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NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME
(NICNAS)

FULL PUBLIC REPORT

Chemical in Larotact LR 9018
(In NA/775, title was Chemical in CYLINK+ 2000 Crosslinking Agent

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Street Address:	334 - 336 Illawarra Road MARRICKVILLE NSW 2204, AUSTRALIA.
Postal Address:	GPO Box 58, SYDNEY NSW 2001, AUSTRALIA.
TEL:	+ 61 2 8577 8800
FAX	+ 61 2 9577 8888.
Website:	www.nicnas.gov.au

Director
Chemicals Notification and Assessment

EX/48
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1. APPLICANT

Original Holder of Assessment Certificate (First Applicant)

An Assessment Certificate for the notified chemical known by the name Chemical in CYLINK+ 2000 Crosslinking Agent was granted to Cytec Australia Holdings Pty Ltd of 7–11 Railway Street BAULKHAM HILLS NSW 2153.

The Assessment Report for Chemical in CYLINK+ 2000 Crosslinking Agent is identified by the sequence number NA/775.

Second Applicant

Since granting of the abovementioned Assessment Certificate, PPG Industries Australia Pty Ltd (ABN: 82 055 500 939) of McNaughton Road, Clayton, VIC 3168, and BASF Australia Ltd (ABN: 62 008 437 867) of 500 Princes Highway, Noble Park, VIC 3174 have jointly submitted a notification statement in support of their application for an extension of the original Assessment Certificate for Chemical in Larotact LR 9018. Cytec Australia Holdings Pty Ltd has agreed to this extension.

Information submitted by PPG Industries Australia Pty Ltd and BASF Australia Ltd pertains to the introduction of the notified chemical for use in crosslinking solution for baking finishes in combination with amino resins. Introduction volumes will be approximately 10 tonnes per year imported as a component of the coating additive Larotact LR 9018.

Under Section 40E of the Act, this modification of the Full Public Report is provided to PPG Industries Australia Pty Ltd and BASF Australia Ltd for consideration. The modified reports will also be provided to the original certificate holder Cytec Australia Holdings Pty Ltd.

No application for Exempt Information was received for the Extension.

Under Section 40G of the Act, the amended Assessment Report for NA/775 will be republished and provided to: the Chief Executive Officer of the National Occupational Health

and Safety Commission and the Secretary of the Department of the Environment and Heritage.

2. IDENTITY OF THE CHEMICAL

The chemical name, CAS number, molecular and structural formulae, molecular weight, spectral data, details of the polymer composition and details of use and customers have been exempted from publication in the Full Public Report and the Summary Report.

The notified chemical is a complex mixture of four minor and major components. Infrared spectroscopy data were submitted for the identification of the notified chemical.

Marketing Name:	Larotact LR 9018 (CYLINK^{+} 2000 Crosslinking Agent in NA/775)
Method of Detection and Determination:	Infrared spectroscopy (IR)

3. PHYSICAL AND CHEMICAL PROPERTIES

The properties reported below are those of the notified chemical unless otherwise stated.

Appearance at 20°C and 101.3 kPa:	White to off white powder in pure form; yellow viscous liquid with butanol odour in imported form
Boiling Point:	161 – 165°C
Melting Point:	131 - 138°C
Density:	1.59 g/mL at 20°C
Vapour Pressure:	3.3 x 10^{-8} kPa at 25°C
Water Solubility:	0.05 g/L at 25°C
Partition Co-efficient (n-octanol/water):	log P_{ow} values are: 0.4, 1.3, 2.1, 2.8, 3.4, 4.2, and 5.5
Hydrolysis as a Function of pH:	$T_{1/2}$ at pH 9.0: 0.51 days at 50°C (triazine component 1) : 0.44 days 50°C (triazine component 2 : 0.43 days at 50°C (triazine component 3)(see comments below)
Adsorption/Desorption:	K_{oc} values are: 0.2, 8, 37, 219, 537 and 3236
Dissociation Constant:	pK_a 3.0 – 7.0 (because of the pyridinium nitrogen atom)

Particle size:	Mean particle size = 1.47μm 100% particles <10μm
Flash Point:	Not applicable for non-flammable solid
Flammability Limits:	Not flammable
Autoignition Temperature:	Does not self ignite
Explosive Properties:	Not explosive
Reactivity/Stability:	Stable; not reactive

Comments on Physico-Chemical Properties

The vapour pressure of the notified chemical, determined by the balance method OECD TG 104, was 3.3 x 10^{-8} kPa indicating that it is not volatile.

The water solubility of the four major components of the notified chemical, determined by the flask shaking method OECD TG 105, were 0.02 g/L, 0.17 g/L, 0.04 g/L and <0.003 g/L, respectively. The calculated solubility of the notified chemical, based on the percent composition of each component is 0.05 g/L.

The stability of the notified chemical in water at pH 4, 7 and 9 at 50ºC was studied in accordance with OECD TG 111. The half-life values of the 3 major components at pH 9 were 0.51, 0.44 and 0.43 days, respectively. At pH 4, the triazine component 1 of the notified chemical declined from 0.44 μg/mL to 0.26 μg/mL after 5 days. At pH 7, it was hydrolytically stable whereas at pH 9, the levels declined to 0.01 μg/mL after 5 days. Triazine component 4 was found to be hydrolytically stable at pH 7 but degraded at pH 4 and 9. At pH 4, levels declined from 5.42 μg/mL to 3.05 μg/mL, while at pH 9, levels declined from 5.98 μg/mL to 2.60 μg/mL. The other major components were hydrolytically unstable at all three pH. It is noted that the notified chemical contains carbamate groups that would be expected to hydrolyse readily.

The partition coefficient for the notified chemical was determined by HPLC in accordance with OECD TG 117. Under the conditions of the test seven components of the notified chemical sample were detected. The log Pow values determined for each component were 0.4, 1.3, 2.1, 2.8, 3.4, 4.2 and 5.5. The range of values suggest that some of the components may be considered as hydrophilic while other components would be hydrophobic. It is noted that the 3 major components which comprise approximately 94.5% of notified chemical, have measured log Pow values of 5.5, 4.2 and 2.8, respectively. Therefore, the majority of the notified chemical would favour the octanol phase.

The adsorption coefficient of the notified chemical was determined by HPLC, using an unspecified draft guideline for the Screening Method for the Determination of the Adsorption Coefficient. The notified chemical resolved into six discrete components with measured Koc values of 0.2, 8, 37, 219, 537 and 3 236. The range of values suggests that some components would have a strong affinity for soil while other components would have a much higher

mobility. It is noted that triazine component 1 and triazine component 2, which comprise approximately 77.5% of the notified chemical, have measured Koc values of 3 236 and 537, respectively. Therefore, the majority of the notified chemical would have a low to slight mobility in soil.

No dissociation constant data was provided for the notified chemical. The notifier states that the chemical contains pyridinium nitrogen atoms that are expected to have pKa values between 3.0 and 7.0. This claim is acceptable and it is noted that the notified chemical would be mostly in its free state at environmental pH.

4. PURITY OF THE CHEMICAL

Degree of Purity: high

Hazardous Impurities: None known

Non-hazardous Impurities (> 1% by weight): None known

Additives/Adjuvants:

Chemical Name	*CAS No.*	*Weight %*
n-Butanol	71-36-3	18 – 53%

5. USE, VOLUME AND FORMULATION

The notified chemical will not be manufactured in Australia. It will be imported as a component of a coating additive Larotact LR 9018 in 181 kg net closed head steel drums or 1 tonne Intermediate Bulk Containers (IBC). It will be blended up to 2.5% with other additives to form automotive coatings containing 1.25% of notified chemical.

Larotact LR 9018 is a crosslinking agent used in the manufacture of automotive coats. It is incorporated at concentrations up to 2.5% in the final automotive coat. This is equivalent to 1.25% notified chemical.

The import volume for Larotact LR 9018 will be approximately 20 to 40 tonnes per year.

6. OCCUPATIONAL EXPOSURE

The imported product Larotact LR 9018 containing the notified chemical is an additive used in the manufacture of automotive coats at concentrations up to 2.5% (0.78 to 2.2 % of the notified chemical). The automotive paints are thinned for use by approximately 10%. The clearcoat is applied by either hand spray gun or automatic spray machine and is then heat cured.

The following table describes the number and category of workers, the nature of work done and the duration of exposure for workers handling the notified chemical from the time of importation until the final application of the product containing the notified chemical.

