File No: STD/1147

April 2005

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

FULL PUBLIC REPORT

ADK STAB NA-20

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Director
Chemicals Notification and Assessment

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1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)
Marubeni Australia Limited (ABN 53 000 329 699)
Level 18
367 Collins Street
Melbourne VIC 3000

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:
Atmospheric monitoring
Dissociation constant
Flammability
Acute inhalation toxicity

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
Commercial Evaluation Permit (CEC/632, permit number 590) issued to the current notifier (2004)

NOTIFICATION IN OTHER COUNTRIES
EU Directive, 1999 (EC No. 430-650-4)
TSCA, 1998
Japan, 1995 (Registration No. 5-6458)

2. IDENTITY OF CHEMICAL

CHEMICAL NAME
Aluminium, hydroxybis[2,4,8,10-tetrakis(1,1-dimethylethyl)-6-(hydroxy-κ*O*)-12H-dibenzo[*d,g*][1,3,2]dioxaphosphocin 6-oxidato]-

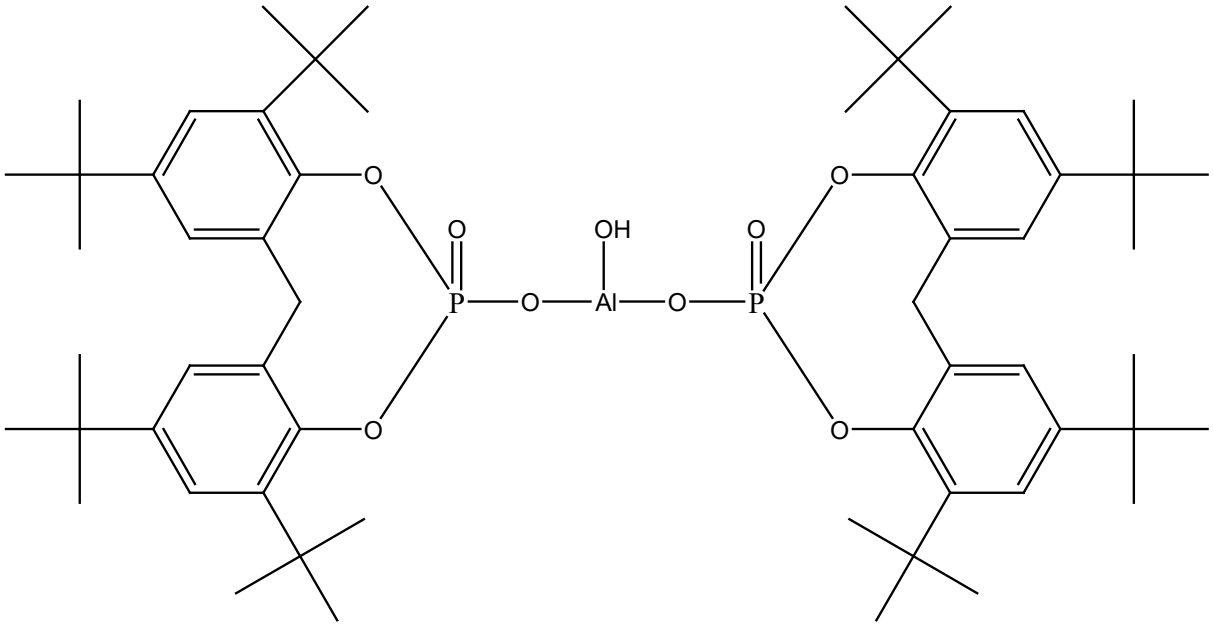
OTHER NAME(S)
Hydroxy aluminium bis(2,4,8,10-tetra-tert-butyl-6-hydroxy-12H-dibenzo[d,g][1.3.2]-dioxaphosphocin-6-oxide
T-301

MARKETING NAME(S)
ADK STAB NA-20

CAS NUMBER
151841-65-5

MOLECULAR FORMULA
$C_{58}H_{85}AlO_9P_2$

STRUCTURAL FORMULA



MOLECULAR WEIGHT
1015.24

SPECTRAL DATA

METHOD	UV/Visible, Infrared and Nuclear Magnetic Resonance spectroscopy
Remarks	Reference spectra were provided.

3. COMPOSITION

DEGREE OF PURITY
99-100% (typically 99.7%)

HAZARDOUS IMPURITIES/RESIDUAL MONOMERS
None.

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

4. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS
The notified chemical will be imported as either 25% or 60% of a powder preparation.

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

Year	*1*	*2*	*3*	*4*	*5*
Tonnes	1	3	5	10	15

USE
An additive used as a clarifying agent in polypropylene plastics.

5. PROCESS AND RELEASE INFORMATION

5.1. Distribution, transport and storage

PORT OF ENTRY
Melbourne.

IDENTITY OF MANUFACTURER/RECIPIENTS
Powder containing 60% notified chemical and granulated powder containing 18% notified chemical will be imported by Marubeni Australia Limited, and distributed to one site in Victoria and one site in NSW.

TRANSPORTATION AND PACKAGING
Powder preparations will be imported in polyethylene bags placed into separate cardboard boxes. The boxes will be transported by road to the distributor's sites.

5.2. Operation description

Imported powder preparations containing plastic powder, the notified chemical and other additives will be transported to polypropylene plants. At the plants the powder is weighed and added to an automatic mixer. The mixture is fed automatically to an extruder, preheated to 220-230ºC, which produces pelletised plastic containing up to 0.5% notified chemical. The pellets are packaged into plastic bags. Bags of pellets are manually transferred into a moulding machine that has been preheated to 220-230ºC. The pellets are melted and moulded to form finished articles, containing up to 0.5% notified chemical.

5.3. Occupational exposure

Number and Category of Workers

Category of Worker	*Number*	*Exposure Duration*	*Exposure Frequency*
Transport and storage	2-3	Not known	<80 days/year
Weighing and loading to mixer	1-2	2 hours/day	80 days/year
Weighing and bagging pellets	1-2	2 hours/day	80 days/year
Loading pellets to moulding machine	1-2	2 hours/day	80 days/year
Disposal	2-3		<1 hour/month

Exposure Details

Transport & Storage

Exposure during transport and storage will only occur in the unlikely event of a serious accident involving breach of import containers. Exposure in such cases is expected to be infrequent and acute.

Mixing

The highest likelihood of exposure to the notified chemical occurs during weighing of powder preparations and loading to the mixing machine. Exposure during these procedures will be limited by local exhaust ventilation (LEV), training personnel in appropriate procedures and personal protective equipment (PPE) including gloves, dust masks and safety glasses.

Extruding & Moulding

Exposure during extruder operations is expected to be negligible as the mixture will be fed automatically to the extruder. Exposure may occur when polymer pellets produced by the extruder are manually transferred to the moulding machine. However, exposure will be substantially limited by LEV, by the low concentration of notified chemical (0.5%) and its lack of bioavailability in the solid pelleted form, and by PPE including gloves, dust masks and safety glasses.

Exposure to the notified chemical in moulded finished articles will be negligible as the notified chemical will be trapped within the polymer. In addition, workers will wear gloves to minimise skin contact.

Disposal

Landfill workers may be involved in disposal of waste pelletised plastic of final plastic products. Exposure will be limited by the lack of bioavailability of the notified chemical in pellets and moulded

articles, and by the low frequency of possible exposure (<1 hour/month).

5.4. Release

RELEASE OF CHEMICAL AT SITE
There will be no release in Australia from manufacture, as the notified chemical will not be manufactured in Australia.

