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April 2000

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME

FULL PUBLIC REPORT

Acid Yellow HT 2803

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Director
Chemicals Notification and Assessment

FULL PUBLIC REPORT

Acid Yellow HT 2803

1. APPLICANT

Ciba Specialty Chemicals of 235 Settlement Road, THOMASTOWN VIC 3074 has submitted a standard notification statement in support of their application for an assessment certificate for Acid Yellow HT 2803.

2. IDENTITY OF THE CHEMICAL

The chemical name, CAS number, molecular and structural formulae, spectral data, details of composition and purity, and details of identity of manufacturing sites have been exempted from publication in the Full Public Report and the Summary Report.

Other Names:	Monoazo Yellow HT 2803; Acid Yellow dye; FAT 45'111/B
Marketing Name:	Acid Yellow HT 2803 (the notified chemical); Erionyl Yellow A-3G (contains typically 70-80% of the notified chemical)
Molecular Weight:	663.5 g/mol; calculated from the molecular formula
Method of Detection and Determination:	ultraviolet/visible (UV/Vis), infrared (IR), nuclear magnetic resonance (NMR) spectroscopy, physical testing and high performance liquid chromatography (HPLC)
Spectral Data:	Spectral data were submitted to characterise the identity of the notified chemical.

Comments on Chemical Identity

Reports with ^{1}H NMR UV/Vis and IR spectrometric data were submitted for the identification of the notified substance.

HPLC analysis determined the composition of the notified substance, including the main component and minor impurity by-products. Potentiometric and the Karl Fischer titration methods determined chloride and water content, respectively.

3. PHYSICAL AND CHEMICAL PROPERTIES

All the physico-chemical studies were performed on the notified chemical Acid Yellow HT 2803.

Appearance at 20°C and 101.3 kPa:	Yellow crystalline powder
Boiling Point/Melting Point:	Decomposes at 218°C
Density:	1.44 g/cm^3 determined using a 10 mL glass pycnometer
Vapour Pressure:	Not determined
Particle Size Distribution:	> 800 μm (3%) > 400 μm (17%) > 200 μm (32%) > 100 μm (43%) > 63 μm (54%) > 40 μm (65%) > 20 μm (89%) < 20 μm (11%) median mass diameter 76 μm
Water Solubility:	19.4 g/L at 20°C
Partition Co-efficient (n-octanol/water):	log P_{ow} 0.99 at 21°C
Hydrolysis as a Function of pH:	$T_{1/2}$ > 1 year tested within the environmental pH range
Adsorption/Desorption:	Not determined (see comments below)
Dissociation Constant:	pK_a 4.4 (see comments below)
Flash Point:	Not flammable
Flammability Limits:	Not flammable; combustible
Autoignition Temperature:	Not auto-flammable (tested to 330°C)
Explosive Properties:	Not considered explosive
Reactivity/Stability:	Not an oxidising substance; Thermally stable
Fixation Rate:	In wool: 95%; In polyamide: 97%

Fat Solubility: 0.22 g/kg at 37ºC

Surface Tension: 46.9 mN/m (aqueous solution of notified chemical)

Comments on Physico-Chemical Properties

Tests were performed according to EEC/OECD test guidelines at facilities complying with OECD Principles of Good Laboratory Practice.

Particle size distribution was determined for the test substance using the sieving method, with details supplied for particles > 20 μm. In the absence of detailed measurement for particles < 20 μm and given that 7 μm is defined as the cut-off particle diameter for respirable dust (NOHSC, 1995), it is considered that a maximum of 11% of the test substance may be in the respirable range.

The maximum water solubility of Acid Yellow HT 2803 was determined to be 19.4 g/L at 20°C using the flask method (OECD TG 105).

The notified chemical was found to be hydrolytically stable with < 10% degradation at 50ºC and pH 4, 7 and at pH 9 as determined by OECD TG 111.

The partition coefficient log P_{OW} of Acid Yellow HT 2803 between n-octanol and water was estimated to be = 0.99 at 21ºC by the flask shaking method (OECD TG 107).

Adsorption/desorption data were not provided. The notifier has indicated that, given the notified chemical's low partition coefficient, log P_{ow} = 0.99, it would be expected to bind strongly to silicates in soils but that the free acid form may bind to organic matter. It is noted that the notified chemical would be expected to bind to silicates in soils due to its negative charge on the sulphonic acid. However, the strength of this binding is unclear since there is only the one negative charge on the chemical and it is reasonably hindered. The free acid would also be unlikely to form at the environmental pH range of 4-9.

The dissociation constant, K_a, of the notified chemical was calculated by the notifier using the Hammett correlation to be 4.0×10^{-5}. It is noted that the notified substance is soluble in water but it would be difficult to estimate an overall dissociation constant, K, for the notified chemical since it contains secondary, tertiary and azo amine groups as well as a sulphonic acid group.

4. PURITY OF THE CHEMICAL

Degree of Purity: high

Hazardous Impurities: None known

Non-hazardous Impurities (> 1% by weight): Identified by-products (2 of) 2.2%;
Unidentified by-products (6 of) 7.7%;
Chloride 2.8%;
Water 4.5%

Additives/Adjuvants: None in the notified chemical. For the commercial product, the chemical name and CAS No. were supplied

and accepted as exempt information.

Synonyms:	Dispersing Agent
Weight percentage:	10-30%

Synonyms:	Anti-dusting Agent
Weight percentage:	< 10%

Synonyms:	water
Weight percentage:	< 10%

5. USE, VOLUME AND FORMULATION

The notified chemical, a water-soluble azo dye, will not be manufactured in Australia, but will be imported by sea in 30 kg polyethylene lined sealed containers as a component (70-80% per weight) of the crystalline dye preparation Erionyl Yellow A-3G. It will be used for colouring polyamide, wool fibres and fabrics by the exhaust application method. The notifier has submitted evidence to show that the dye has a fixation performance of at least 97 and 95% to nylon and wool, respectively. However, at a deeper colour shade, said to be used usually once a month, the former may drop to 93%. The estimated import quantity of Erionyl Yellow A-3G, and therefore the notified substance is:

Year	***Erionyl Yellow A-3G***	***Acid Yellow HT 2803 (assuming 80% content in imported dye)***
1	< 1 tonne	< 800 kg
2 and 3	1-2 tonnes	800-1600 kg
4 and 5	3-4 tonnes	1.6-3.2 tonnes

6. OCCUPATIONAL EXPOSURE

Transport and storage: Only in the event of an accident

Category of workers	***Max. No. exposed***	***Route of exposure***	***Max. duration of exposure***	
			hour/day	***day/year***
Transport & Storage	10-15	Dermal, inhalation	Only in the event of an accident	

The commercial product containing the notified chemical is transported by road from the dock to the notifier's warehouses, where it will be stored under cover in a bunded area for subsequent delivery to customers. No repackaging of the dye will be carried out as it will be supplied to customers in the original containers; the product will initially be sold to 2-4 dye houses within Australia. Waterside, transport and storage workers would only be exposed to the notified chemical in the event of a spill from a transport or handling incident. The nature

of the packaging used for transport minimises the likelihood of accidental release or loss of the chemical. Approximately 10-15 workers will be involved in transportation and storage.

If spillage occurs, the notifier stated that spills would be dampened down and scooped up into containers and disposed of according to state regulations. Final residues will be washed down with detergent and water, only where adequate dilution is possible (1 000 fold). Persons involved in clean up tasks would be required to wear PVC long impervious gloves, overalls, glasses/eye protection and respiratory protection.