Category of worker	Number of workers	Nature of work	Max. potential exposure (hrs/day; days/yr)	Physical form
Waterside, transport and warehouse	4	Loading/unloading trucks	1 – 2 hrs/day; 5 – 10 days/yr	Liquid (imported product)
Paint Formulation Site				
Laboratory and development staff	3	Making up test batches of paint and quality control testing	8 hrs/day; 20 days/yr	Liquid (imported product and paint products)
Paint manufacturing staff	6	Weighing and mixing of the product with other chemicals and solvents	4 hrs/day; 100 days/year	Liquid (imported product and paint products)
Filling operators	3	Filling paint into 200L steel drums	4 hrs/day; 100 days/yr	Liquid (paint products)
Automotive Manufacturers				
End use operators – paint applicators	20	Thinning and addition of paint to circulation tanks Hands spray pick up 90% of paint product applied using automatic electrostatic spray (80% transfer efficiency) and 10% by manual spray (50% transfer efficiency)	2 hrs/day; 200 days/yr 8 hrs/day; 200 days/yr	Liquid (paint products)
End use operator	8	Cleaning of spray equipment 90% of paint product applied using automatic electrostatic spray (80% transfer efficiency) and 10% by manual spray (50% transfer efficiency)	2 hrs/day; 200 days/yr	Liquid (paint products)

Transport and Storage

Larotact LR 9018 will be imported in 181 kg net closed head steel drums or 1 tonne IBCs. The material will be transported from dockside to PPG Industries Australia Pty Limited warehouse where it will be stored in a warehouse before being used for formulation into automotive paints. It is anticipated that waterside workers, transport drivers and warehouse workers would only be exposed to the material in the event of an accident.

Paint Manufacture
At the customer site, a production operator will transfer the required amount of Larotact LR 9018 into a stainless steel mixer of 10 000L capacity either by pump or gravity feed. Larotact LR 9018 will be blended with other paint additives to produce paint products. After mixing, the laboratory personnel will collect and analyse a small sample of paint product from the mixing tank. The filling operator will drum off the formulated paint product by gravity feed into 200L steel drums through a closed filtering system. Filled drums are stored until distributed to paint applicators.

The notifier indicated that paint manufacture is a closed automated process. However, production operators may experience dermal exposure to the substance containing the notified chemical at 31 to 88% (as imported) and 0.78 to 2.2 % (when mixed) when connecting, disconnecting and cleaning the blending equipment. Similarly, filling operators may experience skin contact to spills and drips containing up to 2.2% notified chemical. The laboratory personnel may be exposed dermally to the notified chemical. The notifier states that paint manufacture employs the use of enclosed mixers. Paint manufacture and filling are conducted under exhaust ventilation. The notifier indicated that production operators would wear personal protective equipment such as impervious gloves, overalls and safety glasses as a minimum protection. The manufacturing and storage areas are bunded.

Paint Application
The paint product will be transported by road to automotive paint applicators throughout Australia. The paint will be predominantly applied using automatic electrostatic atomised spray (robotic system), but manual touchups will be necessary. The plant operator will transfer the paint into intermediate tanks by gravity feed. The paint is thinned by approximately 10% then transferred to circulation tanks through an enclosed system. The paint lines will supply both the electrostatic sprayers and the manual spray equipment. The assembly plant area and paint mixing kitchen are under ventilation.

Automated Application
Electrostatic spraying will be carried out using robotic system and no worker exposure is expected. Overspray will collect in a pool of water below the grill floor or in a wet scrubber in the exhaust and will be removed with a filter. The residual solids will be disposed of to secure landfill.

There is potential for dermal exposure to the notified chemical for spray operators connecting and disconnecting pumps and pipes during transfer operations and cleaning the spray equipment. Workers will wear impervious gloves, anti-static coveralls, anti-static footwear and eye protection.

Manual Application
Manual spraying will be carried out in a spray booth fitted with fume extraction system. Dermal and eye contact, and inhalation of vapour or spray mist are possible during touch up. Spray painters will wear as a minimum protection, nylon gloves, anti-static overalls, anti-static foot wear, calico hoods, eye protection and cartridge type respirators.

Curing Process
Following paint application, the paint will be heat cured. The notified chemical reacts with other components in the paint formulation to form an integral part of the paint film. In this form, the notified chemical will not be bioavailable.

7. PUBLIC EXPOSURE

Public exposure is only likely after surface coatings containing the notified chemical have been applied to the exterior of car bodies. Although, there may be dermal contact, there is negligible potential for exposure of the public to the notified chemical since it is strongly bound in polymer matrices and present at low concentrations.

8. ENVIRONMENTAL EXPOSURE

8.1 Release

Paint manufacture
There is potential for release during paint manufacture (formulation) and paint application. Formulation takes place at a customer plant and any spills that occur will be contained by the plant bunding. The notifier estimates that up to 2% (700 kg per year) of waste notified chemical would be generated during formulation at the plant through accidental spillage and cleaning of equipment.

Some residue will remain in the 'empty' drums after formulation. It is estimated that up to 1% of the container contents, 350 kg per annum by year 5, will remain as residue.

The blending equipment and transfer lines will be flushed out at the end of each shift with solvents. There is no release of the notified chemical to natural waterways. PPG Industries have a paint recycling plant_on site to treat waste generated on site. The end products are solvents, which is reused on site and the sludge is removed from site by registered waste disposal contractors for disposal at authorised sites.

Paint application
The paint product is principally applied by automatic electrostatic atomise spray and to a limited extent by manual spray techniques. Paint waste will be generated from three main areas:

1. Overspray from the application process.

2. Flushing and cleaning of application and mixing equipment.

3. Empty paint containers.

Transfer efficiency is estimated as 80% for the combined application. Therefore, as a worst case scenario, 20% of the supplied volume solids is lost as overspray. This will result in a maximum of 2 tonnes notified chemical contained in the overspray. This overspray is collected by the spray booth air and water filtration systems. The paint material removed is treated in water scrubbing systems. The paint material removed by the scrubbers is separated

out using flotation techniques. This separated sludge is then removed to landfill. Cleaning of waste from spray booths is carried out by licensed waste disposal contractors.

Cleaning of application and mixing equipment will generate waste paint and solvent, which is collected and treated in the same way as spray booth waste. It is estimated that 300 kg of notified chemical will be generated in this operation.

Approximately 2% of drum contents remain as residue. Therefore approximately 200 kg of Larotact LR 9018 will remain in the drums. Drums and residue are sent to landfill by licensed waste disposal contractors

8.2 Fate

The notified chemical waste from paint manufacture will be sent to an on-site treatment plant. The customer has developed a process where by waste resin and paint is processed to reclaim solvents. Residues are converted to an inert solid that is disposed of to landfill. There is no release of the notified chemical to natural waterways. The drums and drum residue will also be disposed of to landfill by licensed waste disposal contractors. Leaching of the notified chemical from landfill sites is unlikely, given the expected slight mobility in soils of the substance.

The notified chemical waste from paint application in spray booths and drum residue will be disposed of to landfill by licensed waste disposal contractors. Leaching of the notified chemical from landfill sites is unlikely, given that the paint containing the notified chemical would be expected over time to cure into a high molecular weight inert solid.

Once applied and heat cured to the metal panels of vehicles, the notified chemical will be incorporated in a hard, durable, inert film and would not present an environmental hazard. Fragments, chips and flakes of the lacquer will be of little concern as they are expected to be inert. At the end of their useful life, metal panels coated with the notified chemical are likely to be either recycled for steel reclamation or placed into landfill. During steel reclamation the notified chemical would be destroyed in blast furnaces and converted to water vapour and oxides of carbon and nitrogen.

The ready biodegradation of the notified chemical was determined by exposure to microorganisms from a domestic sewage treatment plant according to OECD TG 301B (Modified Sturm Test). After 28-day exposure to microorganisms the biodegradation rate expressed as a percentage of actual versus theoretical quantities of CO_2 evolved, was 9.00 and 3.47% for the 10 and 20 mg OC/L samples, respectively. After 28-day exposure to microorganisms the control test substance, sodium benzoate, was degraded by 73.03%. Under the conditions of the test the notified chemical was not found to be readily biodegradable.

An additional ready biodegradability test was submitted with the application for Extension, with results as follows.

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	BOD and HPLC.
Remarks – Method	

Results

Test substance		*Reference Substance (Aniline)*	
Day	*% degradation*	*Day*	*% degradation*
28	0% by BOD 7% by HPLC	28	79% by BOD

Remarks – Results

Conclusion	The notified chemical cannot be classed as ready biodegradable.
Test Facility	Kurume Research Laboratories (1996).

The notified chemical is not expected to cross biological membranes because of the moderate solubility and slight mobility in soil. Once the notified chemical becomes part of a cured paint matrix it is not expected to cross biological membranes due to its high molecular weight and low solubility, as such it should not bioaccumulate (Connell, 1990).