Release to the environment during shipping, transport and warehousing will only occur through accidental spills or leaks of the polyethylene bag container. This is expected to be minor due to the packaging of the material.

RELEASE OF CHEMICAL FROM USE
There will be some residual powder left in the empty import bags. This is estimated to be less than 0.1% of the annual import volume (i.e. less than 15 kg annually). Empty bags and any residuals will be disposed of by incineration or landfill.

The process equipment will not be washed between batches. In each batch the first lot of product is discarded. This discarded material, along with any other out-of-specification product or off-cuts will be collected and either disposed of or recycled, if possible. Any spilt material will be collected and placed into sealable containers ready for disposal. It is estimated that these combined releases would account for up to 1% of the import volume of the notified chemical (i.e. up to 150 kg annually)

In the end product the notified chemical is incorporated in an inert matrix and will not be released to the environment.

5.5. Disposal

All the solid wastes generated containing the notified chemical will either be disposed of to landfill or by incineration or will be recycled in the plastics (polypropylene) recycling process. In landfill the notified chemical within the plastic matrix will not be mobile and will slowly undergo abiotic and biotic degradation. Incineration will produce oxides of carbon, phosphorus and aluminium, and water.

5.6. Public exposure

Public exposure to imported powder preparations will only occur in the unlikely event of a transport accident involving breach of import packaging.

Direct exposure of the public during production of plastic articles is also considered unlikely, as any broken products will be swept up and collected for disposal.

The notified chemical will be present in plastic articles for domestic use; however public exposure is expected to be negligible as the notified chemical will be incorporated into the plastic and will not be biologically accessible.

6. PHYSICAL AND CHEMICAL PROPERTIES

Appearance at 20ºC and 101.3 kPa White powder.

Melting Point Approximately 230ºC at 101.3 kPa.

METHOD	OECD TG 102 Melting Point/Melting Range. EC Directive 92/69/EEC A.1 Melting/Freezing Temperature.
Remarks	Metal block method, followed by differential scanning calorimetry. No distinct melting stages were discernible. The notified chemical melted with indications of decomposition at approximately 230ºC.
TEST FACILITY	HLS (1999a)

Boiling Point Not determined.

Remarks Not determinable as the notified chemical decomposed on melting at approximately 230°C.

Density Relative Density = 1.05^{23}_{4}

METHOD OECD TG 109 Density of Liquids and Solids.
EC Directive 92/69/EEC A.3 Relative Density.
Remarks Pycnometer method.
TEST FACILITY HLS (1999a)

Vapour Pressure 4×10^{-7} kPa at 25°C

METHOD OECD TG 104 Vapour Pressure.
EC Directive 92/69/EEC A.4 Vapour Pressure.
Remarks Vapour pressure balance method.

Five runs were performed between 25 and 40°C in steps of 2°C, with the pressure kept at less than 1.3×10^{-6} kPa.
TEST FACILITY HLS (1999a)

Water Solubility 0.0156 g/L at 20°C

METHOD OECD TG 105 Water Solubility.
EC Directive 92/69/EEC A.6 Water Solubility.
Remarks Column Elution Method
Analytical Method: HPLC
The column elution method was chosen after preliminary visual assessment by increasing dilution steps and shaking indicated that water solubility was less than 10 mg/L. In the definitive study, the pH of test samples ranged from 7.19 to 8.49.
TEST FACILITY HLS (1999a)

Hydrolysis as a Function of pH No hydrolysis observed.

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

pH	*T (°C)*	*$t_{½}$ <hours or days>*
4	50	>1
7	50	>1
9	50	>1

Remarks After 5 days at 50°C less than 10% hydrolysis was observed, this equates to a half-life of greater than 1 year, therefore only a preliminary test was done.
TEST FACILITY HLS (1999b)

Partition Coefficient (n-octanol/water) log Pow = 3.4 at 20°C

METHOD OECD TG 107 Partition Coefficient (n-octanol/water).
EC Directive 92/69/EEC A.8 Partition Coefficient.
Remarks A preliminary estimation was based on the solubility ratio in octanol and water. For the main test, aliquots from each of the separated phases were taken for analysis by HPLC, with the n-octanol aliquots diluted prior to analysis.
TEST FACILITY HLS (1999a)

Adsorption/Desorption log K_{oc} = 3.2 (estimation)

METHOD Estimation based on empirical relationship to water solubility and partition coefficient.
Remarks The following empirical equations (Lyman et al, 1982) were used to estimate log

K_{oc}:
1) Based on water solubility (S), log K_{oc} = -0.55 log S + 3.64.
2) Based on partition coefficient (P), log K_{oc} = 0.544 log P + 1.377.
The average of the results of the two equations was then taken as the estimated log K_{oc}.

TEST FACILITY HLS (1999a)

Dissociation Constant Not determined.

Remarks This test is not technically feasible as the notified chemical does not possess ionising groups.

Particle Size

METHOD OECD TG 110 Particle Size Distribution.

Range (μm)	*Mass (%)*
>125	6.2
>105	0.0
60-105	6.4
30-60	35.8
10.4-30	35.4
0.5-10.4	16.2

Remarks Particle size distribution was initially examined by sieve analysis. As >10% by weight was found to pass a 75 micron sieve, it was further examined by image analysis.

16% by mass of the notified chemical is smaller than 10 μm.

TEST FACILITY HLS (1999a)

Flash Point Not determined.

Remarks Test not conducted because the notified chemical is a solid.

Flammability Limits Not highly flammable.

METHOD EC Directive 92/69/EEC A.10 Flammability (Solids).
EC Directive 92/69/EEC A.12 Flammability (Contact with Water).

Remarks Using Method A10, the notified chemical was determined not to be highly flammable.

Using Method A12, the notified chemical was determined to be non-flammable under the conditions of the test.

TEST FACILITY HLS (1999a)

Autoignition Temperature >230ºC

METHOD 92/69/EEC A.16 Relative Self-Ignition Temperature for Solids.

Remarks The notified chemical does not autoignite below its decomposition temperature of 230ºC.

TEST FACILITY HLS (1999a)

Explosive Properties Non-pyrophoric and not explosive.

METHOD EC Directive 92/69/EEC A.13 Pyrophoric Properties of Solids and Liquids
EC Directive 92/69/EEC A.14 Explosive Properties.

Remarks Using Method A13, the notified chemical was determined to be non-pyrophoric.

Using Method A14, the notified chemical was determined to be non-explosive

	under conditions of heat (flame), a fall hammer (shock) and friction test apparatus.
TEST FACILITY	HLS (1999a)

Reactivity

Remarks	The notified chemical is considered to be stable under normal conditions of use.