Weighing and mixing: 30min/person/day for 230 days/year

Category of workers	*Max. No. exposed*	*Route of exposure*	*Max. duration of exposure*	
			Min./day	*day/year*
Weighing & mixing	16	Dermal, inhalation	30	230

At dye houses (2-4), a total of 16 experienced operators (2/shift, 2 shifts/day) will be involved in weighing and mixing the dyes. The dyestuff (approximately 4 kg) is manually scooped from the containers into a sealable weighing vessel, which is charged to a mixing vessel. The dye is dissolved in 50-100 L of water with slow mechanical stirring at 90^{o}C for 10-20 min.

Workers may be exposed to the notified chemical during weighing and mixing of the powdered dyestuff and during disposal of empty used liner bags. Skin contact, eye contact and inhalation are the main routes of exposure to the product, of which a maximum of 11% is in the respirable range. However, the product Erionyl Yellow A-3G contains an anti-dusting agent, which reduces the potential for dust generation when handling the dyestuff.

Dust formation is minimised through the use of local exhaust ventilation over areas where weighing and adding to blending vessels is conducted. Also, batch weigh-men and operators receive induction training and refresher courses on the handling of the notified chemical.

The notifier stated that as part of existing practices, operators are required to wear overalls, protective gloves, glasses and respiratory protection (Class P2 particulate filter) during weighing and when handling the product.

The notifier stated that the notified chemical is a prescribed waste and residual Erionyl Yellow A-3G must be disposed of by approved secure landfill or by incineration in accordance with the Material Safety Data Sheet (MSDS) requirement.

Once Erionyl Yellow A-3G is dissolved, it is metered from the mixing vessel, by gravity using a metering pump, to the enclosed dyeing machine over a specified period. Potential for skin contact with the notified chemical at < 10% in solution may occur during mixing, while the operators connect/disconnect metering pump hoses from the mixing vessel to the dyeing machine, during clean up operations and maintenance of equipment. Inhalation exposure to the dissolved dye is considered negligible because the chemical is not volatile, and any aerosol formation during mixing would be controlled through the use of enclosed systems.

Although the notifier did not provide estimates for exposure from spills and drips during

clean up tasks, exposure to the notified chemical during cleaning operations is possible. Exposure may occur mainly by skin and/or eye contact, however, appropriate engineering controls are in place and personal protective equipment is used.

Dyeing: 20 min/person/day for 230 days/year

Category of workers	*Max. No. exposed*	*Type of exposure*	*Max. duration of exposure*	
			Min./day	*day/year*
Dyeing	64	Dermal	20	230

Typically, eight workers operate the dyeing machines during each shift (2 shifts/day, 230 days/year) at each dye house. A total of 64 operators may be exposed to the notified chemical during dyeing, mainly via the dermal route when handling wet dyed textiles or by eye contact through contaminated hands. Inhalation exposure is negligible since aerosols are contained within the enclosed dyeing system. The initial concentration of chemical in the dye solution was not provided. The dye application process occurs for approximately 20 minutes in every 3 hour dyeing cycle, therefore potential for worker exposure is expected to be limited to short periods.

The cloth to be dyed is manually loaded into the machine via a winch system, which feeds into a series of rollers designed so that the cloth is continuously cycled through the enclosed dyeing machine. Colouring occurs by exhausting the dyestuff in solution onto the fibre surface at 90-100ºC, where it migrates into the fibre and becomes bound. Any unfixed dye is removed from the textile by a cold rinse bath.

Manual handling of the wet dyed textiles may be required if the cloth becomes tangled, which presents potential for short-term exposure to the notified chemical mainly by skin contact. Given the anticipated low concentration of the notified chemical, the high fixation rate and the use of personal protective equipment, workers exposure is expected be low.

Exposure to the notified chemical may also occur during equipment clean up tasks, mainly via the dermal route. Operators regularly clean the filters on the dyeing machines, which are used to collect loose fibres, using a hose. Exposure to the notified chemical during these tasks is expected to be negligible since the dyeing machines are self-cleaning as a result of the washing cycle of the dyeing process. The dyestuff is highly fixed to fibres (95-97% fixation) and any unfixed dye is removed in the rinsing step.

Drying/curing: 45 min/person/day for 230 days/year

Category of workers	*Max. No. exposed*	*Type of exposure*	*Max. duration of exposure*	
			Min./day	*day/year*
Drying/curing	32	Dermal	45	230

A total of 32 workers may be exposed to the notified chemical during drying/curing of dyed textiles at dye houses using Erionyl Yellow A-3G. Minimal handling of the wet dyed textiles is expected, since mechanically operated winches are used to remove the textiles from the

dyeing machines. Moisture is removed from the wet textile by hydro-extraction and the textiles are dried through a stenter at 120-140ºC. This operation is conducted in a partly enclosed system and operators will be briefly exposed to the notified chemical, mainly by skin contact, as they transfer the textiles from the hydro-extractor to the stenter. However, gloves and safety goggles are worn during this operation. Exposure levels are expected to be low since the operation is partly an enclosed system, the notified chemical comprises < 10% of all dyes used, the dyestuff is highly fixed to fibre and any excess dye would have been washed off prior to drying.

Although not detailed by the notifier, operators may also be exposed to the notified chemical when handling dry textiles, for example during reeling and packaging. Dermal exposure during these operations is expected to be low, given the high fixation rate (95-97%) of the notified chemical and the prior rinsing away of unbound dye. As described above for dyeing, inhalation exposure is not expected.

Laboratory: 10 min/person/day for 230 days/year

Category of workers	***Max. No. exposed***	***Route of exposure***	***Max. duration of exposure***	
			Min./day	***day/year***
Laboratory	13	Dermal, inhalation	10	230

It is estimated that 13 laboratory technicians at all dye houses will be involved in the preparation of colour matches, which entail the weighing and mixing of small samples of dye. Exposure to the particulate or dissolved notified chemical during these operations may occur via the dermal, ocular or inhalation routes, but is expected to be low given the small quantities handled, the engineering control measures in place and the use of personal protective equipment.

Education and training

The notifier stated that all users of the notified chemical will be supplied with an MSDS, and engineering controls plus personal protective equipment will be recommended. Also, the notified chemical will be used in a prescribed way in a limited number of locations and will be handled by trained personnel. The notifier stated that seminars on safe handling are conducted, advice to customers and induction training and refresher courses to operators are provided, which are also available to customers upon request.

7. PUBLIC EXPOSURE

The dye containing the notified chemical will not be sold to the public. It is anticipated that the public may come in contact with fabrics dyed with the notified chemical. The dyestuff is highly fixed to fabrics (95-97% fixation) and any unfixed dye is removed in the rinsing step. The fastness to washing and dry cleaning is high and hence negligible residues will be found in cleaning liquors. When handling dry textiles, dermal exposure is expected to be low.

8. ENVIRONMENTAL EXPOSURE

Release

The bulk of the dye will become chemically fixed to the fibres of woollen and polyamide textiles, and in this state it is not expected to impact on the environment.

The major environmental exposure to dye will come from effluent discharge from city and country dye-houses and their wastewater treatment systems. Other releases will be limited to traces remaining from repackaging operations and clean up of any spills, and from trace residues in empty packaging.

All clean up of spills and disposal of empty packaging should be carried out according to the MSDS.

Fate

The bulk of the dye will become chemically fixed to the fibres of textiles with a fixation performance of > 95% at normal shades, while the remainder would be rinsed into wastewater. The fate of the majority of the notified chemical is linked with the fate of the textile and in this state it is not expected to impact on the environment. Eventually the textile will enter the waste disposal stream for either recycling or ultimately for disposal as waste in landfill. It is noted that once in landfill sites, movement of the chemical by leaching is not expected because of its expected high binding affinity to the non-organic component of soil.