The bioaccumulation potential of the notified chemical to Carp was determined in a continuous flow test, by exposure over 8 weeks at two nominal concentrations of test substance, Level 1 and Level 2 of 0.5 and 0.05 mg/L, respectively. The notifier claims that the test method used is essentially the same as that in OECD TG 305C Bioaccumulation. However, it is noted that no depuration phase is indicated in the test carried out by the notifier. Samples extracted from the fish and analysed by HPLC, showed six components of the notified chemical. Bioconcentration factors were calculated from the HPLC results and are summarised below.

	Bioconcentration Factor	
HPLC Peak	***Level 1 (0.5 mg)***	***Level 2 (0.05 mg)***
1	< 5.5	< 54
2	< 3.5	< 36
3	< 0.8	< 7.8
4	< 8.6	< 87
5	< 0.5	< 4.8
6	3.2-7.5	18-43

Under the conditions of the test the notified chemical can be classified as slightly concentrating.

9. EVALUATION OF TOXICOLOGICAL DATA

Tests were performed according to EEC/OECD guidelines (European Economic Community, 1992), (Organisation for Economic Co-operation and Development, 1995-1996) at facilities complying with OECD Principles of Good Laboratory Practice.

The following toxicity studies were conducted on the notified chemical in Larotact LR 9018 (CYLINK^{+} 2000 Cross Linking Agent) except for mouse ear swelling study where the test substance was a product containing 50% notified chemical in n-butanol.

Endpoint	*Assessment Conclusion*
Rat, acute oral	LD50 > 2000 mg/kg; low toxicity
Rat, acute dermal	LD50 > 2000 mg/kg; low toxicity
Rat, acute inhalation	No study available
Rabbit, skin irritation	non-irritating
Rabbit, eye irritation	non-irritating
Guinea pig, skin sensitisation - adjuvant test	inconclusive
Mouse, ear-swelling test	Non-sensitising
Rat, oral repeat dose toxicity - 28 days.	NOEL 15 mg/kg/day
Rat, dermal repeat dose toxicity – 28 days	NOAEL 100 mg/kg/day
Rat, developmental toxicity	NOEL (maternal) 30 mg/kg/day NOEL (developmental) 1000 mg/kg
Genotoxicity - bacterial reverse mutation	non mutagenic
Genotoxicity – in vitro chromosomal aberrations	non genotoxic
Genotoxicity – in vivo mouse micronucleus	non genotoxic

9.1 Acute Toxicity

9.1.1 Oral Toxicity (Wilson JA, 1996d)

Species/strain: Rat/Sprague Dawley

Number/sex of animals: 5/sex

Observation period: 14 days

Method of administration:	Oral (gavage) with a single dose of 2 000 mg/kg in maize oil vehicle
Test method:	OECD TG 401, Limit test
Mortality:	One female was found dead a half-hour after dosing.
Clinical observations:	Survivors showed signs of piloerection, increased activity, ataxia, a hunched appearance and increased breathing. All animals recovered the day after dosing.
Morphological findings:	Necropsy of the premature decedent revealed signs that the death may have occurred as a result of a dosing accident (white creamy substance in the thoracic cavity).
Comment:	No other abnormalities were noted in the premature decedent or survivors. There were no adverse effects on body weight gain.
LD_{50}:	> 2 000 mg/kg
Result:	The notified chemical was of very low acute oral toxicity in rats.

9.1.2 Dermal Toxicity (Wilson JA, 1996b)

Species/strain:	Rat/Sprague Dawley
Number/sex of animals:	5/sex
Observation period:	14 days
Method of administration:	The test substance as a powder moistened with water was applied to the dorsal skin as a single dose of 2 000 mg/kg, under occlusive conditions, for 24 hours.
Test method:	OECD TG 402; Limit test
Mortality:	No premature deaths were observed.
Clinical observations:	A red discharge from the nose was observed in both sexes for a 1-4 hour period after dosing.
Morphological findings:	Males had normal body weight gain. Females had lower body weight gain, with one losing weight.
Comment:	No abnormalities were noted at necropsy.

LD_{50}:	>2 000 mg/kg
Result:	The notified chemical was of low dermal toxicity in rats.

9.1.3 Inhalation Toxicity

The original notifier sought variation on providing data on an inhalation study, based on the low toxicity of the notified chemical in the acute oral and dermal studies, the low vapour pressure of the pure solid form, and the fact that it will be imported in liquid form. The request for variation was accepted on the basis that the solvent has a NOHSC exposure standard therefore the imported product containing the notified chemical is under regulatory exposure control. Should the chemical be imported in powder form, secondary notification and an inhalation study will be required.

9.1.4 Skin Irritation (Wilson JA, 1996a)

Species/strain:	Rabbit/New Zealand White
Number/sex of animals:	3 males
Observation period:	3 days
Method of administration:	The test substance was applied as powder moistened with water. A dose of 0.5 gm was applied as a patch to the dorsal trunk of animals, and secured with tape and bandage. After 4 hours, patches were removed and application sites cleansed.
Test method:	OECD TG 404
Comment:	Skin reactions were assessed 1, 24, 48 and 72 hours after patch removal. No adverse reactions were observed over the 3-day period, with all dermal irritation scores being zero.
Result:	The notified chemical was non-irritating to the skin of rabbits.

9.1.5 Eye Irritation (Wilson JA, 1996c)

Species/strain:	Rabbit/New Zealand White
Number/sex of animals:	3 males
Observation period:	3 days
Method of administration:	0.1 gm of test substance was instilled into the right eye of each animal and the eyelids held together for 1-2 seconds;

	the left eye served as control.
Test method:	OECD TG 405
Comment:	Eyes were examined for reactions at 1, 24, 48 and 72 hours. No adverse reactions were observed for any animal and mean scores for all ocular lesions were zero.
Result:	The notified chemical was non-irritating to the eyes of rabbits.

9.1.6.1 Skin Sensitisation in guinea pigs with the Magnusson-Kligman Maximisation test (Wilson JA, 1996e)

Species/strain:	Guinea pigs/Dunkin-Hartley
Number of animals:	20 test group; 10 control group (females)
Induction procedure:	<u>Test animals</u> Day 1: Intradermal injection (0.1 mL) to scapular region of animals consisted of : 50% aqueous Freund's Complete Adjuvant (FCA); 2% test substance in vehicle (maize oil) 2% test substance in vehicle in 50% aqueous FCA. Because of difficulty with injections, the maximum concentration of test substance that could be successfully administered was 2%. Day 7 Topical application of 0.5mL of 10% sodium lauryl sulphate Day 8 Topical application consisted of 65% test substance applied to the scapular region with a patch covered with aluminium foil and an occlusive tape. Patches were removed after 48 hours. <u>Control animals</u> Control animals were treated identically to test animals but omitting the test substance from the intradermal injection and topical application.
Challenge procedure:	Day 21 1st challenge 65% test substance (0.5 mL) was applied to the flanks of animals under occlusive conditions for 24 hours.

	Day 28 2nd rechallenge 50% test substance was applied to the flanks of animals under occlusive conditions for 24 hours.
Test method:	OECD TG 406
Comment:	Challenge with 65% test substance resulted in 12/20 (60%) responses and in 1/10 (10%) responses in test and control animals, respectively. All reactions consisted of slight erythema (score 1) except the responding animal in the vehicle control group (score 2). Reactions were less frequent upon rechallenge with 50% test substance. In 4/20 (20%) test animals only slight reactions (score 1) were noted and none in the control group. The testing laboratory considered that the high incidence of responses seen in the test group at challenge was almost certainly due to irritation rather than sensitisation. The incidence of reactions seen at 65% test substance was significantly higher than those seen at a marginally lower dose level of 50% test substance. This response pattern was considered to be atypical of sensitisation and indicative of irritation. Based on the evidence, the testing laboratory considered that the rechallenge results gave a more accurate representation of the true sensitisation potential of the test substance. Nevertheless, owing to the technical difficulty of being able to inject only a maximum concentration of 2% test substance for the induction phase of the study, it is considered that the results do not permit a definite conclusion to be made about the sensitising potential of the test substance.
Result:	The notified chemical was a weak sensitiser to the skin of guinea pigs.

9.1.6.2 Skin Sensitisation – Mouse Ear Swelling Study (Cerven DR, 1994)

Species/strain:	Mouse/CF-1
Number of animals:	20 females
Induction procedure:	Day 0 All mice were administered two intradermal injections, in the abdominal area, of a 50% solution of FCA in distilled water. A 0.1 mL volume of a 50% concentration of test substance

	in butanol was applied beneath a patch to the abdominal skin of animals and secured with tape. Patches remained in place for 24 hours. The procedure was repeated on Day 2 and Day 4 of the study.
Challenge procedure:	Day 11 Animals were challenged on both sides of the left ear at a dose of 0.01 mL of a 10% concentration of test substance in vehicle dimethyl sulphoxide (DMSO), and on both sides of the right ear with 0.01 mL of vehicle. Ear thickness of both ears was measured 24 and 48 hours after application.
Test method:	FDA 21 CFR Part 58 and EPA 40 CFR Parts 160 and 792
Comment:	No positive responses were noted in any ear at 24 and 48 hours after challenge. No abnormal physical signs were noted during the observation period.
Result:	The notified chemical was non-sensitising to the skin of mice.