Oxidising Properties

METHOD	EC Directive 92/69/EEC A.17 Oxidizing Properties (Solids).
Remarks	The notified chemical is non-oxidising under the conditions of the test.
TEST FACILITY	HLS (1999)

Surface Tension 58.5 mN/m at 20°C

METHOD	OECD TG 115 Surface Tension of Aqueous Solutions. EC Directive 92/69/EEC A.5 Surface Tension.
Remarks	The harmonised ring method was used. Test solution: 90% saturated solution (14 µg/mL) The notified chemical is marginally surface active.
TEST FACILITY	HLS (1999a)

7. TOXICOLOGICAL INVESTIGATIONS

Endpoint	*Results and Conclusion*
Rat, acute oral	LD50>5000 mg/kg bw low toxicity
Rat, acute dermal	LD50>2000 mg/kg bw low toxicity
Rabbit, skin irritation	slightly irritating
Rabbit, eye irritation	slightly irritating
Guinea pig, skin sensitisation – adjuvant test/non-adjuvant test.	no evidence of sensitisation
Rat, repeat dose oral toxicity – 28 days.	NOEL 80 mg/kg bw/day
Rat, repeat dose oral toxicity – 90 days.	NOEL 50 mg/kg bw/day
Genotoxicity – bacterial reverse mutation	non mutagenic
Genotoxicity – in vitro Chinese hamster lung fibroblasts	non genotoxic
Genotoxicity – in vitro mouse lymphoma cells	non mutagenic

7.1. Acute toxicity – oral

TEST SUBSTANCE — Notified chemical

METHOD — TSCA, Health Effects Test Guidelines, US EPA Office of Pesticide and Toxic Substances, Section 798.1175.

Species/Strain — Rat/CD Sprague-Dawley (Crl:CD BR)

Vehicle — Corn oil

Remarks - Method — None.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
1	5 male	5000	0/5
2	5 female	5000	1/5

LD50 — >5000 mg/kg bw

Signs of Toxicity — One female rat died on day 6. Changes seen at necropsy of the female found dead on day 6 included emaciation, and discolouration of the skin, fur and extremities.

One male rat showed substantial weight loss at day 7 but gained weight between days 7 and 14.

Clinical signs seen in all animals on day 1 included yellow ano-genital stains and watery stools. Yellow staining continued in most animals until day 6, and in some animals until the end of the study. Further signs observed were decreased food consumption, decreased faecal volume, no stools, emaciation and brown stains on snout and forepaws.

Effects in Organs — No abnormalities were observed upon macroscopic examination at the end of the study.

Remarks - Results — Although the test method does not entirely comply with OECD or EC Test Guidelines (not all clinical/functional observations recommended in the OECD or EC guidelines were made), the information it provides is sufficient for classification purposes. Therefore, in the interest of animal welfare, an additional study is not justifiable.

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

TEST FACILITY — Bio/dynamics (1992)

7.2. Acute toxicity – dermal

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 402 Acute Dermal Toxicity. EC Directive 92/69/EEC B.3 Acute Toxicity (Dermal).
Species/Strain	Rat/CD Sprague-Dawley
Vehicle	Corn oil
Type of dressing	Semi-occlusive.
Remarks - Method	None.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
1	5 male	2000	0/5
2	5 female	2000	0/5

LD50	>2000 mg/kg bw
Signs of Toxicity - Local	Dermal reactions were evident in 6 animals following removal of dressings. These reactions comprised slight, transient dermal irritation, and were accompanied in some animals by localised reactions characterised by desquamation of the stratum corneum, spots and/or scabbing, and mechanical damage due to reaction between sticky residue of test material with bandage adhesive.
Signs of Toxicity - Systemic	There were no deaths and no evidence of a systemic response to treatment in any animal.
Effects in Organs	No macroscopic abnormalities were observed at the end of the study.
Remarks - Results	None.
CONCLUSION	The notified chemical is of low toxicity via the dermal route.
TEST FACILITY	HLS (1998a)

7.3. Irritation – skin

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 404 Acute Dermal Irritation/Corrosion. EC Directive 92/69/EEC B.4 Acute Toxicity (Skin Irritation).
Species/Strain	Rabbit/New Zealand White
Number of Animals	3
Vehicle	None.
Observation Period	72 hours.
Type of Dressing	Semi-occlusive.
Remarks - Method	None.

RESULTS

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

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

Remarks - Results	Transient, slight erythema was observed in one rabbit, resolving completely by day 2.
CONCLUSION	The notified chemical is slightly irritating.
TEST FACILITY	HLS (1998b) Huntingdon, UK

7.4. Irritation – eye

TEST SUBSTANCE	Notified chemical.
METHOD	OECD TG 405 Acute Eye Irritation/Corrosion. EC Directive 92/69/EEC B.5 Acute Toxicity (Eye Irritation).
Species/Strain	Rabbit/New Zealand White
Number of Animals	3
Observation Period	3 days
Remarks - Method	None.

RESULTS

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3			
Conjunctiva: redness	0	0.33	0.33	2	2 days	0
Conjunctiva: chemosis	0	0	0	1	1 day	0
Conjunctiva: discharge	0	0	0	0	-	0
Corneal opacity	0	0	0	0	-	0
Iridial inflammation	0	0	0	0	-	0

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

Remarks - Results	Transient hyperaemia of the blood vessels to a diffuse, crimson colouration with or without slight swelling was observed in all 3 animals. These reactions resolved within 1 or 2 days after instillation.
CONCLUSION	The notified chemical is slightly irritating to the eye.
TEST FACILITY	HLS (1998c) Huntingdon, UK

7.5. Skin sensitisation

TEST SUBSTANCE	Notified chemical.
METHOD	OECD TG 406 Skin Sensitisation – Magnusson and Kligman Method. EC Directive 96/54/EC B.6 Skin Sensitisation – Magnusson and Kligman Method.
Species/Strain	Guinea pig/Dunkin-Hartley
PRELIMINARY STUDY	Maximum Non-irritating Concentration: intradermal: Erythema observed at all concentrations tested. topical: 30% (w/v)
MAIN STUDY	
Number of Animals	Test Group: 10 Control Group: 5
INDUCTION PHASE	Induction Concentration: intradermal: 0.1% (w/v) topical: 70% (w/v)

Signs of Irritation: After intradermal injections, slight irritation was observed in animals that received 0.1% notified chemical, and in vehicle control animals.

After topical application, slight to well-defined erythema was observed in animals that received 70% notified chemical, and slight erythema was observed in one vehicle control animal.

CHALLENGE PHASE

1st challenge: topical: 30% and 15% (w/v)

Remarks - Method: None.

RESULTS

Animal	*Challenge Concentration*	*Number of Animals Showing Skin Reactions after:*	
		24 h	*48 h*
Test Group	30%	0	0
	15%	0	0
Control Group	30%	0	0
	15%	0	0

Remarks - Results

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

TEST FACILITY: HLS (1998d) Huntingdon, UK

7.6. Repeat dose toxicity

7.6.1. 28 day study

TEST SUBSTANCE: Notified chemical.

METHOD: OECD TG 407 Repeated Dose 28-day Oral Toxicity Study in Rodents.

Species/Strain: Rat/CD Sprague-Dawley

Route of Administration: Oral – gavage

Exposure Information: Total exposure days: 28 days
Dose regimen: 7 days per week
Post-exposure observation period: 14 days

Vehicle: Corn oil.

Remarks - Method: Post-exposure only 4 animals/sex in Groups 1 and 4 were observed for a further 14 days (recovery phase).

The dose volume was doubled (to 10 ml/kg) to ameliorate the high viscosity at the highest concentration that was considered to be the cause of early deaths attributed to dosing accidents in Group 4.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw/day*	*Mortality*
I (control)	10/sex	0	0/20
II (low dose)	10/sex	80	0/20
III (mid dose)	10/sex	400	0/20
IV (high dose)	10/sex	2000	2/20
V (control recovery)	5/sex	0	0/10
VI (high dose recovery)	5/sex	2000	2/10

Mortality and Time to Death

Two high dose females died early in the study (Day 2 and day 7); death was recorded as "accidental-traumatic" and considered to be the result of dosing accidents. These two females were replaced; one of the

replacement animals was found dead on day 8. Two high dose males were also found dead, on day 11 and day 12. After histopathological examination, the latter 3 deaths were also considered to be the result of dosing accidents.