The dye normally released in water as effluent from the dye-house is expected to be the major environmental exposure. The dye may either partition to the non-organic component of sediment, as expected, or stay in the aqueous compartment. Any dye that binds to the sludge during the waste treatment process would be disposed of through incineration or to landfill. Incineration is the preferred option because of the high water solubility and potential mobility of the material. Incineration of the dye will produce oxides of carbon, sulphur and nitrogen, together with ash and a small amount of hydrogen chloride. Disposal to landfill will be at a secured site, so the risk of leaching to the water table is significantly reduced.

Residues that persist after sewage treatment will enter marine and freshwater environments in solution from city and country wastewater treatment systems. The concentrations are expected to be very low because of the very high fixation rate in the initial process, the expected movement to sediment/sludge and the high dilution rates in the release processes.

Trace residues in empty liner bags are expected by the notifier to amount to approximately 5 g per liner. The bags will be flattened and bailed ready for disposal to an approved secure landfill in accordance with the MSDS requirements.

The dye was not found to be readily biodegradable. After 28-day exposure to micro-organisms from a domestic sewage treatment plant the rate of degradation amounted to 23.7% when estimated using the ratio of the biochemical oxygen demand (BOD) to the theoretical oxygen demand (ThOD) according to OECD TG 301C (Modified MITI Test).

The BOD of the notified chemical was determined in a 5 day closed bottle test at concentrations of the notified chemical of 2, 5 and 10 mg/L according to EEC Directive 84/449 Part C8/9. The chemical oxygen demand (COD) of the notified chemical was determined to be 1.291 mg O_2/mg. After the 5 day incubation period the net BOD of the notified chemical was 0.23, 0.01 and -0.24 mg O_2/L at concentrations of 2, 5 and 10 mg, respectively. Compared to the COD of the notified chemical the BOD was 8%, 0.1% and

-2% at concentrations of 2, 5 and 10 mg of the notified chemical, respectively.
Although the dye appears to be only slightly biodegradable, the potential for bio-accumulation is low due to the low partition coefficient (log P_{ow} = 0.99) of the substance and its expected high adsorption to sludge and other surfaces. Also, hydrophilic dyes with log P_{ow}<3 have been shown not to bioaccumulate (Yen *et al.*, 1991).

9. EVALUATION OF TOXICOLOGICAL DATA

9.1 Acute Toxicity

All toxicity studies were conducted on FAT 45'111/B, which is also referred to as Acid Yellow HT 2803.

Summary of the acute toxicity of FAT 45'111/B (Acid Yellow HT 2803)

Test	*Species*	*Outcome*	*Reference*
acute oral toxicity	rat	LD_{50} > 2 000 mg/kg bw	(Hoff and Althus, 1991a)
acute dermal toxicity	rat	LD_{50} > 2 000 mg/kg bw	(Hoff and Althus, 1991b)
skin irritation	rabbit	Very slight irritant	(Hoff and Lucini, 1991)
eye irritation	rabbit	Slight to moderate irritant	(Hoff, 1991a)
skin sensitisation	guinea pig	Moderate sensitiser	(Arcelin, 1991)

9.1.1 Oral Toxicity (Hoff and Althus, 1991a)

Species/strain:	Rat/Wistar
Number/sex of animals:	5/sex
Observation period:	15 days
Method of administration:	Oral (gavage); single dose of 2 000 mg/kg bw (dose volume 10 mL/kg bw)
Test method:	OECD TG 401; limit test
Mortality:	None
Clinical observations:	None
Morphological findings:	None
LD_{50}:	> 2 000 mg/kg bw
Result:	the notified chemical was of very low acute oral toxicity in a limit test in rats

9.1.2 Dermal Toxicity (Hoff and Althus, 1991b)

Species/strain:	Rat/Wistar
Number/sex of animals:	5/sex
Observation period:	15 days
Method of administration:	Single 24 hours, semi-occluded dermal application to an area of shorn intact skin on the back (equivalent to ~ 10% of total body surface area) at a dose level of 2 000 mg/kg bw (4 mL as a paste using a syringe); after the exposure period, residual test material was removed with lukewarm-tap water and dried with disposable paper towels.
Test method:	OECD TG 402; limit test
Mortality:	None
Clinical observations:	No signs of systemic toxicity were observed during the study period. All animals in both sex groups revealed grade 1 yellowing of the skin of application area, which persisted between days 2 and 8 (through to day 11 for one female) after treatment. Four males and two females developed fully reversible grade 1 scales at the application site, which persisted between days 3 and 9 following treatment. One male developed erythema (score 1) of skin at the application site, which occurred between days 2 and 3 following treatment.
Morphological findings:	No abnormalities observed at necropsy
LD_{50}:	> 2 000 mg/kg bw
Result:	the notified chemical was of low dermal toxicity in rats

9.1.3 Inhalation Toxicity

No inhalation study was submitted.

9.1.4 Skin Irritation (Hoff and Lucini, 1991)

Species/strain:	Rabbit/New Zealand White
Number/sex of animals:	2 males; 1 female
Observation period:	72 hours

Method of administration: 0.5 g of test substance moistened with double-distilled water applied to an area (~ 6 cm^2) of shorn intact skin of the back of each rabbit and held under semi-occlusive dressing. After 4 hours, residual test substance was removed with lukewarm-tap water.

Test method: OECD TG 404

Draize scores (Draize, 1959):

Skin reaction/ Animal				
		Observation Time (hours)		
Erythema	***1***	***24***	***48***	***72***
1	1	1	0	0
2	0	0	0	0
3	1	0	0	0
Oedema				
1	0	0	0	0
2	0	0	0	0
3	0	0	0	0

[a] see Attachment 1 for Draize scales

Comment: No acute clinical symptoms were observed in the animals during the test and observation period; no mortality occurred.

Yellow staining of the treated skin by pigment or colouring of the test substance was observed, which persisted throughout the study period.

No destruction, corrosive effects or irreversible alterations of the treated skin were observed.

The primary irritation index was 0.11

Result: the notified chemical was very slightly irritating to the skin of rabbits

9.1.5 Eye Irritation (Hoff, 1991a)

Species/strain: Rabbit/New Zealand White

Number/sex of animals: 2 males and 1 female

Observation period: 21 days

Method of administration: 0.1 g of undiluted test substance was instilled into the conjunctival sac of the left eye of each animal; the right eye

served as a control

Test method: OECD TG 405

Draize scores (Draize, 1959) of non-irrigated eyes:

	Time after instillation																	
Animal	***1 hr***			***1 day***			***2 days***			***3 days***			***7 days***			***14 days***		
Cornea	***o***		***a***	***o***		***a***	***o***		***a***	***o***		***a***	***o***		***a***	***o***		***a***
1	0		-	[1]		-	1		-	0		-	0		-	0		-
2	1		-	1		-	1		-	1		-	1		-	1		-
3	0		-	0		-	0		-	0		-	0		-	0		-
Iris																		
1	0			0			0			0			0			0		
2	0			0			1			1			0			0		
3	0			0			0			0			0			0		
Conjunctiva	***r***	***c***	***d***	***r***	***c***	***d***	***r***	***c***	***d***	***r***	***c***	***d***	***r***	***c***	***d***	***r***	***c***	***d***
1	1	1	3	1	1	1	1	0	0	1	0	0	0	0	0	0	0	0
2	1	2	3	2	2	3	2	1	3	2	1	3	1	0	1	1	0	0
3	1	1	1	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0

[1] see Attachment 1 for Draize scales
o = opacity a = area r = redness c = chemosis d = discharge - = not measured

Mean scores (24, 48, 72 hours observation):

Animal	***Corneal opacity***	***Iridial inflammation***	***Conjunctival redness***	***Conjunctival chemosis***
1	0.7	0	1	0.3
2	1	0.7	2	1.3
3	0	0	0.7	0.3

Comment:

No acute clinical symptoms were observed in the animals during the test and observation period, and no mortality occurred.