9.2.1 Repeated Dose Oral Toxicity – one week range finding study (Martin T, 1996)

Species/strain:	Rat/Sprague Dawley
Number/sex of animals:	Four groups of 5/sex
Method of administration:	Oral (gavage)
Dose/Study duration:	0, 25 (low), 150 (intermediate) and 1000 (high) mg/kg/day for 7 days
Test method:	OECD TG 407

Clinical observations

High dose animals had salivation, piloerection, fast and/or shallow breathing and staining of the coat, slight decrease in weight gain in males and transient decrease in body weight in females. Similar clinical signs were seen in intermediate dose animals, but of lower incidence and severity.

There were no intergroup differences in food consumption.

Result

The range finding study was able to determine that the suitable dose levels for the 28 day study are 0, 15, 150 and 1000 mg/kg/day.

9.2.2 Repeated Dose Oral Toxicity – 28 day study (Wilcox S, 1996)

Species/strain:	Rat/Sprague Dawley
Number/sex of animals:	5/sex/group, including one control and high dose 14-day recovery group
Method of administration:	Oral (gavage)
Dose/Study duration:	0, 15 (low dose), 150 (intermediate dose) and 1 000 (high dose) mg/kg/day of test substance in maize oil vehicle; 28 consecutive days
Test method:	Compliant with EU, EPA and Japanese MITI

Clinical observations

No neurotoxic effects at any dose were determined by Functional Observation Battery (FOB) tests. One control animal died on Day 42 reportedly because of eye damage sustained during the bleed.

There was an increased incidence of salivation and staining of the coat in all high dose animals. The staining of the coat was considered to be likely due to the vehicle.

There was a reduction in body weight gain and food consumption for both sexes at the high dose and a slight reduction in body weight gain at the intermediate dose throughout the dosing period. Equivocal evidence of toxicity at the intermediate dose included a slight decrease in food consumption in the female animals.

During the recovery period, high dose animals had gained significantly more weight compared with control animals.

Clinical chemistry/Haematology

White blood cells, monocytes and basophils were increased in males and lymphocytes and white blood cells were increased in females at the high dose after 4 weeks treatment. No notable changes were seen after the 2-week recovery period.

Urea and creatinine were increased in all animals at the high dose, with no notable changes seen after the 2-week recovery period. Specific gravity of urine was decreased in the intermediate and high dose males and urinary pH was decreased in the high dose males.

Pathology

Kidney weight was increased in the main and recovery study males at the high dose, and in the main study females at the high dose. At necropsy, there were no notable intergroup differences in either sex at any dose levels tested.

Histopathology:

Tubular nephrosis in the kidney and diffuse cell hyperplasia of the transitional bladder epithelium were observed in all high dose animals, with the male animals being more severely affected than the female animals. No similar findings were seen in animals at 150 or 15 mg/kg/day, or in the control animals. In the recovery study, there was an increased incidence of basophilic tubules which was consistent with the increase in kidney weight for both sexes. Inflammatory cell foci were also increased in both sexes of the high dose animals suggestive of regenerating and repair phase following nephrotoxic insult.

A second histopathology study of the urinary bladders of the low and intermediate dose animals was undertaken after the completion of the main study. The study showed simple diffuse cell hyperplasia of the transitional bladder epithelium in all males and 2 females in the intermediate group. This lesion was not observed in any of the low dose animals.

Result:

The histological findings in the high and intermediate dose groups suggest that the kidney may be a target organ. Kidney damage was seen as tubular nephrosis with altered haematology, clinical chemistry and urinalysis parameters in both sexes of the high dose groups. Diffuse cell hyperplasia of the transitional bladder epithelium was also seen in both high and intermediate dose groups. High dose males and females previously examined in the main study suggested a low-grade treatment-related toxicity which was considered to be associated with urinary excretion of the test substance or its metabolites.

Basophilic tubules and inflammatory cell foci were increased in high dose recovery animals suggesting the reversibility of kidney effects seen at the end of exposure period.

Body weight gain and food consumption parameters were also reduced in high dose and to a lesser degree in intermediate dose groups.

The No Observed Effect Level (NOEL) is 15 mg/kg/day based on cell hyperplasia of the transitional bladder epithelium observed at 1 000 mg/kg and 150 mg/kg, and tubular nephrosis with an impairment in kidney function resulting in altered hematology, clinical chemistry and urinalysis parameters observed at 1 000 mg/kg.

9.2.3 Pilot developmental toxicity study in rats (Turck PA, 1999b)

Species/strain:	Rat/Sprague Dawley
Number/sex of animals:	10 females/group
Method of administration:	Oral (gavage) of test substance in corn oil
Dose/Study duration:	Test substance in corn oil administered at 0, 30, 100, 300, 500 and 1000 mg/kg/day on Day 6 of gestation and continued through to Day 19. Treated animals were examined for clinical observations from Day 6 to Day 20.

On Day 20 of gestation, animals were subjected to laparohysterectomy.

Test method: US EPA, Health Effects Test guideline

Clinical observations:

No animals died during the study. The only dose-related clinical observation noted was red discoloured urine observed in animals at 300 mg/kg/day and higher. Body weight and body weight gain were comparable between control and treated groups during the study. Possible test-related findings were noted at necropsy in the spleen at 500 and 1 000 mg/kg/day and kidney at 1 000 mg/kg/day. No effects on uterine parameters were observed in the treated groups when compared with controls. Numbers of corpora lutea, implantations, live foetuses and resorptions were similar in all groups, including the control.

Result:

A high dose of 1 000 mg/kg/day was recommended for the main developmental toxicity study.

9.2.4 Main developmental toxicity study in rats (Turck PA, 1999a)

Species/strain: Rat/Sprague Dawley

Number/sex of animals: 30 time-mated females/group

Method of administration: Oral (gavage) of test substance in corn oil

Dose/Study duration: Test substance in corn oil administered at 0 (control), 30 (low dose), 300 (intermediate dose) and 1000 (high dose) mg/kg/day on Day 6 of gestation and continued through to Day 19.

On Day 20 of gestation, animals were subjected to laparohysterectomy.

Test method: US EPA, Health Effects Test guidelines

Maternal observations

Clinical observations:

There were 28/30 rats used for uterine examinations in the control and low dose groups and 30/30 animals in the intermediate and high dose groups.

No mortalities occurred during the study. Discoloured red urine was noted in 47% (14/30 rats) and 67% (20/30 rats) in the intermediate and high dose animals, respectively. This

was considered to be the result of test article administration. There were no other clinical observations.

Body weight reductions seen in the intermediate and high dose animals were considered to be treatment-related and associated with the corresponding decrease in food consumption.

Necropsy findings:

No abnormal findings were noted in dams at necropsy.

Uterine examinations:

There were no treatment-related effects on uterine parameters, including numbers of corpora lutea, implantations, live or dead foetuses, and resorptions. Post-implantation loss was lower at intermediate dose group when compared with the control group.

Foetal observations

Clinical observations

Foetal body weight was comparable between controls and treatment groups and no treatment-related changes were noted. Adjusted body weight gain in the high dose group was statistically lower than the control group, a finding considered to be test substance-related.

External examination

One foetus from the intermediate dose group had anascara (generalised oedema) and a second foetus from a different litter in this group had exencephaly and ablepharia (reduction or absence of eyelids). These findings were reported to be within the laboratory's historical control incidence.

Visceral examination:

One foetus from the high dose group had undeveloped renal papillae and distended ureters, and one foetus from the control group had distended ureters. These findings were reported to be within the laboratory's historical control incidence.

Skeletal examination

The incidences of all findings were similar among all groups.

Comment:

Test substance-related maternal toxicity, as evidenced by reductions in body weight change and food consumption and increased incidences of discoloured urine, was observed in dams at 300 and 1000 mg/kg/day during gestation. Based on these results, the NOEL for maternal effects was 30 mg/kg/day. Based on the lack of effects on uterine parameters or foetal development at the highest tested dose, the NOEL for developmental effects was 1000 mg/kg/day.

Result:

The notified chemical was not found to be teratogenic in rats.