Clinical Observations

One high dose male showed yellow ano-genital staining, red snout staining, chromodacryorrhea, laboured breathing, unformed stool, and was subsequently found dead. These symptoms were considered consistent with a dosing accident.

Oily ano-genital stains were observed on 4 females and 3 males in the high dose group. This was considered to be due to the high concentration of test material in the vehicle.

Mid- and high-dose groups showed reduced body weight and body weight gains. This was statistically significant for mid- and high-dose males in weeks 3-4 (4-8% body weight reduction) and weeks 2-4 (7-10% body weight reduction), respectively. This effect was shown to be reversible in the high dose group during the 2-week recovery period.

Notably, these body weight findings were accompanied by statistically significant increases in food consumption for both female and male mid- and high-dose animals.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

Haematology

Statistically significant rises (compared to controls) in prothrombin time and activated partial thromboplastin time were observed in mid- and high-dose males. High-dose females showed a statistically significant rise in prothrombin time. High-dose females also showed statistically significant rises in prothrombin time and activated partial thromboplastin time compared to controls following the recovery phase. However, this was not considered biologically significant, as the ranges of values were comparable to historical ranges for control animals.

Statistically significant rises in haemoglobin concentration, haematocrit and erythrocyte count were also observed for high dose groups. However these were not considered to be related to administration of test material.

Clinical Chemistry

High dose animals showed higher levels of aspartate and alanine aminotransferase and alkaline phosphatase. This was statistically significant for alanine aminotransferase and alkaline phosphatase for females; and for all three parameters in males. These effects were observed to be reversible during the recovery phase.

Urinalysis

All data for treatment groups was comparable to control data or within the normal range of variability for this species and strain.

Effects in Organs

Macroscopic

High and low dose (but not mid dose) females showed a slight increase in liver weight. In the absence of a dose response this was not considered treatment related. There was a statistically significant rise in relative liver weight for high dose females.

High dose males showed statistically significant increases in relative brain, adrenal and testes/epididymides weights; however absolute weights were not affected, so this was considered a secondary effect of the significant drop in body weights noted above. Likewise, statistically significant decreases in kidney and liver weights in mid- and high-dose males (including the high-dose recovery group) were considered to be associated with significantly lower body weights in these groups.

Histopathology

Female and male high-dose animals showed a higher incidence of hyperchromatic single hepatocytes.

A single small focus of coagulative necrosis was observed in 3 high-dose females and 3 high-dose males, in 3 mid-dose males and in 2 low-dose males. This was considered an incidental finding, as this observation is typically spontaneous in origin, and there was no evidence of a dose response.

Remarks – Results
The number of dosing accidents causing death in the high dose groups was considered to be due to the high viscosity of the highest dose of test material. No further deaths were recorded after the dose volume was doubled in week 3.

CONCLUSION
The No Observed (Adverse) Effect Level (NO(A)EL) was established as 80 mg/kg bw/day in this study, based on significantly lower body weights and body weight gains, and significantly higher prothrombin time and activated partial thromboplastin time, in male rats treated with 400 mg/kg bw/day.

TEST FACILITY Pharmaco LSR (1994a)

7.6. Repeat dose toxicity

7.6.2. 90 day study

TEST SUBSTANCE Notified chemical.

METHOD OECD TG 408 Repeated Dose 90-day Oral Toxicity Study in Rodents.
EC Directive 87/302/EEC, Method B.26

Species/Strain	Rat/CD Sprague-Dawley
Route of Administration	Oral – gavage
Exposure Information	Total exposure days: 90 days Dose regimen: 7 days per week Post-exposure observation period: None
Vehicle	Corn oil.
Remarks - Method	None.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw/day*	*Mortality*
I (control)	20/sex	0	
II (low dose)	20/sex	50	1 male
III (mid dose)	20/sex	150	2 males
IV (high dose)	20/sex	450	2 females and 2 males

Mortality and Time to Death
In the high-dose group, 2 females and 2 males were found dead, one in week 2 and the remainder in weeks 11 and 12. In the mid-dose group, 2 males were found dead, in weeks 7 and 10. In the low-dose group, one male was found dead in week 9. In the control group, one female died of accidental trauma on day 49.

Abnormal antemortem signs were seen only in one female and one male in the high-dose group. Observations included ano-genital staining and/or red stains on the ventral surface.

Death was attributed to kidney infection in one high-dose male, gavage error in one low-, one mid- and two high-dose animals (based on microscopic findings in the lung), and the cause of death was not evident in one high-dose female and one mid-dose male.

Clinical Observations
Yellow ano-genital staining and/or red stains on the ventral surface were observed in two of the high-dose animals that died and in one other high-dose female. Excessive salivation was observed in mid- and high-dose males in week 1, and in one high-dose male on day 79.

Dose-dependent drops in body weight and body weight gains were observed in treated males. In the high-dose group there were statistically significant drops in body weight gain in weeks 3 to 13, and in body weight in weeks 6 to 13. At study termination, body weight in the high-dose male group was 15% lower than controls. In the mid-dose group there were statistically significant drops in body weight gain in weeks 6 and 8 to 13, and in body weight in weeks 9 to 13. At study termination, body weight in the mid-dose male group was 9% lower than controls. This was, notably, in conjunction with statistically significant rises in food consumption among

mid- and high-dose males.

Water consumption was also statistically significantly higher for all male treatment groups, most notably in the mid- and high-dose groups, and for high-dose females. A statistically significant increase in water consumption in the low-dose female group was attributed to normal variation, as this group had demonstrated slightly elevated water consumption in the pre-test period.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

Haematology

At week 7 all male treatment groups and high-dose females had statistically significantly elevated haemoglobin, haematocrit, red blood cell count and/or platelet count compared to controls. At study termination, high-dose males still had significantly higher red blood cell counts, while high-dose females had significantly higher neutrophil counts. These changes were considered transient and within the normal range for these parameters, and therefore not indicative of a toxic treatment effect.

Clinical Chemistry

At week 7 high-dose males showed statistically significant rises in aspartate aminotransferase and alanine aminotransferase activity. These changes were reversed by the end of the study.

At the end of the study, all treated groups had statistically significantly lower concentrations of total protein, albumin and/or globulin compared to controls. However, the values were within normal ranges for these parameters and were therefore not considered toxicologically significant.

Further statistically significant changes at the end of the study were observed as follows. High-dose females and males had high alkaline phosphatase activity. Cholesterol levels were lowered in mid- and high-dose females and high-dose males. High-dose males also showed lowered glucose and elevated blood urea nitrogen levels.

Urinalysis

Urinalysis parameters showed normal variability, with no consistent differences between treated and control groups.

Effects in Organs

Macroscopic

Mid- and high-dose males had statistically significant changes in absolute and relative organ weights consistent with the significantly lower body weights in these groups noted above. One low-dose male found to have oedema and thoracic adhesions showed very high relative lung weight; this was not considered to be a consistent finding of toxicological significance. High-dose females had significantly higher relative liver and lung weights.

Histopathology

Microscopic findings occurred sporadically or with comparable incidence and severity in control and high-dose groups.

Remarks – Results

None.

CONCLUSION

The No Observed (Adverse) Effect Level (NO(A)EL) was established as 50 mg/kg bw/day in this study, based on significantly lower body weight and body weight gains in male rats treated with 150 mg/kg bw/day.

