % of the mean of the measured concentrations. The depuration phase was not reported in the test report.
CONCLUSION	The notified chemical is not considered to be bioaccumulative.
TEST FACILITY	Kurume (2003)

B.2. Ecotoxicological Investigations

B.2.1. Acute toxicity to fish

TEST SUBSTANCE	Notified chemical
METHOD	Japanese Industrial Standard (JIS K 0102-1998-71): Testing methods for industrial waste water, Acute toxicity test with fish.
Species	*Oryzias latipes* (Orange-red killifish)
Exposure Period	96 hours
Auxiliary Solvent	None
Water Hardness	78.5 mg $CaCO_3$/L
Analytical Monitoring	Atomic absorption spectrometry
Remarks – Method	This test was performed together with the bioaccumulation study above (Appendix B.1.2). The test was conducted under semi static conditions (test water was renewed every 8-16 hours). The test item and dispersant (HCO-20) (50 times the amount of test item) were kneaded together. Ion-exchanged water was added to the mixture to prepare 1,000 mg/L stock solution. The test was conducted in accordance with the test guideline above, with no significant deviation in protocol reported.
RESULTS	
LC50	>100 mg/L at 96 hours.
NOEC	Not reported
Remarks – Results	The toxicity of the dispersant was taken into account. The 96 hour LC50 of the dispersant (HCO-20) was > 50,000 mg/L. As the concentration of the dispersant during the test was 5,000 mg/L, higher concentration of the test item (>100 mg/L) was not performed. The 96 hour LC50 for fish was determined to be >100 mg/L.
CONCLUSION	The notified chemical is not harmful to fish
TEST FACILITY	Kurume (2003)

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