9.2.5 Repeated Dose Dermal Toxicity – 28 day study

Test Substance	Notified chemical.
Method	OECD TG 410 Repeated Dose Dermal Toxicity: 28-day Study.
Species/Strain	Rat/Crl:CD(SD) IGS BR
Route of Administration	Dermal – occluded.
Exposure Information	Total exposure days: 28 days; Dose regimen: 7 days per week; Duration of exposure (dermal): 6 hours/day; Post-exposure observation period: 2 weeks
Vehicle	1-butanol.
Remarks – Method	Doses were selected based on a 1-week range finding study where doses up to 1000 mg/kg/day did not reveal any toxic effects (Inveresk 2000a). Histopathological examinations initially on control and high dose animals for all tissues. Further histopathological examinations of urinary bladder from low and mid-dose groups – in separate report.

Results

Group	*Number and Sex of Animals*	*Dose mg/kg bw/day*	*Mortality*
I (control)	8/sex	0	None
II (low dose)	"	100	"
III (mid dose)	"	300	"
IV (high dose)	"	1000	"
V (control recovery)	"	0	"
VI (high dose recovery)	"	1000	"

Clinical Observations
Desquamation, oedema, erythema and eschar noted in all groups, but at a higher incidence in females, was attributed to the procedure of dermal dosing under occlusive dressing. There were no treatment-related effects on body weight, body weight gain, or food consumption. Water consumption was higher in the first week in high dose animals and during the recovery period in males. There were no clinical observations associated with this increase.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis
There were no treatment-related effects on haematology and urinalysis. Decreases in brain cholinesterase were noted in males in all treated groups, however, wide variations were observed and some doubt exists in the significance of the decreases at 100 and 300 mg/kg/day. Levels were normal in female animals and recovery males. There was no

correlation with plasma or red blood cell cholinesterase levels.

Pathology – effects in organs, macroscopic findings, histopathology

Effects in organs

There were no treatment-related effects on organ weights.

Macroscopic findings

There was no macroscopic findings on necropsy.

Histopathology

Minimal to mild transitional cell hyperplasia (simple type) of the urinary bladder was observed in animals of all treated groups. One animal of each sex from the low dose group was affected at the minimal severity level. At mid-dose, all males and 4/5 females were affected at the minimal or mild severity level. A similar incidence was observed at the top dose, with the effect observed in recovery animals of both sexes. Evidence of reversibility was observed by a decrease in the incidence of the finding in recovery animals.

Remarks – Results

Conclusion

A No Observed Effect Level (NOEL) was not established in this study, based on microscopic effects in the urinary bladder of treated animals in all groups. Based on low incidence and minimal severity of the effect at the low dose, the No Observed Adverse Effect Level (NOAEL) is 100 mg/kg/day.

Test Facility Inveresk (2000b)

9.3 Genotoxicity

9.3.1.1 *Salmonella typhimurium* Reverse Mutation Assay (San R, 1994)

Strains:	*Salmonella typhimurium* TA1535, TA1537, TA1538, TA98 and TA100
Concentration range:	100, 333, 1000, 3333 and 10000 μg/plate in ethanol Each concentration was tested in triplicate, with or without metabolic activation. Appropriate strain specific positive control reference substances were used.
Metabolic activation:	10% rat liver S9 fraction (Aroclor 1254-induced) in standard cofactors
Test method:	Ames Test
Comment:	Precipitation was evident at concentrations at and above 1000 μg/plate.

	Under the conditions of the study, the notified chemical caused no substantial increase in revertant colony numbers over control counts at any concentration in either the presence or absence of rat liver microsomal enzymes. All positive and negative controls responded appropriately and all criteria for a valid study were met.
Result:	The notified chemical was considered to be non-mutagenic under the conditions of the assay.

9.4.1.1 *Salmonella typhimurium* and *Escherichia coli* Reverse Mutation Assay (Willington SE, 1996)

Strains:	*Salmonella typhimurium* TA1535, TA1537, TA98 and TA100 *Escherichia coli* strain*:* WP2uvrA$^-$
Concentration range:	Experiment 1: 6.7, 20, 66.7, 200, 666.8 and 2000 μg/plate in DMSO Experiment 2: 62.5, 125, 250, 500, 1000 and 2000 μg/plate in DMSO Each concentration was tested in triplicate, with or without metabolic activation using a preincubation period of 20 minutes before addition of top agar. Appropriate strain specific positive control reference substances were used.
Metabolic activation:	10% rat liver S9 fraction (Aroclor 1254-induced) in standard cofactors
Test method:	Japanese MHW Guidelines on Toxicity Studies
Comment:	Precipitation was evident at concentrations at and above 1000 μg/plate. Under the conditions of the study, the notified chemical caused no substantial increases in revertant colony numbers over control counts at any concentration in either the presence or absence of rat liver microsomal enzymes. All positive and negative controls responded appropriately and all criteria for a valid study were met.
Result:	The notified chemical was considered to be non-mutagenic

under the conditions of the assay.

9.3.2 Chromosomal Aberration Assay in Chinese Hamster Ovary Cells (Murie E, 1996)

Cells: Chinese Hamster Ovary cells

Metabolic activation system: liver fraction (S9 mix) from rats pretreated with Aroclor 1254

Experimental design: Two independent experiments were conducted in duplicate. The experimental design and concentrations tested are tabulated below.

Metabolic Activation	Experiment	Test substance concentration (µg/mL)	Controls
-S9	Experiment 1	24 hour harvest: 0, 0.20, 0.39, 0.078*, 0.156, 0.313, 0.625, 1.250, 2.50* and 5.0*	Positive: 0.01-0.04 mg/mL methyl methane-sulfonate (MMS) Negative: 500 mg/mL DMSO
	Experiment 2	24 hour harvest: 0, 0.39*, 0.078, 0.156, 1.25, 2.5* and 5.0* 48 hour harvest: 0, 0.20*, 0.39, 0.078, 0.156, 1.250*, 2.50* and 5.0	Positive: 0.01-0.04 mg/mL MMS Negative: 500 mg/mL DMSO
+S9	Experiment 1	8 hour harvest: 0, 0.20, 0.39, 0.078, 0.156, 0.313, 0.625, 1.250*, 2.50* and 5.0*	Positive: 0.02-0.6 mg/mL cyclophosphamide Negative: 500 mg/mL DMSO
	Experiment 2	24 hour harvest: 0, 0.625*, 1.25, 2.5* and 5* mg/mL 48 hour harvest 0, 0.625*, 1.25*, 2.5 and 5* mg/mL	Positive: 0.02-0.6 mg/mL CP Negative: 500 mg/mL DMSO

* cultures selected for metaphase analysis

Test method: OECD TG 473

Comment: *Experiment 1*
The notified chemical was toxic and non clastogenic.

Experiment 2
There were several dose points, both with and without metabolic activation that produced borderline results. Since these were not reproduced in duplicate culture or were not

dose-related, they were considered to be sporadic.

In the presence of S9, cultures harvested at 48 hours had a significantly increased incidence of polyploidy at the high concentration of 5 mg/mL, indicating that the test substance was capable of inducing a moderate increase in numerical aberrations.

Positive controls caused marked increases in the incidence of aberrant cells and the activity of the S9 fraction was found to be satisfactory. All criteria for a valid assay were met.

Result: The notified polymer was not considered to be clastogenic under the conditions of the chromosomal aberration assay.

9.3.3 Micronucleus Assay in the Bone Marrow Cells of the (Holmstrom LM, 1997)

Species/strain: Mouse/CD-1

Number and sex of animals: 5/sex/group

Doses: 250, 500 and 1000 mg/kg test substance in maize oil, dosed at 0 and 24 hours and sampled at 48 hours;
vehicle and positive control (50 mg/kg CP) animals were treated in parallel

Method of administration: Intraperitoneal injection

Test method: OECD TG 474

Comment: Clinical signs of subdued behaviour, hunched appearance, piloerection, laboured breathing, wet perigenital area, unable to use right hindlimbs, swollen abdomen, eyes half closed, abdomen dark in colour, rolling gait, tremors, discharge (eyes), eyes closed and agitated were recorded. Due to the severity of the clinical signs observed in high dose group, these animals were not treated on Day 2.

Three males in the high dose group and one female in the mid dose group died, and one female in the vehicle control group was killed on Day 2 because of clinical signs of distress.

The test substance did not induce micronuclei in the bone marrow of mice when tested to the maximum tolerated dose of 1000 mg/kg/day.

All positive and negative controls responded appropriately

and all criteria for a valid study were met.

Result: The notified chemical was considered to be non-clastogenic in the *in vivo* mouse bone marrow test.

9.4 Overall Assessment of Toxicological Data

Toxicity Assessment
The notified chemical was of very low acute oral toxicity (LD_{50}>2 000 mg/kg) and low acute dermal toxicity (LD_{50}>2 000 mg/kg) in rats. It was non-irritant in the skin and eye of rabbits.