Yellowish staining of the lidhair by pigment or colouring of the test substance was observed in all animals; in one male and the female this persisted for 3 days following treatment, and for 7 days in the other male.

Draize scores on day 21 were zero for all animals.

The primary irritation index was 2.67.

According to the corneal opacity and the duration of

	findings observed, a possible irritation potential of the eye could not be excluded.
Result:	the notified chemical was a slight to moderate irritant to the eyes of rabbits

9.1.6 Skin Sensitisation - Magnusson and Kligman Maximisation Method (Arcelin, 1991)

Species/strain:	Guinea pigs/Himalayan spotted
Number of animals:	Pretest sighting study: 6 females Main study: 20 females (test group), 10 females (naïve control group)
Induction procedure:	
Pre-test	
Intradermal (i.d.)	0.1 mL of 1, 3 and 5% w/w dilutions of the test substance in double distilled water were injected intradermally;
Epidermal application	filter paper covered with a thin layer of the test substance in vaselinum album at 5, 10, 15, and 25% w/w dilutions were applied to the shaved flanks under occlusive dressing for 24 hours. After removal of the bandage, the skin was depilated to clean from yellow staining produced by the test substance.
Results of pretest	Very slight erythema and oedema were observed at all concentrations used for i.d. injections. Accordingly, the concentration of the test substance selected for the main study was 5% w/w; No oedema was observed at all concentrations used for epidermal application in all four animals tested. Very slight erythema was observed at 15 and 25% w/w concentrations in all four animals, at 10% w/w in two animals. No reactions were observed at 5% w/w concentration of the test substance. Accordingly, 10% and 5% were the concentrations selected for topical induction and challenge procedures, respectively.
Comment:	One pre-test animal from epidermal application group died spontaneously on day 20 of test.

Main Study

test group:	
day 1	three pairs of intradermal injections (0.1 mL) into the dorsal skin of the scapular region: Freund's complete adjuvant (FCA) 50:50 in physiological saline; the test substance, diluted to 5% w/w in double distilled water; the test substance diluted to 5% w/w emulsified in a 50:50 mixture of FCA and physiological saline;
day 7	filter paper covered with a thin layer of 10% w/w of the test substance in vaselinum album was applied to the treated area and held under occlusive dressing for 48 hours. After removal of occlusive dressing, the test sites were depilated to clean them from yellow staining produced by the test substance.
day 21	filter paper covered with a thin layer of 5% w/w of test substance in vaselinum album, or with vaselinum album only, was applied to sites on the left and right flank respectively, and held under occlusive dressing; after 24 hours the test sites were depilated to clean them from yellow staining produced by the test substance.
control group:	treated similarly to test animals omitting the test substance from the intradermal injections and topical application
Skin reactions following, Intradermal (i.d.) and Topical Induction:	
i.d. induction	Erythema and oedema were observed at all injection sites in the test group animals from day 2 to 5 following i.d. induction. Necroses were also observed from day 6 to 11. In addition, injection sites of test substance showed staining from day 2 to 5 of test. Erythema and oedema were observed at all sites of i.d. induction from day 2 to 5 in control group animals; necroses were observed in control group animals after i.d. induction at application sites with FCA.
topical induction:	Very slight oedema was observed in seven test group animals at the 24 hours observation. Erythema reactions

could not be observed (see comments below).

Also, encrustations were observed from day 12 to 14 and exfoliation was noted from day 15 onwards at all sites.

No erythema or oedema reactions were observed at the topical induction sites of control group animals. Encrustations and exfoliation were also observed at induction sites with FCA in control group animals.

Comments: Evaluation of erythema reactions were prevented in test group animals as a result of yellow staining produced by the test substance.

No systemic symptoms were observed.

Test method: OECD TG 406- Magnusson and Kligman Maximisation Test.

Challenge outcome:

	Test animals		***Control animals***	
Challenge concentration	***24 hours****	***48 hours****	***24 hours***	***48 hours***
5%	11**/20	10/20	0/10	0/10

* time after patch removal
** number of animals exhibiting positive response

Comment: Slight erythema was observed in the test group animals following challenge with the test substance.

Neither erythema nor oedema was observed in control group animals following challenge with the test substance.

Yellow staining of application sites was observed in both test and control groups. Exfoliation from day 15 to 25 (termination of the test) was also noted (see above).

Result: the notified chemical was moderately sensitising to the skin of guinea pigs.

9.2 Repeated Dose Toxicity (Hoff, 1991b)

Species/strain: Rat/Wistar

Number/sex of animals: 5/sex/group (treatment and control groups);
Two additional animal groups (5/sex/group) were used in a recovery study at high dose level and control

Method of administration:	Oral (gavage); vehicle: double distilled water; dose volume: 10 mL/kg bw
Dose/Study duration:	0, 50, 200, 1 000 mg/kg bw /day for 28 consecutive days; Duration of treatment-free period: 14 days (termination after 14-day recovery period post 28-day treatment)
Test method:	OECD TG 407
Mortality	One female at the high dose level (1 000 mg/kg bw/day) died spontaneously on treatment day 27. No definitive cause of death could be established from autopsy and tissue examination; there was no evidence of any treatment-related findings. No other premature deaths occurred at this or other doses.

Clinical observations:

No treatment related clinical findings were observed in test or control animals during the study period or during the treatment-free recovery period. One female in the medium dose level (200 mg/kg bw/day) showed alopecia on its fore-legs from day 23 to study termination. The author of the study considered this to be treatment unrelated and toxicologically irrelevant.

Males at the high dose level (1 000 mg/kg bw/day) ate statistically significantly, absolute and relatively, more food than controls between treatment days 8 and 15. The author considered these findings to be of no toxicological relevance and within the normal biological variations for the animals used.

When compared to the controls, females at the high dose level showed statistically lower body weights starting with treatment day 22, which persisted till day 1 of treatment-free recovery period. The author considered these findings insignificant and incidental as no confirmatory findings in the other sex were observed.
Apart from the presence of corneal debris in one male at high dose level on recovery day 13, which was considered incidental, no ophthalmic abnormalities were observed.

Clinical chemistry

Males at the high dose treatment level had marginally decreased blood glucose, urea, potassium and chloride concentrations. Females at the high dose treatment level had decreased blood chloride and total protein and globulin concentrations. Marginally increased aspartate aminotransferase and lactate dehydrogenase activities were observed in females at the medium and high dose treatment levels. Marginal increase in alanine aminotransferase activity was also noted in females at the high dose treatment level.

Although the author considered these changes of no toxicological significance, it was indicated that they reflect an increased metabolic functional load on the liver with the test substance. These findings were reversible and comparable to the control group animals at the termination of the treatment-free recovery period.

Haematology

At the high dose level treatment, both sexes had marginally decreased haemoglobin concentration, and marginally increased Heinz bodies and slightly increased methaemoglobin formation. Marginal increase in the methaemoglobin formation was also observed in males at the medium dose level. Males at the high dose level treatment revealed marginal reduction in haematocrit and marginal increase in reticulocyte count (relative). Haematological parameters were normal in recovery animals at the end of the 14-day treatment-free period.

