File No: NA/653

August 1999

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME

FULL PUBLIC REPORT

FAT 41020/A

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Director
Chemicals Notification and Assessment

FULL PUBLIC REPORT

FAT 41020/A

1. APPLICANT

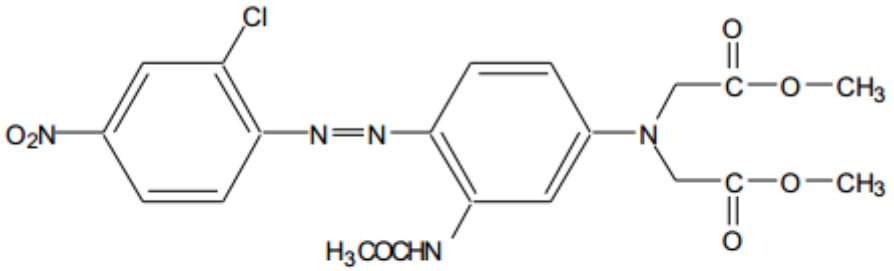
Ciba Specialty Chemicals of 235 Settlement Road THOMASTOWN VIC 3074 has submitted a standard notification statement in support of their application for an assessment certificate for FAT 41020/A. The applicant has not applied for the any information relating to FAT 41020/A to be exempt from publication in the Full Public and Summary Reports.

2. IDENTITY OF THE CHEMICAL

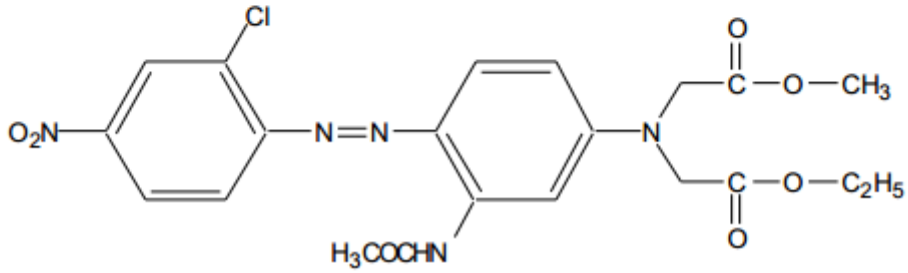
Chemical Name:	glycine, N-[3-(acetylamino)phenyl-N-(carboxymethyl)-, mixed ethyl and methyl diesters, reaction products with diazotised 2-chloro-4-nitrobenzenamine
Chemical Abstracts Service (CAS) Registry No.:	188070-47-5
Other Names:	Scarlet CLA 881
Trade Name:	Terasil Red W-RS
Molecular Formula:	$C_{20}H_{20}ClN_5O_7$ main component 1 $C_{21}H_{22}ClN_5O_7$ main component 2

Structural Formula:

Main component 1



Main component 2



Molecular Weight:	477 491	Main component 1 Main component 2
Spectral Data:	ultraviolet/visible spectrum: main peaks were - 236.8, 295.3 and 499.9 nm (neutral solution) - 237.4, 294.8 and 499.8 nm (acid solution) - 239.3, 297.3 and 554.3 nm (alkaline solution) infrared and ^{1}H-nuclear magnetic resonance spectra used to derive structure were provided	
Method of Detection and Determination:	spectroscopy, as noted above	

3. PHYSICAL AND CHEMICAL PROPERTIES

Appearance at 20°C and 101.3 kPa:	red powder
Melting Point:	approximately 187-194°C (decomposes above 221°C)
Specific Gravity:	1.5 at 20°C
Vapour Pressure:	4.2 x 10^{-5} kPa

Water Solubility:	less than 1 mg/L at 20°C
Partition Co-efficient (n-octanol/water):	log P_{ow} = 3.8 at 20.6 to 21°C (for major component)
Hydrolysis as a Function of pH:	not performed due to low water solubility
Adsorption/Desorption:	could not be performed QSAR calculation K_{oc} = 1 507
Dissociation Constant:	not performed due to low water solubility
Particle Size:	< 2 μm: 31.2% 2-5 μm: 35.6% 5-10 μm: 16.4% < 10 μm: 83.2% 10-20 μm: 16.8%
Flammability Limits:	not flammable
Autoignition Temperature:	greater than 400°C
Explosive Properties:	not explosive
Reactivity/Stability:	not an oxidising agent; does not form dangerous gas on contact with water; not pyrophoric
Surface Tension:	72.2 mN/m at 1 g/L and 20°C (90% saturated solution)

Comments on Physico-Chemical Properties

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

Hydrolysis, Adsorption/Desorption and Dissociation constant data could not be provided because of the measured low water solubility. Low water solubility and a high partition coefficient indicate high affinity for soil or sediment. The notifier has indicated the notified chemical is likely to bind/adsorb strongly to soil and not be available in the water partition. The calculated K_{oc} of 1 507 would class the notified substance as having a low potential for soil movement (Gawlik, 1997). It is expected that the chemical will bind to sediment and soils.