In a skin sensitisation test using the maximisation method in guinea pigs, challenge with 65% test substance resulted in 12/20 responses and in 1/10 responses in test and control animals, respectively. All reactions consisted of slight erythema (score 1) except the responding animal in the vehicle control group (score 2). In a rechallenge with 50% test substance, 4/20 test animals with slight reactions (score 1) were noted and none in the control group. The testing laboratory considered that the rechallenge results gave a more accurate representation of the true sensitisation potential of the test substance. Another skin sensitisation study, employing swelling of the mouse ear as the endpoint, did not demonstrate any sensitisation potential by the notified chemical. This test can only reliably detect moderate to strong sensitisers. The notifier sought expert opinions to resolve the inconclusive outcome of the maximisation test in guinea pigs. The Health and Safety Executive (UK) (Partridge H, 1998) considered that, on balance, the reactions seen in the reported study (Wilson JA, 1996e) were consistent with irritation. Professor Hess of Registration and Consulting Company Ltd, concluded that on account of the equivocal results on the guinea pig maximisation test and the clearly negative result obtained in the mouse ear swelling assay, the present evidence is insufficient to classify the notified chemical as a sensitiser (Hess R, 1998).

In a 4-week oral rat study at 15, 150 and 1 000 mg/kg/d, tubular nephrosis and diffuse cell hyperplasia of the transitional epithelium of the bladder were observed in all animals receiving the high dose treatment. In the recovery study, there was an increased incidence of basophilic tubules in both sexes of the high dose. Equivocal evidence of toxicity at the intermediate dose included a slight decrease in food consumption in the female animals and a decrease in urine specific gravity in males. A second histopathology study was undertaken after the completion of the main study, to investigate the urinary bladders of the low and intermediate dose animals. The study showed simple diffuse cell hyperplasia of the transitional epithelium of the bladder in all males and 2 females in the intermediate group. Based on these findings, the notified chemical was considered to have a NOEL of 15 mg/kg/day.

In a 4-week dermal rat study at 30, 100 and 1 000 mg/kg/d, diffuse cell hyperplasia of the transitional epithelium of the bladder was observed in all treated animals, with the finding also observed in recovery animals. A second histopathology study was undertaken after the completion of the main study, to investigate the urinary bladders of the low and intermediate dose animals. The study showed simple diffuse cell hyperplasia of the transitional epithelium of the bladder at mild to minimal severity in males and females in the low and intermediate groups. Based on low incidence and minimal severity of the effect at the low dose, the No Observed Adverse Effect Level (NOAEL) is 100 mg/kg/day, with no NOEL established.

An oral gavage prenatal development toxicity study employed doses of 30, 300 and 1000 mg/kg/day and found similar kidney effects on dams as in the pilot study. There were no treatment-related mortalities, or abnormal effects on uterine parameters, foetal development, or skeleton and viscera. The NOEL for maternal effects was 30 mg/kg/day, and 1000 mg/kg/day for developmental effects. It was concluded that the notified chemical was not a teratogen under the conditions of the study.

The notified chemical was not considered mutagenic in two independent bacterial reverse mutation assays. An *in vitro* chromosome aberration assay in Chinese hamster ovary cells did not detect clastogenic activity of the notified chemical, but there was clear evidence of numerical aberrations (polyploidy). This finding was considered to be of little significance because of the failure to detect clastogenic activity with the *in vivo* mouse micronucleus assay. The overall conclusion therefore is that the notified chemical is not genotoxic and the observed polyploidy activity may probably have arisen from culture artifacts relating to pH and osmotic effects that are often seen in *in vitro* assays at higher test doses.

Hazard Classification
Based on the data provided, the notified chemical is not classified as hazardous according to the NOHSC *Approved Criteria for Classifying Hazardous Substances* (National Occupational Health and Safety Commission, 1999a).

10. ASSESSMENT OF ENVIRONMENTAL EFFECTS

The notifier has supplied ecotoxicity studies on the notified chemical, which are summarised in the following table: Additional studies provided in the application for an Extension to the Original Assessment Certificate are denoted with an asterisk.

Test	Species	Test concentrations (nominal) mg/L	Results mg/L
Acute Toxicity - (Semi-Static Test) (OECD TG 203)	Rainbow trout (*Oncorhynchus mykiss*)	0.75, 1.5, 3, 6, 12 & 24	96 h LC_{50} = 4.36
Acute Toxicity - (Semi-Static Test)	Orange killifish (*Oryzias latipes*)		48 h LC_{50} = 163
Acute Toxicity -Immobilisation (Static Test) (OECD TG 202 part 1)	Water Flea (*Daphnia magna*)	12.5, 25, 50, 100 & 200	48 h EC_{50} = 19.37
Growth Inhibition - Growth (μ) & Biomass (b) (Static Test) (OECD TG 201)	Green Algae (*Selenastrum capricornutum*)	6.25, 12.5, 25, 50 & 100	72 h $E\mu C_{50}$ = 114.79 72 h E_bC_{50} = 18.68 72 h NOEC = 5.5
Chronic toxicity	Rainbow trout (*Oncorhynchus mykiss*)	0.068 to 2.18	MATC 1.61 (compared to solvent control) MATC 0.67 (water control)
Chronic toxicity (OECD TG 211)	Water Flea (*Daphnia magna*)	0.02 to 10	21 d NOEC 1.357
Activated Sludge, Respiration Inhibition (OECD TG 209)		10 to 1000	NOEC 31.6

MATC = Maximum acceptable toxicant concentration

The tests were performed in compliance with OECD/EEC Test Methods and according to OECD Principles of Good Laboratory Practice.

10.1 Fish

The acute toxicity of the notified chemical to Rainbow trout was determined in a 96 hour semi-static test. To ensure the maintenance of satisfactory environmental conditions and near nominal exposure levels the test medium was renewed daily. The test media were reported as solution/suspensions with undissolved material noticed at the base of the test tanks but with clear water columns. All samples were taken from the water column and filtered prior to analysis. The arithmetic mean calculated concentrations of soluble notified chemical were determined by the notifier to be 0.32, 0.71, 1.46, 2.98, 6.37 and 10.16 mg/L, with the triazine component 1 much lower than in the test material.

The 96 hour LC_{50} of the notified chemical was determined by the notifier using Probit analysis to be 4.36 mg/L and the highest concentration tested without toxic effects was 2.98 mg/L. The notifier used Berkson's modification of Probit analysis and no confidence limits were reported. Also, no raw exposure data was provided, precluding independent confirmation. Marked reactions to exposure, other than death, included swimming at the bottom, loss of equilibrium and erratic swimming.

The acute toxicity of the notified chemical to Orange killifish was also briefly reported by the notifier in a 48 hour semi-static test which was reported preliminary to the bioaccumulation study. The 48 hour LC_{50} of the notified chemical was determined by the notifier to be 163 mg/L. The test sample concentrations ranged from 39.5 to 300 mg/L but no raw data or method of calculation were given. Also, the 48 hour LC_{50} 163 mg/L is well above the reported water solubility of the notified chemical, though no observations on the clarity of the solutions are given.

The following study was submitted with the application for Extension.

Chronic toxicity to rainbow trout embryos and larvae

Test Substance	Notified chemical.
Method	USEPA, OPPTS Ecological Effects Test Guidelines, OPPTS 850.1400 Fish Early Life Stage Toxicity Test.
Species	Rainbow Trout (*Oncorhynchus mykiss)* embryos
Exposure Period	60 days post-hatch (93 days overall).
Auxiliary Solvent	Methanol (50µl/L).
Water Hardness	41.3 – 51.3 mg $CaCO_3$/L
Analytical Monitoring	Test concentrations were determined by HPLC.
Remarks – Method	A dynamic (flow through) test system was used for this study.

Results

Concentration mg/L	
Nominal	*Actual*

Dilution Water Control	Solvent Control
0.068	-
0.136	0.089
0.273	0.204
0.545	0.412
1.09	1.083
2.18	2.407

NOEC (mg/L) and LOEC (mg/L) with dilution water and solvent controls pooled:

Hatch		Survival		Length of larvae at 60 days post hatch		Weights of Larvae at 60 days post hatch	
NOEC	LOEC	NOEC	LOEC	NOEC	LOEC	NOEC	LOEC
2.407	2.407	1.083	2.407	0.412	1.083	1.083	2.407

Remarks – Results	Percentage hatch ranged from 74 to 82%, percentage survival at 60 days from 69 – 88%, larval length at 60 days from 41 to 48 mm and larval weight at 60 days from 1.19 to 1.74 g for pooled replicates.
Conclusion	The Maximum Acceptable Toxicant Concentration (MATC) expressed as a geometric mean of NOEC and LOEC was 1.61 mg/L based on a comparison to the solvent control and 0.67 mg/L based on comparisons to the dilution water control or to pooled controls.
Test Facility	Inveresk (2000c)

10.2 Aquatic Invertebrates

The acute toxicity of the notified chemical to *Daphnia magna* was determined in a 48 hour static test. The 200 and 100 mg/L nominal test samples are well above the water solubility reported for the notified chemical. Test media were reported as solution/suspensions with undissolved material noticed at the base of the test tanks but with clear water columns. Ultrasonication was used to aid dispersion of the test material. All samples were taken from the water column and filtered prior to analysis. The arithmetic mean calculated concentrations were determined by the notifier to be 0.04, 9.65, 13.39, 20.66, 31.95 and 58.99 mg/L.