The findings were indicative of a borderline compensated haemolytic anaemia with a likely effect on splenic haematopoiesis.

All other differences in the results of clinical biochemical, haematology and urinalysis parameters were considered incidental by the author and due to normal biological variation for the animal used.

Pathology:

Organ weights
At 28 day (termination), the absolute and relative thyroid weights and the kidney to body weight ratios were found to be statistically significantly higher in the females at the high dose treatment level when compared to those of the control animals. In contrast, the absolute heart weights and heart to brain weight ratios in the same test group were significantly lower than those measured for the control animals. The author considered these findings to be incidental and of no toxicological relevance as no confirmatory findings were observed in the male animals of the same group, nor were confirmatory macroscopical or microscopical findings observed (see below).

Necropsy
The mucosa of the stomach, intestine and tongue in most animals at low, medium and high dose levels showed yellowish discolouration; only exceptionally were these observed in the control animals. No microscopic findings corresponded to the yellowish discoloured mucosa.

Three females at the low dose level, two males and three females at the medium dose level, and two males and two females at the high dose level revealed isolated/several reddish/dark or red/black foci at the lowest part (fundus) of the stomach. These were suspected to be due to treatment-related toxicity on the intestines, and tissues from all animals were subjected to microscopic evaluations.

Microscopic examination of the foci in one male treated with the high dose level of the test substance revealed that it represented a focus of mucosal fibrosis. Focal glandular cell necrosis was observed in the stomach mucosa of one male at the high dose level treatment and in three females at the low and medium dose levels. Sub-mucosal oedema was observed in two females at the medium and high dose level treatment.

Also observed within the same area of the stomach were dark red/black-solitary crateriform retractions in two females at the high dose treatment level. Microscopically, these consisted of oedema and necrosis in one animal in addition to fibrinoidal scab in the other.

The author concluded that these alterations could not be considered treatment-related toxico-pathological lesions due to the random distribution throughout all groups. Other findings recorded were considered to be within the normal range of background lesions, which may be observed in the animals used.

Comment: Test substance administration at 1 000 mg/kg bw/day resulted in a treatment-related effect which may be responsible for a borderline compensated haemolytic anaemia with a likely effect on splenic haematopoiesis. These adverse effects were found to be reversible following termination of treatment.

Organ weight changes (kidney, thyroid, heart) were observed in females at 1 000 mg/kg bw/day. A number of changes in clinical biochemistry parameters, indicative of an increased metabolic load on the liver, were also observed at this dose, with some marginal changes in these parameters at 200 mg/kg bw/day.

Result: Based on haematological effects at 1 000 mg/kg bw/day, the NOAEL was determined to be 200 mg/kg bw/day; based on minor enzyme changes at the mid-dose, the NOEL was determined to be 50 mg/kg bw/day.

9.3 Genotoxicity

9.3.1 *Salmonella typhimurium* Reverse Mutation Assay (Poth, 1991)

Strains: TA1535, TA1537, TA1538, TA98 and TA100

Concentration range: Two independent experiments were conducted at the following concentrations:

Experiment 1:
- TA1535, TA100, TA98

0, 10, 33.3, 100, 333.3, 1 000 and 5 000 µg/plate; with/without S9 mix

- TA1537, TA1538

0, 10, 33.3, 66.6, 100, 333.3, 666.6 and 1 000 µg/plate; without S9 mix

- TA1537, TA1538

0, 10, 33.3, 100, 333.3, 1 000 and 5 000 µg/plate; with S9 mix

Experiment 2:
- TA1535, TA100, TA98

0, 10, 33.3, 100, 333.3, 1 000, 2 500 and 5 000 µg/plate; with/without S9 mix

- TA1537, TA1538
0, 10, 33.3, 66.6, 100, 333.3, 666.6 and 1 000 μg/plate; without S9 mix

- TA1537, TA1538
0, 10, 33.3, 100, 333.3, 1 000, 2 500 and 5 000 μg/plate; with S9 mix

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

Test method: OECD TG 471- plate incorporation method

Comment: Toxic effects, evidenced by a partial or complete reduction in the number of revertants, were observed at the higher concentrations tested with and without metabolic activation in experiment 1 and 2 in nearly all strains used. These effects were more prominent in strains TA1537 and TA1538 without S9 mix.

No significant or reproducible increase in the frequency of revertant colonies was observed in any of the bacterial strains, at any concentration, with or without S9 metabolic activation.

A slight increase in revertant colonies was observed in strain TA98 at 333.3 μg/plate in the presence of metabolic activation in experiment 1. However, this effect was irreproducible and was thus considered by the author to be irrelevant.

All positive controls used in the study confirmed the sensitivity of the various strains and the efficacy of the S9-mix. Colony counts in the vehicle controls were within historical limits.

Result: The notified chemical was considered non-mutagenic in the bacterial strains tested in the presence or absence of metabolic activation.

9.3.2 *Salmonella typhimurium* Reverse Mutation Assay (Poth, 1993)

Strains: TA1535, TA1537, TA1538, TA98 and TA100

Concentration range: Two independent experiments, using identical procedures, both with and without S9 mix were conducted at the following concentrations:

10, 33.3, 100, 333.3, 1 000 and 5 000 μg/plate;

Metabolic activation:	liver fraction (S9 mix) from Syrian golden hamsters
Test method:	OECD TG 471- preincubation method
Comment:	Test article toxicity was observed in strain TA 1535 in the absence of metabolic activation (experiment 2) and in strain TA 98 in the presence of metabolic activation in the two experiments as well as in the absence of metabolic activation at the highest dose (experiment 2). The test article was also toxic to strain TA 100 at 1 000 in the absence of S9 mix and at 5 000 µg/plate with and without S9 in both experiments. The test substance did not induce substantial increases in revertant colonies in any of the strains tested at any of the doses. All positive controls used in the study confirmed the sensitivity of the various strains and the efficacy of the S9-mix. Colony counts in the vehicle controls were within historical limits.
Result:	The notified chemical was considered non-mutagenic in the bacterial strains tested in the presence or absence of metabolic activation.

9.3.3 Chromosomal Aberration Assay in Chinese Hamster V79 Cells *in vitro* (Heidemann, 1991)

Cell line:	Chinese Hamster V79 cells
Doses:	Duplicate cultures were used to test each concentration, with or without metabolic activation (S9); asterisk* indicates cultures selected for metaphase analysis without S9, 0*, 3, 10*, 30, 100*, 200* and 340 µg/mL; treatment/harvest time: 4/18 hours; 0, 30, 100, 200*, and 340 µg/mL; treatment/harvest time: 4/28 hours; Positive control: Ethylmethanesulphonate, 1* mg/mL with S9: 0*, 10, 30*, 100*, 150, 200 and 340* µg/mL; treatment/harvest time: 4/18 hours; 0, 100, 150, 200 and 340* µg/mL; treatment/harvest time: 4/28 hours; Positive control:

	Cyclophosphamide, 4.2* μg/mL; In all cases the vehicle control was dimethyl sulphoxide (DMSO) at 1% v/v
Metabolic activation system:	liver fraction (S9 mix) from rats pretreated with Aroclor 1254
Test method:	OECD TG 473
Comment:	Toxicity, measured by reduced colony forming ability, in the absence and presence of S9 activation after treatment with concentrations higher than 10 μg/mL (without S9) and 150 μg/mL (with S9) was distinctly increased. The mitotic index was clearly reduced after treatment with the test substance at concentrations > 30 μg/mL in the absence of S9 activation, and > 100 μg/mL in the presence of activation. The test substance increased the frequency of aberrant cells in the absence of S9 (10%) and presence of S9 (11%) at fixation interval 28 hours after treatment with 200 and 340 μg/mL, respectively. In addition, 5% (-S9) and 8.5% (+S9) of the cells had chromosome exchanges as compared to 0.5% and 0% in the corresponding solvent controls. Polyploid metaphases in cultures treated with the test substance did not deviate from that for control cultures. Concentrations of the test substance higher than 340 μg/mL precipitated strongly in the medium. All vehicle control cultures had frequencies of chromosome aberrations within the expected range. Positive controls induced distinct increases in the incidence of aberrant cells and the activity of the S9 fraction was found to be satisfactory.
Result:	the notified chemical was clastogenic in V79 CHL cells.