The notified chemical is not surface active at a concentration of 1 g/L. By definition, a chemical has surface activity when the surface tension is less than 60 mN/m (European Economic Community, 1992b).

4. PURITY OF THE CHEMICAL

Degree of Purity: 85.5% (72.6-98.3%)

Toxic or Hazardous Impurities: the notified chemical, containing a variety of impurities, is a skin sensitiser and, therefore, would be determined to be a hazardous substance according to NOHSC *Approved Criteria for Classifying Hazardous Substances* (National Occupational Health and Safety Commission, 1994a); it consists of 2 main components and a range of known and unknown coloured and uncoloured impurities; it is not known which of these impurities are sensitising; the unknown components comprise 1.1% of the notified chemical; the known components are as follows:

Chemical Name	*Weight %*
[(3-acetylamino-phenyl)-methoxycarbonylmethyl-amino]-acetic acid methyl ester	0.9
[(3-acetylamino-phenyl)-ethoxycarbonylmethyl-amino]-acetic acid methyl ester	0.2
[[5-acetylamino-2-(2-chloro-4-nitro-phenylazo)-phenyl]-methoxycarbonylmethyl-amino]-acetic acid methyl ester	8.1
[[5-acetylamino-2-(2-chloro-4-nitro-phenylazo)-phenyl]-ethoxycarbonylmethyl-amino]-acetic acid methyl ester	2.7
[[5-acetylamino-2-(2-chloro-4-nitro-phenylazo)-phenyl]-methoxycarbonylmethyl-amino]-acetic acid methyl ester	1.9

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

Additives/Adjuvants: none

5. USE, VOLUME AND FORMULATION

The notified chemical is to be used for colouring polyester fibres and textiles. The dyestuff to be imported is in ready-to-use form and contains the notified chemical at a concentration of up to 40%. The remainder of the mixture is made up of stabilising agents, inhibitors and other additives. The notified chemical is to be imported at up to 2 tonnes in the first year, 2-4 tonnes in the second and third years and 4-6 tonnes in the fourth and fifth years. It will be used in dyehouses only, mainly in NSW and Victoria. The dye is applied by the exhaust dyeing method and has a fixation performance of 97.5%.

6. OCCUPATIONAL EXPOSURE

The notified chemical is to be imported in 20 kg polythene-lined fibreboard boxes. Exposure of waterside, transport and storage workers should only occur in the event of accidental spillage.

The notifier states that repacking is unlikely to be necessary as the 6 customers order full drum quantities. If necessary, up to 100 kg would be repacked at the notifier's warehouse by 2 people on 10 days per year for 15-20 minutes per day. Repacking takes place in a down-flow booth with the air flow away from the operator.

The imported dyestuff is stated to be non-dusting. Therefore, dust clouds should not accumulate and persist in the weighing area. Based on data from a US air monitoring study, worker exposure to the notified chemical during weighing was calculated by the notifier. The notifier calculated that no more than 0.0008 mg/kg/day would be absorbed. The notifier did not provide the complete list of assumptions on which the dose was calculated. Inhalation exposure only was assumed.

Five weighing operators at each dyehouse add the dyestuff to warm water in a blending vessel to a concentration of not more than 2.5%, in a dispensary, under the influence of local exhaust ventilation. The notifier states that weighing operators wear elbow-length PVC gloves, safety glasses, face-shield and protective overalls. The dyestuff is immediately wetted and dispersed and the solution pumped through a closed system to a high temperature exhaust dyeing machine. The notifier states that the operator need not come into contact with the solution when it is fed to the machine. Weighing a mixing is expected to occur on 75 days per year for 40 minutes per day over 2 – 3 shifts.

Twenty dyeing operators, 12 stenter operators (involved in drying the cloth after dyeing) and possibly 6 laboratory technicians per dyehouse are involved in dyeing, washing off the unfixed dye (2.5% of dye added) and drying of the dyed cloth. The cloth to be dyed is handled into the mechanism that passes the cloth through the dyeing machine. The concentration of the dye in the dye bath is no more than 2.5%. Following exhaust of dye on to the fabric, it is lead to the wash-off baths where it is washed free of unfixed dye and passed to the drier. The notifier states that gloves are worn when handling the cloth after dyeing. Over 2 – 3 shifts addition of chemicals at the start of the dyeing process is expected to take 10 minutes per day on 75 days per year. In the event of a tangle, it may be necessary to cool and open the machine for 20 minutes on 5 days per year. Wet dyed cloth is handled for 2 minutes when unloading the machines. For curing and drying over 3 shifts, wet cloth is handled at the start of the process for 5 minutes on 75 days per year. Laboratory analysis over 1 – 2 shifts involves handling 1 gram or less of the dyestuff when dyeing samples for 5 – 10 minutes on 150 days per year.