The 24 and 48 hour EC_{50} of the notified chemical were determined by the notifier using Probit analysis to be 15.03 mg/L and 19.37 mg/L, respectively. The highest concentration tested without toxic effects was 9.65 mg/L. The lowest concentration tested causing 100% immobilisation was 20.66 mg/L. No raw exposure data was provided, precluding independent confirmation.

The following study was submitted with the application for Extension.

Effects of survival and reproduction of aquatic invertebrates

Test Substance	Notified chemical.
Method	OECD TG 211 and US EPA TSCA Guideline 40 CFR Section 797.1330.
Species	*Daphnia magna*
Exposure Period	21 days
Auxiliary Solvent	Methanol.
Water Hardness	158 - 172 mg $CaCO_3$/L
Analytical Monitoring	HPLC
Remarks – Method	Test solutions were renewed at 48 hr intervals throughout the 22 test period.

Results

Concentration mg/L		*Number of Adult D. magna immobilised*
Nominal	*Actual*	*22 d*
0	-	
0.02	-	
0.063	-	1 (2.5%)
0.20	0.162	
0.633	0.552	
2.00	1.357	
6.33	4.149	9 (22.5%)
10	6.924	16 (40%)

The EC_{50} for survival of adults is >6.924 mg/L and NOEC for immobilisation of adult *daphnia* is 1.357 mg/L.

Effects on *Daphnia* Reproduction:

Time (days)	EC50 Reproduction (mean measured concentrations of notified chemical mg/L)
14	3.721
21	4.360
22	4.604

NOEC for reproduction	1.357 mg/L at days 14, 21 and 22.
Remarks – Results	The first offspring were observed at all test concentrations, with the exception of 6.924 mg/L at 9 days after initiation of the study. Off spring from the 6.924 mg/L concentration group were observed on day 10.
Conclusion	The notified chemical has a significant effect on *Daphnia* survival and reproduction at concentrations greater than 1.357 mg/L over a 22 day period under the test conditions indicated.
Test Facility	Inveresk (2000d)

10.3 Algae

The acute toxicity of the notified chemical to Algae was determined in a 72 hour static test. The 100 mg/L nominal test sample is well above the water solubility reported for the notified chemical and the test media were reported as solution/suspensions. Ultrasonication was used to aid dispersion of the test material. All samples were taken from the water column and filtered prior to analysis. The arithmetic mean calculated concentrations were determined by the notifier to be 5.5, 10, 12.5, 27.3and 42.9 mg/L.

The 72 hour inhibition rates calculated for both algal biomass and growth rate were determined by the notifier using Probit analysis to be E_bC_{50} = 18.68 mg/L and $E\mu C_{50}$ = 114.79 mg/L, respectively. The no-observed effect concentration was determined to be 5.5 mg/L. No raw exposure data was provided.

10.4 Microorganisms

No data for the inhibitory effect of the notified chemical on aerobic wastewater bacteria from a domestic wastewater treatment plant, was supplied. However, a statement was supplied as part of the ready biodegradation study that an inhibition test showed that the notified chemical was not inhibitory to bacteria in activated sludge at a concentration of 46 mg/L.

The following study was submitted with the application for Extension.

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.
Exposure Period	3 hours
Concentration Range Nominal	10 - 1000 mg/L
Remarks – Method	
Results	
EC50	> 1000 mg/L
NOEC	31.6 mg/L
Remarks – Results	No respiration inhibition was observed at 1000 mg/L but a maximum inhibition of 34.7% was observed at 316 mg/L. This indicated a limited solubility of the notified chemical at higher concentrations.
Conclusion	The EC50 for a 3 h contact time, based on respiration inhibition, was > 1000 mg/L MBC. The results indicated a poor solubility of the test item at the top concentration, limiting the effects observed. The EC20 was estimated at 156.74 mg/L.
Test Facility	Inveresk (2003).

Conclusion

The original ecotoxicity data for the notified chemical suggest that it is moderately toxic to fish and harmful to aquatic invertebrates and algae, though deficiencies in the tests should be noted. The additional data provided with the application for an Extension indicated that the notified chemical is slightly toxic to fish and Daphnia, non-inhibitory to microorganisms and is not ready biodegradable.

It is noted that the two MSDS submitted for Larotact LR 9018 in the application for Extension state that the notified chemical is classified as a hazardous substance in the EU with the following risk phrases: R51/53 – Toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment.

11. ASSESSMENT OF ENVIRONMENTAL RISK

The notified chemical cross-links with other paint components to form a very high molecular weight and stable film that adheres firmly to the primer layer to which it is applied. The chemical, as part of this surface coating will share the fate of the vehicle panel. The paint will slowly deteriorate under the action of UV light, but this deterioration will be negligible over the life of the motor vehicle. When the vehicle panel is recycled, the polymer would be destroyed through incineration.

The majority of notified chemical associated with waste from the application of the coating to the automotive surface should not enter the environment until it is disposed of to landfill. Movement of the chemical by leaching from landfill sites is not expected because of the lack of mobility due to either the low water solubility, high binding affinity to soil or cross-linking in the cured coating.

In the event of accidental spillage of the chemical solution into waterways, the notified chemical is not expected to disperse into the water, but settle out onto sediments. If the chemical is spilt on land, it is expected to become immobilised in the soil layer. Contaminated soil can then be collected and disposed to landfill.

Given the above, environmental exposure and the overall environmental risk are expected to be low.

12. ASSESSMENT OF PUBLIC AND OCCUPATIONAL HEALTH AND SAFETY EFFECTS

Hazard assessment

The notifier provided toxicological studies in support of their application for an assessment certificate. The notified chemical exhibited very low acute oral toxicity (LD_{50}>2 000 mg/kg) and low acute dermal toxicity (LD_{50}>2 000 mg/kg) in rats. Acute inhalation studies have not been conducted on the notified chemical. Claims were made and accepted for variation of the schedule requirements for this toxicological end point. In rabbits, the notified chemical was not a skin and eye irritant.

In a skin sensitisation test using the maximisation method in guinea pigs, slight erythema was observed in 12/20 and 4/20 animals challenge with 65% and 50% notified chemical, respectively. Another skin sensitisation study employing swelling of the mouse ear as the end point did not demonstrate any sensitisation potential by the notified chemical. Because of the equivocal results on the guinea pig maximisation test and the clearly negative result obtained in the mouse ear swelling assay, the present evidence is insufficient to classify the sensitisation potential of the notified chemical.

In a 28-day repeated dose oral study, histological findings in the high and intermediate groups suggest that the kidney may be a target organ. Kidney damage was seen as tubular nephrosis resulting in altered haematology, clinical chemistry and urinary parameters in all animals receiving the high dose treatment. In high and intermediate dose, diffuse cell hyperplasia of the transitional epithelium of the bladder was observed. Basophilic tubules and inflammatory cell foci were increased in high dose recovery animals suggesting the reversibility of kidney effects seen at the end of exposure period. Based of the above findings, the notified chemical was considered to have a NOEL of 15 mg/kg/day.

In a 28-day repeated dose dermal study submitted with the application for Extension, similarities with the findings of the oral study were noted, namely, diffuse cell hyperplasia of the transitional epithelium of the urinary bladder. The effect was observed at all doses, however, based on low incidence and minimal severity of the effect at the low dose, the No Observed Adverse Effect Level (NOAEL) is 100 mg/kg/day, with no NOEL established (low dose 30 mg/kg/day).

There were no treatment-related mortalities, or abnormal effects on uterine parameters, foetal development, or skeleton and viscera reported in an oral prenatal development toxicity study. The NOEL for maternal effects was 30 mg/kg/day, and 1000 mg/kg/day for developmental effects. It was concluded that the notified chemical was not a teratogen under the conditions of the study.

The notified chemical was not considered mutagenic in two independent bacterial reverse mutation assays. An *in vitro* chromosome aberration assay in Chinese hamster ovary cells did not detect clastogenic activity of the notified chemical, but there was clear evidence of numerical aberrations (polyploidy). The overall conclusion is that the notified chemical is not genotoxic.

Hazard classification

Based on the toxicological data submitted, the notified chemical would not be classified as a hazardous substance under the NOHSC Approved Criteria for Classifying Hazardous Substances (National Occupational Health and Safety Commission, 1999a). However, it is noted that the two MSDS submitted for Larotact LR 9018 in the application for Extension state that the notified chemical is a skin sensitiser with the risk phrase R43 – May cause sensitisation by skin contact.