9.3.4 Micronucleus Assay in the Bone Marrow Cells of the Mouse (Volkner, 1991)

Species/strain:	Mice/NMRI
Number and sex of animals:	6/sex 24 hours exposure: vehicle control, positive control and test substance dose groups; 6/sex 48 and 72 hours exposure: vehicle control and test substance dose groups.

Doses:	5 000 mg/kg bw; vehicle control- deionized water; Positive control- cyclophosphamide- 30 mg/kg bw
Method of administration:	Oral; single dose volume of 20 mL/kg bw for test substance and vehicle control and a dose volume of 10 mL/kg bw for positive control.
Test method:	OECD TG 474
Comment:	Four animals (2/sex) were dosed with 5 000 mg/kg bw in a pre-experiment to assess the toxicity of the test substance. Slight toxic reactions including reduction of spontaneous activity, eyelid closure, and apathy were observed in some animals up to 24 hours post-treatment. The test substance was not cytotoxic as determined by the lack of increase, relative to negative controls, in the mean number of normochromatic erythrocytes (NCEs) following treatment. Also, the ratio of polychromatic erythrocytes (PCEs)/NCEs did not vary significantly between negative control groups and test substance groups at all time intervals. Higher doses were not tested because the homogeneity and viscosity of the test substance could not be achieved at > 250 mg/mL and application volumes higher than 20 mL/kg bw were not justifiable for mice. When compared to negative controls, no significant enhancement in the frequency of the detected micronuclei was observed following administration of the test substance at all exposure periods tested. The positive control induced a distinct increase in micronucleus frequency.
Result:	The notified chemical was considered non-genotoxic in the *in vivo* mouse micronucleus assay.

9.3.4 DNA Damage and Repair- *In Vivo*/*In Vitro* Unscheduled DNA Synthesis in Rat Hepatocytes (Fautz, 1993)

Species/strain:	Rat/Wistar-WU
Number and sex of animals:	4 males/group
Doses:	Group 1= 1 000 mg/kg bw, 2 hours treatment; Group 2= 100 and 1 000 mg/kg bw, 16 hours treatment; vehicle control- Carboxymethylcellulose (1% aqueous solution);

	Positive control- 2-Acetylaminofluorene (2AAF)- 100 mg/kg bw
Method of administration:	Oral; single dose volume of 10 mL/kg bw for test substance, vehicle control and positive control.
Test method:	No OECD or EEC guidelines available; similar to OECD TG 482
Comment:	The test substance was not toxic to the animals at any of the concentrations or treatment periods examined. Similarly, the test substance did not significantly affect the viability of the hepatocytes, recovered from the pre-treated animals. *In vivo* treatment of the animals with the test substance for 2 and 16 hours did not induce unscheduled DNA synthesis in the recovered hepatocytes at any doses. No enhancement in the nuclear grains or net grains per nucleus was observed following treatment with the test substance. In contrast, *in vivo* treatment with the positive control revealed distinct increases in the number of nuclear and net grain counts.
Result:	The notified chemical did not induce DNA-damage leading to repair synthesis in the hepatocytes of the treated rats.

9.4 Overall Assessment of Toxicological Data

The notified chemical was of very low acute oral toxicity in rats with LD_{50} > 2 000 mg/kg bw. The notified chemical was of low dermal toxicity in rats (LD_{50} > 2 000 mg/kg bw). The notified chemical was a very slight irritant to the skin of rabbits and a slight to moderate irritant to the eyes of rabbits. The notified chemical was moderately sensitising to the skin of guinea pigs.

Oral administration of the notified chemical to rats in a 28 day repeated dose toxicity study resulted in haematological effects at the highest dose of 1 000 mg/kg bw/day, with a likely effect on splenic hematopoiesis. These effects were found to be reversible after a 14-day recovery period. Minor changes in clinical biochemistry parameters, indicative of an increased metabolic load on the liver, and organ weight changes were also observed at 1 000 mg/kg bw/day, with some marginal changes in biochemical parameters also observed at 200 mg/kg bw/day. Based on these results, the NOAEL for this 28-day study was determined to be 200 mg/kg bw/day; the NOEL was determined to be 50 mg/kg bw/day.

In two independent *Salmonella* test reports, the notified chemical was considered non-mutagenic to the bacterial strains tested; it was non-genotoxic in an *in vivo* mouse micronucleus assay. However, it was considered to be clastogenic *in vitro* in a chromosomal aberration assay. In further investigations of mutagenicity, it was negative in an *in vivo*/*in vitro* unscheduled DNA synthesis in rat hepatocytes.

The notified chemical is classified as a hazardous substance according to the NOHSC *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 1999) based on the

findings of the potential for skin sensitisation observed in an adjuvant type test in guinea pigs. The overall classification is Irritant (Xi) and the risk phrase R43 - May Cause Sensitisation by Skin Contact, is assigned. The safety phrase S25 – Avoid Contact with Eyes, is suitable as a warning of possible eye irritation.

10. ASSESSMENT OF ENVIRONMENTAL EFFECTS

The notifier has supplied ecotoxicity studies on the notified dye. The tests were performed in compliance with OECD/EEC Test Methods and according to OECD Principles of Good Laboratory Practices, and are summarised in the following table:

Test	***Species***	***Test concentrations[a] mg/L***	***Results mg/L***
Acute Toxicity (Semi-Static Test) (OECD TG 203)	Zebra Fish (*Brachydanio rerio*)	3.0, 5.6, 10, 18 & 32	96 h LC_{50} = 10.4
Acute Toxicity - Immobilisation (Static Test) (OECD TG 202 part I)	Water Flea (*Daphnia magna*)	1.25, 2.5, 5.0, 10 & 20	48 h EC_{50} = 3.8
Growth Inhibition - Growth (μ) & Biomass (b) (Static Test) (OECD TG 201)	Green Algae (*Scenedesmus subspicatus*)	1.0, 3.2, 10, 32 & 100	96 h $E\mu C_{50}$ = 136 96 h E_bC_{50} = 21.9 96 h LOEC[b] = 3.2
Respiration Inhibition (OECD TG 209)	Activated Sludge-Aerobic Waste Water Bacteria	3.2, 10, 32, 100, 320 & 1000	3 h IC_{50} = 568

[a] nominal concentration
[b] LOEC – Lowest Observed Effect Concentration

Fish (Memmert, 1991a)

The acute toxicity of the notified dye to Zebra fish was determined in a 96 hour semi-static test. The analytically determined test substance concentrations in the test media samples varied in the range from 80% to 89.5% of the nominal values. Although the test media were freshly prepared each day of the study, and despite the high water solubility, the test substance precipitated at the two highest test concentrations. The samples of these test media concentrations were measured corresponding to 58.4% to 74.2% of nominal values. In the two lowest nominal concentrations, 3.0 and 5.6 mg/L, either no mortality or symptoms of intoxication were observed. However, the effect of the notified chemical is quite steep where nearly all the fish, 9 out of 10, were dead after 96 hours at the 18 mg/L nominal concentration. The 96 hour LC_{50} of the notified dye was determined by the notifier using Moving Average Interpolation analysis to be 10.4 mg/L and the highest concentration tested without toxic effects was 4.7 mg/L. The 96 hour LC_{50} of the notified dye was also determined for this assessment using Probit analysis to be 10.4 mg/L which was calculated by applying Toxcalc 5.0 for Microsoft Excel (1992-1994) to the measured concentrations of 2.5, 4.7, 8.6, 12.5 and 18.8 mg/L.