TEST FACILITY Pharmaco LSR (1994b)

7.7. Genotoxicity – bacteria

TEST SUBSTANCE Notified chemical.

METHOD OECD TG 471 Bacterial Reverse Mutation Test.
EC Directive 2000/32/EC B.13/14 Mutagenicity – Reverse Mutation Test using Bacteria.

	Plate incorporation procedure
Species/Strain	*S. typhimurium*: TA1535, TA1537, TA98, TA100, *E. coli*: WP2uvrA (pKM101)
Metabolic Activation System	Aroclor 1254-induced rat liver S9 fraction.
Concentration Range in Main Test	a) With metabolic activation: 5-5000 µg/plate b) Without metabolic activation: 5-5000 µg/plate
Vehicle	Dimethyl sulfoxide
Remarks - Method	None.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	No toxicity observed	No toxicity observed	None observed	None
Test 2	No toxicity observed	No toxicity observed	None observed	None
Present				
Test 1	No toxicity observed	No toxicity observed	None observed	None
Test 2	No toxicity observed	No toxicity observed	None observed	None

Remarks - Results	Concurrent positive controls demonstrated the sensitivity of the assay and the metabolising activity of the liver preparations. Negative controls were within historical limits.

CONCLUSION — The notified chemical was not mutagenic to bacteria under the conditions of the test.

TEST FACILITY — HLS (1999c)

7.8. Genotoxicity – in vitro

7.8.1. Chromosomal Aberration Test

TEST SUBSTANCE — Notified chemical.

METHOD — EC Directive 2000/32/EC B.10 Mutagenicity - In vitro Mammalian Chromosome Aberration Test.

Cell Type/Cell Line	Chinese Hamster lung fibroblasts
Metabolic Activation System	Aroclor 1254-induced rat liver S9 fraction.
Vehicle	0.5% methylcellulose
Remarks - Method	

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	75, 150, 300	24	24
Test 2	50, 100, 200	48	48
Present			
Test 1	375, 750, 1500	6	24
Test 2	375, 750, 1500	6	48

All concentrations were 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		50	Precipitation was	None

Test 2	50	observed at all concentrations tested	None
Present			
Test 1	50	Precipitation was	None
Test 2	50	observed at all concentrations tested	None

Remarks - Results: Positive control plates demonstrated the sensitivity of the assay. Negative controls were within historical limits.

CONCLUSION: The notified chemical was not clastogenic to Chinese hamster lung fibroblasts treated in vitro under the conditions of the test.

TEST FACILITY: Hita (1993)

7.8. Genotoxicity – in vitro

7.8.2. Mammalian Cell Mutation Assay

TEST SUBSTANCE: Notified chemical

METHOD: OECD TG 476 In vitro Mammalian Cell Gene Mutation Test.
EC Directive 2000/32/EC B.17 Mutagenicity - In vitro Mammalian Cell Gene Mutation Test.

Cell Type/Cell Line: Mouse lymphoma cells/L5178Y
Metabolic Activation System: Aroclor 1254-induced rat liver S9 fraction.
Vehicle: Suspended in culture medium.
Remarks - Method: Methyl methanesulfonate (MMS) was used as a positive control in the absence of S9 mix. 20-Methylcholanthrene was used as a positive control in the presence of S9 mix.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Expression Time*
Absent			
Test 1	0, 10, 25, 50, 100, 125, 150*, 200*, 300*, 400*	3 hours	48 hours
Test 2	0, 5, 10, 25*, 40*, 50*, 60*, 80*, 100, 125	24 hours	48 hours
Present			
Test 1	0, 10, 25, 50, 100, 125*, 150*, 200*, 300*, 400	3 hours	48 hours
Test 2	0, 10, 25, 50, 100*, 125*, 150*, 200*, 300	3 hours	48 hours

*Cultures selected for analysis of cloning efficiency and induced mutation.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/mL) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	500	200	400	200*
Test 2	125	25	None observed	None observed
Present				
Test 1	125	10	400	None observed
Test 2		200	None observed	None observed

Remarks - Results: *The statistically significant rise in mutant frequency observed at 200 µg/mL in the absence of S9 mix (Test 1) was not reproduced at higher concentrations in Test 1, or in Test 2. Therefore it was not considered to be of biological significance.

Highly significant rises in mutant frequency were observed after treatment with MMS or 20-Methylcholanthrene (positive controls). Negative controls were within historical limits.

CONCLUSION	The notified chemical was not mutagenic to mouse lymphoma cells treated in vitro under the conditions of the test.
TEST FACILITY	HLS (1999d)

8. ENVIRONMENT

8.1. Environmental fate

8.1.1. Ready biodegradability

TEST SUBSTANCE: Notified chemical

METHOD: Method for testing the Biodegradability of Chemical Substances by Micro-organisms in the Testing Methods for New Chemical Substances, July 1974, Japan).

Inoculum: Activated sludge (mixture prepared from samples from 10 locations across Japan).

Exposure Period: 28 days

Auxiliary Solvent: None

Analytical Monitoring: BOD – closed system oxygen consumption measuring apparatus with soda lime as CO_2 absorbent.
Test substance concentration - HPLC

Remarks - Method: The method is essentially the same as OECD TG 301 C Ready Biodegradability: Modified MITI Test (I)

Reference Substance – aniline
Treatments:

- Vessel 1: 29.5 µL of aniline + 300 mL of basal culture medium + inoculum.
- Vessel 2: 30 mg of test substance + 300 mL of water.
- Vessel 3: 300 mL of basal culture medium + inoculum.
- Vessels 4, 5 & 6: 30 mg of test substance + 300 mL of basal culture medium + inoculum.

Concentration of suspended solids was 30 mg/L.

Solutions in vessels 2, 4, 5 and 6 all contained visible undissolved test substance.

The recovery rates below were used as correction factors for the determination of the test substance on the analytical samples.
Recovery HPLC rate for water + test substance – 95.6% average
Recovery HPLC rate for sludge + test substance – 96.6% average

RESULTS

Test substance		*Aniline*	
Day	*% Degradation based on BOD*	*Day*	*% Degradation based on BOD*
	Average of vessels 4, 5 and 6		*Vessel 1*
7	0	7	56
14	0	14	66
21	0	21	67
28	0	28	68

Remarks - Results: Since the degradation of the reference substance exceeded 60% by day 10, the study conditions were validated.
Based on BOD, there was no degradation of the test substance over the 28 days. The HPLC analysis also showed 0% degradation over the 28 days.

CONCLUSION: Under the study conditions, the notified chemical was not readily biodegradable.