7. PUBLIC EXPOSURE

The dye will not be sold to the public. Since the dye is strongly fixed to the fibre it is used to treat, public exposure during washing and dry cleaning of the fabric is expected to be negligible.

8. ENVIRONMENTAL EXPOSURE

Release

The bulk of the dye will become chemically fixed to the cellulose textiles, and in this state is not expected to impact on the environment. The result of fastness performance tests shows that a high order of fastness rating is achieved in all cases. After application to fabrics, the dye undergoes a chemical change involving the chemical bonding with hydroxy groups on the polyester fibres.

The major environmental exposure to dye will come from effluent discharge from dyehouses and waste water treatment systems. Other releases will be limited to traces remaining from repacking operations and clean-up of any spills, and from trace residues in empty packaging (estimated at a maximum of 0.1% based on previous similar notifications by the notifier).

Fate

The dye normally released in water as effluent from the dyehouse is expected to be the major environmental exposure. The dye may either partition to sediment or stay in the aqueous compartment. Hobbs (Hobbs, 1988) reports that reactive dyes have been found not to adsorb to sludge in model systems. Any dye that binds to the sludge during the waste treatment process would be disposed of through incineration or 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, nitrogen and sulfur, together with sodium salts in the ash and a small amount of hydrogen chloride. Disposal by landfill will be at a secured site, so the risk of leaching to the water table is significantly reduced.

The biochemical oxygen demand (BOD) of the dye was tested and the five-day study showed the BOD_5 was 2 mg O_2/g. The chemical oxygen demand (COD) was determined to be 759 mg/g O_2. The dye was found not to be readily biodegradable. Measured as dissolved organic carbon (DOC) and expressed as percentage elimination, biodegradation amounted to 2% at the end of the 28-day exposure to micro-organisms from a domestic sewage treatment plant) in the OECD 301F Test (Manometric Respirometry Test) for ready biodegradability. No inhibition on the activity of the bacteria was observed in this test. The dye's inherent biodegradability was -3% after 28 days according to the test procedure that followed OECD guideline 302B (Zahn-Wellens/EMPA Test).

Although the dye is not readily biodegradable, the potential for bioaccumulation is low due to the low partition coefficient (log P_{OW} < -2.0) and very high water solubility of the substance. Hydrophilic dyes with log P_{OW} < 3 have been shown not to bioaccumulate (Yen et al., 1991). Also, biological membranes are not permeable to chemicals of very large molecular size and therefore bioaccumulation of the notified polymer is not expected (Anliker et al., 1988; Gobas et al., 1986).

Residues that persist after sewage treatment will enter marine or freshwater environments in solution (from city and country waste water treatment systems, respectively). A possible route of entry of the dye to the sediment is by the precipitation of its calcium salts, as several calcium salts of sulphonic dyes are known to be insoluble at modest concentrations (Gawlik, 1997). Degradation of such dyes in sediment water systems proceeded with a half-life of 2-16 days. Accordingly, no significant increase in dissolved concentrations over time is predicted, while residues bound to sediment are expected to undergo reductive degradation.

9. EVALUATION OF TOXICOLOGICAL DATA

9.1 Acute Toxicity

Summary of the acute toxicity of FAT 41020/A

Test	*Species*	*Outcome*	*Reference*
acute oral toxicity	rat	LD_{50} > 5 000 mg/kg	(Busschers, 1997b)
acute dermal toxicity	rat	LD_{50} > 2 000 mg/kg	(Pels Rijcken, 1997a)
skin irritation	rabbit	not irritant	(Busschers, 1997c)
eye irritation	rabbit	slight irritant	(Busschers, 1997a)
skin sensitisation	guinea pig	sensitiser	(Pels Rijcken, 1997b)

9.1.1 Oral Toxicity (Busschers, 1997b)

Species/strain:	rat/Wistar
Number/sex of animals:	5/sex
Observation period:	14 days
Method of administration:	gavage; vehicle: 1% aqueous carboxymethyl cellulose
Clinical observations:	red staining of the faeces between days 2 and 4; red staining of the front paw and hunched posture in two animals on days 1 or 2
Mortality:	none
Macroscopic findings:	none

Test method:	OECD guideline TG401 (Organisation for Economic Co-operation and Development, 1995-1996)
LD_{50}:	> 5 000 mg/kg
Result:	the notified chemical was of very low acute oral toxicity in rats

9.1.2 Dermal Toxicity (Pels Rijcken, 1997a)

Species/strain:	rat/Wistar
Number/sex of animals:	5/sex
Observation period:	14 days
Method of administration:	the notified chemical in 1% aqueous carboxymethyl cellulose was applied under occlusive dressing for 24 hours
Clinical observations:	red staining of the skin caused by the notified chemical in all animals disappeared with 10 to 11 days in 8 animals and persisted to termination in 2 animals
Mortality:	none
Macroscopic findings:	none
Test method:	OECD guideline TG402 (Organisation for Economic Co-operation and Development, 1995-1996)
LD_{50}:	> 2 000 mg/kg
Result:	the notified chemical was of low acute dermal toxicity in rats

9.1.3 Inhalation Toxicity

Study not performed.