Aquatic Invertebrates (Memmert, 1991b)

The acute toxicity of the notified dye to *Daphnia magna* was determined in a 48 hour static test. The analytically determined test substance concentrations in the test media samples 1.25 and 5.0 mg/L varied in the range from 82.9% to 88.5% of the nominal values. In the highest test concentration 20 mg/L the mean measured concentration was determined to be 70.9% of nominal. Again, despite the high water solubility the test substance precipitated in the highest test concentration. After 48 hours 10% of the daphnia were either dead or immobilised in the lowest measured test concentration of 1.3 mg/L which gradually increased to the point where all daphnia were either dead or immobilised in the highest measured test concentration of 14.2 mg/L. The 24 and 48 hour EC_{50} of the notified dye were determined to be 14.4 mg/L and 3.8 mg/L, respectively, which indicates a sharp rise in toxicity over time and that equilibrium may not have been reached.

It is noted that it is unclear as to how the notifier determined the LC_{50} value of the notified dye. The 48 hour EC_{50} of the notified dye determined for this assessment using Probit analysis was 4.1 mg/L, calculated by applying Toxcalc 5.0 for Microsoft Excel (1992-1994) to the nominal concentrations of 1.25, 2.5, 5.0, 10 and 20 mg/L.

Algae (Memmert and Knoch, 1991)

The acute toxicity of the notified dye to Algae was determined in a 96 hour static test. The measured test substance concentrations varied from 78.8 to 90.8% of the nominal values. The 96 hour inhibition rates calculated for both algal biomass and growth rate were 21.9 mg/L and 136 mg/L, respectively. The lowest concentration of the notified dye tested with significant toxic effects (LOEC) was determined to be 3.2 mg/L. All test media had some turbidity initially and were coloured by the test substance. The notifier indicates that any observed inhibition effect of the notified chemical on algal growth maybe caused by either a toxic effect on the algal cells or due to the reduced light intensity or change of light quality in the coloured test media. It is agreed that inhibition maybe caused by both toxicity and reduced light quality. However, the notifier did not carry out a comparison test where algal growth was measured in either the presence or absence of light.

Microorganisms (Memmert, 1991c)

The inhibitory effect of the notified substance on aerobic wastewater bacteria, activated sludge from a domestic wastewater treatment plant, was investigated in a respiration test. The notified substance showed no inhibition on the respiration rate at concentrations ranging from 3.2 mg/L to 32 mg/L. At a nominal concentration of 1 000mg/L a respiratory inhibition of 57.1% was observed. The final 3 hour IC_{50} was determined to be 568 mg/L. It is noted that all reported results are related to the nominal concentrations of the test substance, thus, the IC_{50} may possibly be below 568 mg/L.

Conclusion

The ecotoxicity data for the notified substance indicates that it is slightly toxic to fish and algae, moderately toxic to aquatic invertebrates and practically non-toxic to microorganisms.

11. ASSESSMENT OF ENVIRONMENTAL HAZARD

The environmental hazard from the dye, when fixed to textile fibres is rated as low. The majority of exposure of the notified chemical to the environment will come from effluent released from dyehouses containing unfixed dye.

An estimation of the Predicted Environmental Concentration (PEC) for use in polyamide and wool textiles is provided in the following tables:

Polyamide Textiles

Calculation Factor	*City dyehouse*	*Country dyehouse*
Commercial dyestuff consumed per day	8 kg	8 kg
Notified chemical consumed per day based on 80% of commercial dyestuff	6.4 kg	6.4 kg
Fixation rate	97%	97%
Substance not fixed to fibres	192 g	192 g
Typical dyehouse effluent per day	2 ML	260 000 L
Dye concentration in effluent	0.096 mg/L	0.74 mg/L
Dilution factor in STP	1:100	1:36
Concentration after dilution in STP	9.6×10^{-4} mg/L	0.02 mg/L
Typical dilution factor in receiving waters	1:10	1:3
Predicted Environmental Concentration	9.6×10^{-5} mg/L	6.8×10^{-3} mg/L
Safety factor for exposure of most sensitive aquatic organism *Daphnia magna* 48 h EC_{50} = 3.8 mg/L	> 39500	> 550

Wool Textiles

Calculation Factor	*City dyehouse*	*Country dyehouse*
Commercial dyestuff consumed per day	8 kg	8 kg
Notified chemical consumed per day based on 80% of commercial dyestuff	6.4 kg	6.4 kg
Fixation rate	95%	95%
Substance not fixed to fibres	320 g	320 g
Typical dyehouse effluent per day	2 ML	260 000 L
Dye concentration in effluent	0.16 mg/L	1.23 mg/L
Dilution factor in STP	1:100	1:36
Concentration after dilution in STP	1.6×10^{-3} mg/L	0.034 mg/L
Typical dilution factor in receiving waters	1:10	1:3
Predicted Environmental Concentration	1.6×10^{-4} mg/L	1.1×10^{-2} mg/L
Safety factor for exposure of most sensitive aquatic organism *Daphnia magna* 48 h EC_{50} = 3.8mg/L	> 23700	> 330

It is noted that the potential environmental concentrations estimated above are conservative. No biodegradation and adsorption to solids at sewage treatment plants, for example, has been assumed. It is unlikely that much biodegradation would occur, but it can be assumed that at least 50% adsorption may occur at a typical sewage treatment plant. It is noted, specifically,

that the wastewater treatment plant at a country dye-house has a handling capacity of 9.4 ML per day and is currently operating at approximately 7.0 ML per day. All sewage is handled on site through irrigation over an area of 2 000 acres and no effluent is released into the environment. If these factors are taken into account, safety margins are expected to be much greater than the lowest expected safety margin of 330, and the hazard from use at the proposed sites is acceptable.

The only other source of environmental contamination is from accidental spills and disposal of packaging. The MSDS contains sufficient information to enable operators to limit the environmental exposure and, therefore, limit the environmental effects.

In the event of accidental spillage of the dyestuff into waterways, the chemical is expected to disperse into the water due to its high water solubility but also settle out onto sediments. If the dyestuff is spilt on land, either during usage or transport, it is expected that the chemical would become immobilised in the soil layer. Contaminated soil can then be collected and disposed of to landfill.

Solid waste consigned to landfill, either from spillages or residues in packaging, would be expected to be retained at the landfill sites and not be mobile. Movement of the chemical by leaching from landfill sites is not expected because of its high binding affinity to soil.

Given the above, environmental exposure and the overall environmental hazard is expected to be low.

Conclusions
The dye is not likely to present a hazard to the environment when it is stored, transported and used in the typical manner.

12. ASSESSMENT OF PUBLIC AND OCCUPATIONAL HEALTH AND SAFETY EFFECTS

The notified chemical was of very low acute oral toxicity and low acute dermal toxicity. The notified chemical was a very slight skin irritant in rabbits and a slight to moderate irritant to the eyes of rabbit. The notified chemical was a moderate skin sensitiser in guinea pigs.