TEST FACILITY: Kurume Research Laboratories (1996a)

8.1.2. Bioaccumulation

TEST SUBSTANCE	Notified chemical
METHOD	Method for testing the Degree of Accumulation of Chemical Substances by Micro-organisms in the Testing Methods for New Chemical Substances, July 1974, Japan
Species	Carp (*Cyprinus carpio*)
Exposure Period	Exposure: 56 days (8 weeks) Depuration: Not done
Auxiliary Solvent	None
Concentration Range	Nominal: Level 1 - 1 mg/L Level 2 - 0.1 mg/L Actual: more than 90% of nominal Level 1 ranged 0.942 – 0.971 mg/L Level 2 ranged 0.0953 – 0.0968 mg/L
Analytical Monitoring	HPLC
Remarks - Method	This method is essentially the same as OECD TG 305 Bioconcentration: Flow-through Fish Test. Based on a preliminary acute toxicity test with orange-red killifish (*Oryzias latipes*) which gave a 48-hour $LC_{50} \geq 500$ mg/L, the bioaccumulation test concentrations of 1 and 0.1 mg/L were chosen. Conditions for bioaccumulation study: 11 fish were placed in each concentration. Water temperature 25±2°C Dissolved oxygen concentration: Level 1, 6.9 – 7.4 mg/L Level 2, 7.5 – 7.7 mg/L Control, 7.4 – 7.9 mg/L. Fish were analysed every second week. Water was analysed twice a week The recovery rates below were used as correction factors for the determination of the test substance on the analytical samples. Recovery HPLC rate for water + test substance – 90.9% average Recovery HPLC rate for fish homogenate + test substance – 70.6% average.
RESULTS	
Bioconcentration Factor	Level 1, BCF ≤ 1.9 Level 2, BCF ≤ 20.
Remarks - Results	No abnormal behaviour was observed during the study.
CONCLUSION	Under the conditions of the study, the notified chemical did not bioaccumulate.
TEST FACILITY	Kurume Research Laboratories (1996b)

8.2. Ecotoxicological investigations

8.2.1. Acute toxicity to fish

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 203 Fish, Acute Toxicity Test – Semi-static conditions. EC Directive 92/69/EEC C.1 Acute Toxicity for Fish - Semi-static conditions.
Species	Rainbow trout (*Oncorhynchus mykiss*)
Exposure Period	96 hours
Auxiliary Solvent	Acetone

Water Hardness	192 mg $CaCO_3$/L
Analytical Monitoring	HPLC
Remarks – Method	To ensure that the fish were exposed to the maximum attainable concentration of the test substance, the three highest concentrations selected intentionally exceeded the stated water solubility of the test substance. Test concentrations were prepared by the combining of measured amounts of test substance, acetone (1.1 mL/L) and diluent water (1.5L) in a volumetric flask, then treated by ultrasound for 20 minutes, stirred for 18 hours and subsequently left to stand for 3 hours prior to the addition of fish. The test medium was hazy with visible solid material at all concentrations but with the amount increasing with concentration. The measured filtered concentrations were between 10 and 89% of the nominal concentrations and were maintained at between 95 and 122% of the initial concentrations. Initial static loading – 0.52 g body weight/L Daily medium renewal was undertaken. Temperature was maintained at 15±2°C, with a photoperiod of 16 hours light and 8 hours dark and supplementary aeration was provided. Dissolved oxygen and pH remained within acceptable limits.

RESULTS

Concentration mg/L			*Number of Fish*	*Mortality*				
Nominal	*Actual*			*4 h*	*24 h*	*48 h*	*72 h*	*96 h*
	unfiltered	*filtered*						
0	0	0	7	0	0	0	0	0
4.27	4.17	3.81	7	0	0	0	0	0
9.39	9.02	7.85	7	0	0	0	0	0
20.7	13.3	9.58	7	0	0	0	1	1
45.5	16.4	10.1	7	0	0	0	0	0
100	28.1	9.67	7	0	0	0	1	2

LC50	> 10.1 mg/L actual filtered concentration at 96 hours.
NOEC (or LOEC)	<3.81 mg/L actual filtered concentration at 96 hours.
Remarks – Results	Abnormal behaviour (including hyperventilation erratic behaviour, change in pigmentation, lethargy, aggression and loss of orientation) was observed at all concentrations after 15 minutes exposure to the test medium. However, in the lowest concentration (3.81 mg/L) only 1 of the 7 fishes showed any abnormality and this was at 15 min, 2 hours and 48 hours only. In the other concentrations abnormal behaviour was observed in 2 or more fish at all observation times (15 min, 2, 4, 24, 48, 72 and 96 hours).
CONCLUSION	Under the study conditions the notified chemical is harmful to fish.
TEST FACILITY	HLS (1998e)

8.2.2. Acute/chronic toxicity to aquatic invertebrates

8.2.2.1. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 202 Daphnia sp. Acute Immobilisation Test and Reproduction Test – Static conditions.

	EC Directive 92/69/EEC C.2 Acute Toxicity for Daphnia - Static conditions.
Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	Acetone
Water Hardness	222 mg $CaCO_3$/L
Analytical Monitoring	HPLC
Remarks - Method	To ensure that the Daphnia were exposed to the maximum attainable concentration of the test substance the three highest concentrations selected intentionally exceeded the stated water solubility of the test substance. Test concentrations were prepared by the combining of measured amounts of test substance, acetone (0.2 mL) and diluent medium (Elandt M4) in a volumetric flask (2 L), then treated by ultrasound for 20 minutes, stirred for 16 hours, left to stand for 4 hours, then the mid-portion of the solution was taken for use in the study. Initially the test medium was clear except for the highest concentration, which was hazy with visible solid material, but with the amount increasing with concentration. After 24 hours the three highest concentrations had solid material on the bottom of the tanks and 100 mg/L also had material on the surface of the water. The measured filtered concentrations were between 16 and 78% of the nominal concentrations and were maintained at between 78 and 110% of the initial concentrations. No medium renewal was undertaken throughout the study. Temperature was maintained in the range 19.9 to 22.3°C, along with a photoperiod of 16 hours light and 8 hours dark and there was no supplementary aeration provided. Dissolved oxygen and pH remained within acceptable limits.

RESULTS

Concentration mg/L			*Number of D. magna*	*Number Immobilised*	
Nominal	*Actual*			*24 h*	*48 h*
	unfiltered	Filtered			
0	0	0	20	0	0
0.882	0.632	0.594	20	0	0
1.94	1.53	1.34	20	2	2
4.27	3.37	3.39	20	3	4
9.39	6.93	7.01	20	3	5
20.7	12.6	11.7	20	5	17
45.5	17.5	13.9	20	3	16
100	22.5	16.3	20	8	19

LC50	>16.3 mg/L actual filtered concentration at 24 hours 8.62 mg/L actual filtered concentration at 48 hours (95% CL 7.01 - 11.7 mg/L)
NOEC (or LOEC)	0.594 mg/L actual filtered concentration at 48 hours
Remarks - Results	Due to the presence of solid material, which increased with increasing concentration, the effects may have been a physical. Analysis was done by probit.

CONCLUSION — Under the study conditions the notified chemical is toxic to aquatic invertbrates.

TEST FACILITY — HLS (1998f)

8.2.2.2. Chronic toxicity to aquatic invertebrates

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 211 Daphnia magna. Reproduction Test – Semi-static
Species	*Daphnia magna*
Exposure Period	21 days
Auxiliary Solvent	Acetone
Water Hardness	234 to 260 mg $CaCO_3$/L
Analytical Monitoring	HPLC
Remarks - Method	A preliminary study indicated that the definitive study should use concentrations between 1 and 10 mg/L. To ensure that the Daphnia were exposed to the maximum attainable concentration of the test substance the three highest concentrations selected intentionally exceeded the stated water solubility of the test substance. Test substance stock solution of 100 mg/mL was prepared using acetone. Then the desired test concentrations were prepared by the combining of measured amounts of test stock solution, acetone and Elandt M4, which were treated by ultrasound for 20 minutes, then stirred for 16 hours and subsequently left to stand for 4 hours, then the mid-portion of the solution was taken for use in the study. Test solutions appeared clear with no precipitated suspended solid material. The measured filtered concentrations were between 82 and 96.4% of the nominal concentrations in fresh solutions and 74.4 and 94% in expired solutions, thus giving an average actual concentration percentage range of 81 to 90% of nominal concentration. Ten (10) replicates were done for each concentration and 20 for the blank control and the solvent control. Medium was renewed on days 2, 4, 7, 9, 11, 14, 16, and 18 and the Daphnia were fed once daily. Dissolved oxygen and pH remained within acceptable limits.