Larotact LR 9018 (CYLINK$^+$ 2000 Crosslinking Agent) is classified as a hazardous substance in accordance to the NOHSC *Approved Criteria for Classifying Hazardous Substances* (National Occupational Health and Safety Commission, 1999a) because of the presence of n-butanol at ≥ 25%. The classification for n-butanol is Harmful (Xn) in accordance to the NOHSC *List of Designated Hazardous Substances* and warrants the risk phrase: Harmful by inhalation (R20) (National Occupational Health and Safety Commission,

1999b). The EEC Council Directive has recently updated the EC classification for n-butanol and the new classification is expected to be adopted by NOHSC. The new classification for n-butanol are: Harmful (Xn) and Irritant (Xn) with risk phrases: Harmful if swallowed (R22); Irritating to respiratory system and skin (R37/38); Risk of serious damage to eyes (R41); and Vapours may cause drowsiness and dizziness (R67).

Occupational Health and Safety Risk

Transport and Storage
Exposure to the notified chemical is not expected during transport or storage as long as the packaging remains intact. Exposure after a spill would be controlled by use of the recommended practices for spillage clean up given in the MSDS supplied by the notifier. The risk of adverse health effects for transport and storage workers is considered low.

In accordance with the Australian Code for the Transport of Dangerous Goods by Road and Rail (Federal Office of Road Safety, 1992), Larotact LR 9018 is classified as Dangerous Goods (Class 3) because of the solvent content. The required precautions should be taken during transport, storage and handling.

Paint Manufacture
The paint manufacture is a closed automated process. There is potential for production operators to be exposed by skin contact to the notified chemical at 33 – 88% (as imported) and up to 2.2% (when mixed) when connecting, disconnecting and cleaning the blending equipment, therefore, there is risk of skin sensitising effects. Similarly, filling operators may experience dermal exposure to spills and drips containing up to 2.2% notified chemical. Paint manufacture and filling are conducted under exhaust ventilation and personal protective equipment including impervious gloves, overalls and safety glasses is recommended. Laboratory personnel may be dermally exposed to the notified chemical when collecting test samples. These workers also wear protective equipment as described above. The manufacture and storage areas are bunded. The overall risk of adverse health effects arising from exposure to the notified chemical during paint manufacture is low because of the relatively low toxicity of the notified chemical and the control measures provided to minimise exposure, however, precautions are required to minimise the risk of allergic skin reactions.

Due to the presence of n-butanol in the imported product, employers must to ensure that the NOHSC exposure standard for n-butanol of 50 ppm peak limitation is not exceeded in the workplace (National Occupational Health and Safety Commission, 1995). N-butanol has been assigned with skin notation (National Occupational Health and Safety Commission, 1995) indicating that skin absorption could occur. The required control measures should be taken to prevent absorption through the skin.

Paint Application
The paint will be predominantly applied using automatic electrostatic atomised sprayer (robotic systems) and no worker exposure is anticipated during this process. There is potential for dermal exposure to the notified chemical for spray operators connecting and disconnecting pumps and pipes during transfer operations and cleaning the spray equipment. Workers will wear impervious gloves, anti-static coveralls, anti-static footwear and eye protection. Touch up using manual spraying will be carried out in a spray booth fitted with fume extraction system. Dermal and eye contact, and inhalation of vapour or spray mist are

possible. Spray painters will wear as a minimum protection, nylon gloves, anti-static overalls, anti-static foot wear, calico hoods, and eye protection and cartridge type respirators. The risk of adverse health effects due to exposure to the notified chemical is low during paint application due to the low concentration of notified chemical in the paint and the high level of engineering control.

Curing Process
During the curing process the notified chemical reacts with other components in the paint formulation to form an integral part of the paint film. Once curing is complete, the notified chemical is not bioavailable.

Public Health Risk

Surface coating products containing the notified chemical will be used only in the automotive industry. Although members of the public will make contact with articles coated with products containing the notified chemical, exposure will be negligible because of the low concentrations of the notified chemical in these products and the notified chemical being bound within the coatings.

Based on the toxicity profile and use pattern of the notified chemical, it is considered that the notified chemical will not pose a significant risk to public health.

13. RECOMMENDATIONS

Health surveillance

- As potential for skin and respiratory sensitisation exist, the notifier's MSDS should be provided to the authorised medical practitioner responsible for health surveillance in the workplace. Sensitised persons should not continue to handle the notified chemical and should be transferred to another workplace.

Occupational health and safety
To minimise occupational exposure to the notified chemical and the product containing the notified chemical the following guidelines and precautions should be observed:

- Employers should implement the following engineering controls to minimise occupational exposure to the notified chemical:
  - Enclose processes as much as possible
  - Use local exhaust ventilation where process cannot be enclosed

- Spray painting booths should conform to AS/NZS 4114 (Standards Australia/Standards New Zealand, 1994d) and spraying should be conducted in accordance with the NOHSC ;

- Employers should implement the following safe work practices to minimise occupational exposure during handling of the notified chemical::
  - Avoid skin and eye contact

- Employers should ensure that the following personal protective equipment is used by workers to minimise occupational exposure to the notified chemical::

- Chemical protective clothing, e.g. coveralls
- Chemical resistant gloves
- Goggles or safety glasses
- Safety footwear

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

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

- If products and mixtures containing the notified chemical are classified as hazardous to health in accordance with the 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.

- Spillage of the notified chemical should be avoided. Spillages should be cleaned up promptly with absorbents which should be put into containers for disposal;

- Good personal hygiene should be practised to minimise the potential for ingestion;

Employers should ensure that the NOHSC exposure standard for n-butanol of 50 ppm peak limitation is not exceeded during all phases where worker exposure to the product containing the notified chemical may occur. Control measures should be taken to prevent absorption through the skin.

If the conditions of use are varied, greater exposure of the public to the product may occur. In such circumstances, further information may be required to assess the hazards to public health.

14. MATERIAL SAFETY DATA SHEET

The MSDS for the notified chemical were provided in a format consistent with the *National Code of Practice for the Preparation of Material Safety Data Sheets* (National Occupational Health and Safety Commission, 1994).

This MSDS were provided by the applicant as part of the notification statement. They are reproduced here as a matter of public record. The accuracy of this information remains the responsibility of the applicant.

15. REQUIREMENTS FOR 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 subsection 64(1) of the Act; if
- the importation volume exceeds one tonne per annum notified chemical; or
- the notified chemical will be imported in solid form;

- an inhalation study for the notified chemical becomes available
- the method of use changes in such a way as to greatly increase the exposure of the notified chemical
- additional information becomes available on adverse health or environmental effects of the chemical; or

(2) Under subsection 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.

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Attachment 1

The Draize Scale for evaluation of skin reactions is as follows:

Erythema Formation	*Rating*	*Oedema Formation*	*Rating*
No erythema	0	No oedema	0
Very slight erythema (barely perceptible)	1	Very slight oedema (barely perceptible)	1
Well-defined erythema	2	Slight oedema (edges of area well-defined by definite raising	2
Moderate to severe erythema	3	Moderate oedema (raised approx. 1 mm)	3
Severe erythema (beet redness)	4	Severe oedema (raised more than 1 mm and extending beyond area of exposure)	4

The Draize scale for evaluation of eye reactions is as follows:

CORNEA

Opacity	*Rating*	*Area of Cornea involved*	*Rating*
No opacity	0 none	25% or less (not zero)	1
Diffuse area, details of iris clearly visible	1 slight	25% to 50%	2
Easily visible translucent areas, details of iris slightly obscure	2 mild	50% to 75%	3
Opalescent areas, no details of iris visible, size of pupil barely discernible	3 moderate	Greater than 75%	4
Opaque, iris invisible	4 severe		

CONJUNCTIVAE

Redness	*Rating*	*Chemosis*	*Rating*	*Discharge*	*Rating*
Vessels normal	0 none	No swelling	0 none	No discharge	0 none
Vessels definitely injected above normal	1 slight	Any swelling above normal	1 slight	Any amount different from normal	1 slight
More diffuse, deeper crimson red with individual vessels not easily discernible	2 mod.	Obvious swelling with partial eversion of lids	2 mild	Discharge with moistening of lids and adjacent hairs	2 mod.
		Swelling with lids half-closed	3 mod.	Discharge with moistening of lids and hairs and considerable area around eye	3 severe
Diffuse beefy red	3 severe	Swelling with lids half-closed to completely closed	4 severe		

IRIS

Values	*Rating*
Normal	0 none
Folds above normal, congestion, swelling, circumcorneal injection, iris reacts to light	1 slight
No reaction to light, haemorrhage, gross destruction	2 severe