Haematological effects at 1 000 mg/kg bw/day were observed in 28-day oral (gavage) repeated dose toxicity study in rats, with a likely effect on splenic hematopoiesis. Also observed were minor changes in clinical biochemistry and organ weights at the highest dose and marginal changes in biochemical parameters at the mid-dose. The NOAEL was 200 mg/kg bw/day and the NOEL was 50 mg/kg bw/day.

In two independent *Salmonella* test reports, the notified chemical was considered non-mutagenic to the bacterial strains tested; it was non-genotoxic in an *in vivo* mouse micronucleus assay. However, it was considered to be clastogenic *in vitro* in a chromosomal aberration assay. In further investigations of mutagenicity, it was negative in an *in vivo*/*in vitro* unscheduled DNA synthesis in rat hepatocytes.

The notified chemical is classified as a hazardous substance according to the NOHSC *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 1999) based on skin sensitisation observed in an adjuvant type test. The overall classification is Irritant (Xi) and

the risk phrase R43 - May Cause Sensitisation by Skin Contact, is assigned. The safety phrases S22 - Avoid Breathing Dust, is suitable as a warning against inhalation of the particulate dye, and S25 – Avoid Contact with Eyes, is suitable as a warning of possible eye irritation.

The submission indicates that cases of skin and respiratory sensitisation have been observed with reactive dyes and care should be taken to avoid skin contact and inhalation. However, no work related injuries or diseases related to the notified chemical have been reported by the notifier's overseas plants.

Occupational Health and Safety

Transport and Storage
Exposure to the notified chemical is not expected during transport or storage as long as the packaging remains intact and would only occur in the event of a spill. 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.

Formulation and Dyeing
The greatest potential for exposure to the notified chemical is during weighing and mixing of the powdered chemical and during disposal of empty used liner bags. There exists potential for exposure by inhalation and/or skin and eye contact with dust particles with the associated health effects of skin sensitisation, possible respiratory sensitisation and possible eye irritation. The notified chemical comprises 70-80% by weight of Erionyl Yellow A-3G, of which a maximum of 11% is in the respirable range. Mixtures containing Acid yellow HT 2803 at concentrations of $\geq$ 1% are hazardous substances. Although significant absorption through the skin is expected to be low, given the molecular weight of 663, the hazardous nature of the chemical necessitates strict controls during handling.

As there is potential for inhalation exposure, the level of dust in the workplace should be controlled to as low as possible. Given the risk of adverse health effects during the handling of the particulate dye, local exhaust ventilation and dust extraction need to be maintained over mixing areas to capture dust and aerosols at source, and minimise exposure to airborne particulates generated from the notified chemical and any other ingredients. Good hygiene and work practices are required in these areas to minimise the generation and subsequent settling of dusts on work surface areas and floors; this is also recommended in the MSDS for Erionyl Yellow A-3G.

Given that atmospheric monitoring cannot be related to the health effects anticipated, that is, skin and respiratory sensitisation, the wearing of an air purifying dust respirator (with P2 particulate filter) and other protective equipment such as overalls, protective gloves, and goggles during these operations, is needed.

Exposure to Acid Yellow HT 2803 at < 10% may occur after dissolution, when mixing with other dyes, during connection/disconnection of metering pump hoses from the mixing vessel to the dyeing vessel, during the dyeing process, and during cleaning and maintenance of equipment. Inhalation exposure is not expected as any aerosols would be within enclosed automated operation systems. Skin and/or eye contact will be the main routes of exposure. As the notified chemical is hazardous, a risk of skin sensitisation exists during dye

preparation and textile dyeing. The chemical is an eye irritant, though not sufficient to warrant classifying as such, however, wearing of safety glasses, protective gloves and overalls are needed to reduce the risk of irritation and sensitisation when handling the dyestuff and wet dyed textiles. The notifier stated that workers will be trained in the safe handling of hazardous substances.

Exposure to the notified chemical may also occur during the drying and curing processes, however, exposure will be negligible because the operation is partly an enclosed system, the chemical has a high fixation rate to textile/fibres and excess non-fixed dye would have been washed off in the rinse cycle. Nonetheless, workers involved in this process will need to wear gloves and safety goggles.

Exposure to dusts and aerosols may also occur during laboratory testing, however, given the smaller quantities handled, the potential for skin sensitisation effects and eye irritancy is reduced. Local exhaust ventilation and the routine wearing of laboratory coats, impervious gloves and safety glasses would be expected to further reduce these risks.

Measures should also be implemented in the disposal of the notified chemical to ensure that exposure is avoided.

Public health
Public exposure is through touching the fabric dyed with the notified chemical. The dyeing process fixes the dye firmly to the fabric. While the fabric is used for clothing, the dyestuff is wetfast and will not be removed by contact with the skin or with water. This renders the notified chemical biologically unavailable. Hence, the potential for public exposure to the notified chemical is considered to be low.

Based on the above information, it is considered that Acid Yellow HT 2803 will not pose a significant hazard to public health when used in the proposed manner.

13. RECOMMENDATIONS

Acid Yellow HT 2803 is a skin sensitiser; workers handling it or products containing it will need to be strictly protected against skin contact. To minimise occupational exposure to Acid Yellow HT 2803 the following guidelines and precautions should be observed:

- Safety goggles should be selected and fitted in accordance with Australian Standard (AS) 1336 (Standards Australia, 1994) to comply with Australian/New Zealand Standard (AS/NZS) 1337 (Standards Australia/Standards New Zealand, 1992); industrial clothing should conform to the specifications detailed in AS 2919 (Standards Australia, 1987) and AS 3765.1 (Standards Australia, 1990); impermeable gloves or mittens should conform to AS 2161.2 (Standards Australia/Standards New Zealand, 1998); all occupational footwear should conform to AS/NZS 2210 (Standards Australia/Standards New Zealand, 1994); respiratory protection should conform to AS/NZS 1715 (Standards Australia/Standards New Zealand, 1994), and AS 1716 (Standards Australia/Standards New Zealand, 1994);

- Local exhaust ventilation should conform to AS 1668.2 (Standards Australia, 1994);

- Dust levels in the workplace should be maintained as low as possible;

- 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;

- As potential for skin and respiratory sensitisation exists, the notifier's MSDS should be provided to the authorised medical practitioner responsible for health surveillance in the workplace. Sensitised persons should be transferred to another workplace;

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

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

- The notified chemical may be recommended to the National Occupational Health and Safety Commission for consideration for inclusion in the NOHSC List of Designated Hazardous Substances with the risk phrase R43 - May Cause Sensitisation by Skin Contact.

14. MATERIAL SAFETY DATA SHEET

The MSDS for Erionyl Yellow A-3G containing the notified chemical was provided in a format consistent with the *National Code of Practice for the Preparation of Material Safety Data Sheets* (NOHSC, 1994).

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

15. REQUIREMENTS FOR SECONDARY NOTIFICATION

Secondary notification under Section 64 of the Act will be required if:

i) the method of use changes in such a way as to greatly increase the environmental exposure of the notified chemical; or

ii) if additional information becomes available on adverse environmental effects of the chemical. In particular, notification will be needed if a proposed use of the dye is at country dyehouses where effluent dilution rates and subsequent environmental safety factors are expected to be quite low through release to a local creek, or where the deeper shade is proposed to be regularly employed. In both cases the hazard assessment would need to be revised and submission of a chronic daphnia test is highly recommended; or

iii) any of the circumstances stipulated under subsection 64(2) of the Act arise. No other specific conditions are prescribed.

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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.
Diffuse beefy red	3 severe	Swelling with lids half-closed	3 mod.	Discharge with moistening of lids and hairs and considerable area around eye	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