RESULTS

Concentration mg/L		*Number of D. magna*	*Number of Surviving Adults*	
Nominal	*Actual*		*14 d*	*21 d*
0 (blank control)	0	20	19	16
0 (solvent control)	0	20	20	20
0.63	0.55	10	10	9
1.3	1.1	10	10	10
2.5	2.2	10	10	9
5	4.5	10	2	2
10	8.6	10	1	0

Parental survival EC50	3.5 mg/L actual filtered concentration at 21 days
Parental survival NOEC	2.2 mg/L actual filtered concentration at 21 days
Parental reproduction EC50	>4.5 mg/L actual filtered concentration at 21 days
Parental reproduction NOEC	4.5 mg/L actual filtered concentration at 21 days
Parental Growth EC50	>4.5 mg/L actual filtered concentration at 21 days
Parental growth NOEC	2.2 mg/L actual filtered concentration at 21 days
Remarks - Results	All study validity criteria were met. While there was a clear impact on the survival of adult Daphnia, parental growth and timing of the release of the first brood, there did not appear to be any reproduction inhibition in the surviving adults.
CONCLUSION	Under the study conditions the notified chemical is toxic to aquatic

invertebrates with long lasting effects.

TEST FACILITY HLS (2004)

8.2.3. Algal growth inhibition test

TEST SUBSTANCE Notified chemical

METHOD OECD TG 201 Alga, Growth Inhibition Test – Static under non-axenic conditions.
EC Directive 92/69/EEC C.3 Algal Inhibition Test.

Species	*Selenastrum capricornutum*
Exposure Period	72 hours
Concentration Range	Nominal: 1.94, 4.27, 9.39, 20.7, 45.5 and 100 mg/L Actual: 1.21, 2.55, 5.23, 11.5, 18.9 and 24.1 mg/L (filtered).
Auxiliary Solvent	Acetone
Water Hardness	Not Specified
Analytical Monitoring	HPLC
Remarks - Method	To ensure that algae were exposed to the maximum attainable concentration of the test substance the three highest concentrations selected intentionally exceeded the stated water solubility of the test substance. Test concentrations were prepared by the combining of measured amounts of test substance, acetone (150 µL) and sterile culture medium in a glass bottle (2 L), then treated by ultrasound for 20 minutes, then stirred for 18 hours and subsequently left to stand for 2 hours, then the mid-portion of the solution was taken for use in the study. At all test concentrations, the medium was clear at the start of the study except for the two highest concentrations, which were off white, hazy emulsions. The measured filtered concentrations were between 23 and 68% of the nominal concentrations and were maintained at between 85 and 114% of the initial concentrations. There were 5 replicates of each concentration and 6 for the controls. The initial cell of $1X10^4$/mL. During the incubation period there was continuous illumination at 8110 lux, the temperature was maintained at 23±2°C and the flasks were shaken continuously at 150 cycles/min. Temperature and pH remained within acceptable limits.

RESULTS

Biomass		*Growth*	
E_bC_{50} mg/L at 72 h	*NOEC* mg/L	E_rC_{50} mg/L at 72 h	*NOEC* mg/L
7.84 (95% CL 7.07 & 8.70)	2.55	14.9 (95% CL 13.6 & 16.3)	2.55

Remarks - Results The results are based on the measured filtered concentrations.

The NOEC was determined by a Dunnett's multi-comparison test to compare the percentage inhibition on the test group with that for the solvent control cultures.

CONCLUSION Under the study conditions the test substance is toxic to aquatic life. (United Nations, 2003)

TEST FACILITY HLS (1998g)

8.2.4. Inhibition of microbial activity

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 209 Activated Sludge, Respiration Inhibition Test. EC Directive 88/302/EEC C.11 Biodegradation: Activated Sludge Respiration Inhibition Test
Inoculum	Activated sludge from Oakley Sewage Treatment Plant (predominantly treating domestic effluent)
Exposure Period	3 hours
Water Hardness	200 - 250 mg $CaCO_3$/L
Concentration Range	Nominal: 1, 10 and 100 mg/L Actual: Not determined.
Remarks – Method	Reference substance – 3,5-dichlorophenol at 3.0, 10.0 and 32.0 mg/L. Temperature range 17.7 to 18.9°C.
RESULTS	
EC50	>100 mg/L
NOEC	100 mg/L
Remarks – Results	With increasing concentration of the reference substance the microbial respiration decreased, giving an EC_{50} of 23.0 mg/L (95% CI 19.3-28.9 mg/L) calculated by the moving average method. There was no observed respiration inhibition at any of the test substance concentrations. The study conditions were validated by the findings of the reference substance and since the respiration rates in the control at the start and end of the study were within 15%.
CONCLUSION	Under the study conditions the test substance is not toxic to microorganisms.
TEST FACILITY	HLS (1998h)

9. RISK ASSESSMENT

9.1. Environment

9.1.1. Environment – exposure assessment

The proposed use and disposal pattern for the notified chemical suggests that direct release to the aquatic and terrestrial environmental compartments is unlikely and therefore no predicted environmental concentration (PEC) has been estimated for the notified chemical.

Wastes containing the notified chemical generated during pellet formulation and end-product moulding are expected to be disposed of to landfill or incinerated. Up to 165 kg per annum of the notified chemical could be disposed of to landfill, including as residues in empty containers. Most of this waste would be cured product in which case the chemical will be incorporated into an inert matrix and will be unavailable to the environment. It is unlikely that the notified chemical will leach into the water compartment due to its low solubility.

At the end of their useful lives articles made containing the notified chemical would be disposed of to landfill or recycled.

From the study undertaken, it is apparent that the notified chemical is unlikely to bioaccumulate.

9.1.2. Environment – effects assessment

The aquatic toxicity data submitted for the 4 taxa (fish, invertebrates, algae and micro-organisms) indicates that the chemical is toxic to aquatic invertebrates and algae and harmful to fish. The most sensitive species was algae with a reported EC50 of 7.84 mg/L at 72 hours. A predicted no effect concentration for aquatic organisms ($PNEC_{aquatic}$) of 78.4 µg/L has been derived by dividing the lowest acute EC50 value by a safety factor of 100.

9.1.3. Environment – risk characterisation

The notified chemical does not pose a significant risk to the environment based on its reported use pattern because there will be very low environmental exposure. The majority of the notified chemical will be contained in a cured polymeric matrix and will eventually be disposed of to landfill in the final products at the end of their useful lives.

Despite the low PNEC, there is unlikely to be any release of the chemical into the aquatic environment under the proposed use patterns and levels are expected to be well below the safety margin.

Tests show that the notified chemical has a low potential to bioaccumulate and that it is not readily biodegradable. Abiotic or slow biotic processes are expected to be largely responsible for the eventual degradation of the notified chemical.

9.2. Human health

9.2.1. Occupational health and safety – exposure assessment

Transport & Storage

Occupational exposure to the notified chemical during transport and storage of powder preparations containing 60% or 18% notified chemical is only likely in the event of accidental container spillage involving breach of import packaging. Exposure in these circumstances is expected to be infrequent and acute, and can be limited by use of appropriate PPE during clean-up operations.

Mixing

During manual weighing of powder preparations and loading to the mixing machine, dermal exposure is the most likely route. Ocular and inhalation exposure may occur as a result of powder spills. Exposure will be limited by LEV, personnel training and PPE including gloves, dust masks and safety glasses.

Exposure during mixing operations is expected to be minimal, as closed systems will be used.

Inhalation exposure during manual handling of powder preparations was estimated using the EASE model (HSE, 1994). Assuming 16% of the dust is respirable (as 16% of particles of the notified chemical in powder form are <10μm), and assuming LEV is present, the estimated inhalation exposure during manual handling is 0-1 mg/m^3. Therefore, for a 70 kg worker with an inhalation rate of 1.3 m^3/hour and 2 hours of exposure/day, systemic exposure is estimated to be 0-0.04 mg/kg bw/day.

Estimated dermal exposure during manual handling of powder preparations, assuming non-dispersive use and LEV present, is rated as "very low" according to the EASE model.

Extruding & Moulding

Exposure during extruder operation is expected to be minimal as closed systems will be used.

During manual transfer of extruded polymer pellets to the moulding machine, and during handling of finished plastic articles, dermal exposure is the only likely route. Exposure will be limited by LEV and PPE, by the low concentration of notified chemical in pellets and finished articles (0.5%) and by the lack of bioavailability of the notified chemical in the solid pelleted and finished form.

Disposal

During disposal of waste pellets and plastic products, dermal exposure is the only likely route. Exposure will be limited by the low concentration of notified chemical in pellets and finished articles (0.5%), by the lack of bioavailability of the notified chemical in the solid pelleted and finished form, and by the low frequency of exposure.

9.2.2. Public health – exposure assessment

Public exposure during transport of imported powder preparations and production of plastic articles is only likely in the event of a major accident or industrial spill.

The public will be exposed to finished plastic articles containing the notified chemical; however the notified chemical will be not biologically accessible.

Overall, public exposure is expected to be very low.

9.2.3. Human health – effects assessment

The notified chemical is of low acute oral and dermal toxicity in rats. Acute inhalation toxicity data were not provided. The notified chemical has low volatility, although in its powdered form 16% of particles are in the respirable range and 94% of particles are in the inspirable range.

The notified chemical is slightly irritating to rabbit skin and eyes. There was no evidence of sensitisation in an adjuvant study in guinea pigs.

The notified chemical was not mutagenic in a bacteriological test or a mammalian cell mutation test, and was not clastogenic in Chinese hamster lung fibroblasts in vitro.

In rats, a 28-day repeat dose oral toxicity study showed the NOAEL to be 80 mg/kg bw/day, while a 90-day repeat dose oral toxicity study showed the NOAEL to be 50 mg/kg bw day. Higher doses of the notified chemical were shown to cause reduced body weight and body weight gain (at 400 mg/kg bw/day in the 28-day study and at 150 mg/kg bw/day in the 90-day study) and elevated prothrombin time and activated partial thromboplastin time (at 400 mg/kg bw/day in the 28-day study).

Based on the available data, the notified chemical is not classified as a hazardous substance in accordance with the NOHSC *Approved Criteria for Classifying Hazardous Substances* (NOHSC 2004).

9.2.4. Occupational health and safety – risk characterisation

Mixing

During manual handling of powder preparations for weighing and adding to the mixing machine, inhalation exposure was estimated to be 0-0.04 mg/kg bw/day. The margin of exposure (MOE) for chronic toxicity is based on a NOAEL of 50 mg/kg bw/day. MOE greater than or equal to 100 are considered to be acceptable to account for intra- and inter-species differences. For inhalation exposure, the MOE is calculated to be >1000. Therefore, the risk of chronic systemic toxicity using modelled worker data is acceptable for manual handling of powder preparations in the presence of LEV. Dust masks would further reduce inhalation exposure.

Dermal exposure during manual handling of powder preparations was determined to be too low for a quantitative estimate. Therefore the risk of chronic systemic toxicity due to dermal exposure is acceptable for manual handling of powder preparations in the presence of LEV. PPE including gloves and protective clothing would further reduce dermal exposure.

Available toxicological data show that the notified chemical is not sensitising and only slightly irritating. Therefore the risk of irritation or sensitisation following dermal or ocular exposure is slight to negligible. However, PPE including gloves, safety glasses and protective clothing would further limit this risk.

Extruding & Moulding; Disposal

Exposure during the rest of the processes involving solid pellets and plastic articles is expected to be very low due to the low concentration of notified chemical in solid products and its lack of bioavailability. Therefore the risk of adverse health effects during extruding, moulding and disposal operations is considered to be negligible.

9.2.5. Public health – risk characterisation

Based on the low likelihood of public exposure to the notified chemical, and the available toxicological data, the public risk from exposure to the notified chemical through all phases of its life cycle is considered to be low.

10. CONCLUSIONS – ASSESSMENT LEVEL OF CONCERN FOR THE ENVIRONMENT AND HUMANS

10.1. Hazard classification

Based on the available data the notified chemical is not classified as hazardous under the NOHSC *Approved Criteria for Classifying Hazardous Substances*.

As a comparison only, the environmental classification of the notified chemical using the Globally Harmonised System for the Classification and Labelling of Chemicals (GHS) (United Nations 2003) is Chronic Hazard, Category 2: Toxic to aquatic life with long lasting effects. This system is not mandated in Australia and carries no legal status but is presented for information purposes.

10.2. Environmental risk assessment

The chemical is not considered to pose a risk to the environment based on its reported use pattern.

10.3. Human health risk assessment

10.3.1. Occupational health and safety

There is Low Concern to occupational health and safety under the conditions of the occupational settings described.

10.3.2. Public health

There is Negligible Concern to public health when used as a clarifying agent in polypropylene plastics.

11. MATERIAL SAFETY DATA SHEET

11.1. Material Safety Data Sheet

The MSDS of the notified chemical provided by the notifier was in accordance with the NOHSC *National Code of Practice for the Preparation of Material Safety Data Sheets* (NOHSC, 2003). It is published here as a matter of public record. The accuracy of the information on the MSDS remains the responsibility of the applicant.

11.2. Label

The label for the notified chemical provided by the notifier was in accordance with the NOHSC *National Code of Practice for the Labelling of Workplace Substances* (NOHSC, 1994). The accuracy of the information on the label remains the responsibility of the applicant.

12. RECOMMENDATIONS

CONTROL MEASURES

Occupational Health and Safety

- 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 NOHSC *Approved Criteria for Classifying Hazardous Substances*, workplace practices and control procedures consistent with provisions of State and Territory hazardous substances legislation must be in operation.

Environment

- The following control measures should be implemented by reformulators and plastic manufactures to minimise environmental exposure during use of the notified chemical:
  - Ensure all process areas are bunded with all drains going to collection pits or on-site treatment plants.

Disposal

- The notified chemical should be disposed of by recycling, landfill or incineration.

Emergency procedures

- Spills/release of the notified chemical should be contained, collected and stored in a sealable labelled container ready for disposal.

12.1. Secondary notification

The Director of Chemicals Notification and Assessment must be notified in writing within 28 days by the notifier, other importer or manufacturer:

(1) Under Section 64(2) of the Act:
  - if any of the circumstances listed in the subsection arise.

The Director will then decide whether secondary notification is required.

No additional secondary notification conditions are stipulated.

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