9.1.4 Skin Irritation (Busschers, 1997c)

Species/strain:	rabbit/New Zealand White
Number/sex of animals:	3/male
Observation period:	72 hours
Method of administration:	0.5 g of notified chemical moistened with water applied under semi-occlusive dressing for 4 hours
Test method:	OECD guideline TG404 (Organisation for Economic Co-operation and Development, 1995-1996)
Result:	no scoring for erythema at 1 hour post-treatment due to red staining of the skin; no Draize scores (ref) (see Attachment 1 for details) above zero were recorded for either erythema or oedema up to 72 hours post-treatment; light red staining was apparent during this period the notified chemical was not a skin irritant in rabbits

9.1.5 Eye Irritation (Busschers, 1997a)

Species/strain:	rabbit/New Zealand White
Number/sex of animals:	3/male
Observation period:	72 hours
Method of administration:	40 mg of the notified chemical was instilled into one eye of each of three rabbits
Test method:	OECD guideline TG405 (Organisation for Economic Co-operation and Development, 1995-1996)
Result:	no corneal or iridal effects were observed in any animal up to 72 hours post-instillation; conjunctival redness could not be scored at 1 hour due to red staining; no conjunctival effects were observed at 72 hours in any animal; at the other time points, Draize (ref) scores (see Attachment 1 for details) for conjunctival redness, chemosis and discharge at 1, 24 and 48 hours were as follows:

Animal No.		*1hr*	*24hr*	*48hr*
	r	-	1	1
1	**c**	2	1	0
	d	1	1	0
	r	-	2	1
2	**c**	2	1	0
	d	2	0	0
	r	-	1	1
3	**c**	2	1	0
	d	2	1	0

the only persistent staining noted in the study was colouration of the head and paws at 72 hours

the notified chemical was a slight eye irritant in rabbits

9.1.6 Skin Sensitisation (Pels Rijcken, 1997b)

Species/strain: guinea pig/Himalayan, albino

Number of animals: 10 test males, 5 control males

Induction procedure:

day 1
three pairs of intradermal injections (0.1 mL/site), one of each pair on each side of the midline from cranial to caudal, as follows:

- Freund's Complete Adjuvant (FCA), 1:1 with water;
- 2% notified chemical in 1% carboxymethyl cellulose;
- 2% notified chemical in 1% carboxymethyl cellulose in FCA

day 7
scapular area between the injection sites treated with 10% sodium dodecyl sulphate (SDS) in vaseline

day 8
the area treated with SDS treated with 0.5 mL of 20% notified chemical

Challenge procedure:

day 22
one flank of all animals treated by application of 20% notified chemical and 1% carboxymethyl cellulose (0.5 mL each) under a Metalline patch mounted on medical tape under an elastic bandage

for 24 hours; after bandage removal, the site was treated with a depilatory cream to remove staining caused by the notified chemical

Challenge outcome:

	Control animals		***Test animals***	
Challenge concentration	***24 hours****	***48 hours****	***24 hours***	***48 hours***
20%	0/5**	0/5	10/10	7/10

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

Test method: OECD guideline TG406 (Organisation for Economic Co-operation and Development, 1995-1996)

Result: the notified chemical was a skin sensitiser in guinea pigs

9.2 Repeated Dose Toxicity (Schoenmakers, 1997)

Species/strain: rat/Wistar

Number/sex of animals: 5/sex/dose group; 5/sex/recovery group

Method of administration: gavage; vehicle: 1% carboxymethyl cellulose

Dose/Study duration:: 0, 50 (low dose), 200 (mid dose), 1 000 (high dose) mg/kg/day for 28 days; recovery groups at 0 and 1 000 mg/kg/day kept for 14 days following the last dose prior to necropsy

Clinical observations: extensive treatment-related alopecia in all females at the high dose during the treatment and recovery periods; red discolouration of skin/fur and faeces in treated animals; high dose females showed reduced body weights and body weight gain from day 8 of treatment up until day 1 of the recovery period

Clinical chemistry: at the high dose, cholesterol levels were elevated by 28% and 57% in males and females, respectively; after the recovery period, values were not significantly different than the control

Haematology: in high dose females, reductions in red blood cell count (9%), haemoglobin (11%) and haematocrit (9%) were observed; after the recovery period, these parameters had returned to control levels

	while the red cell distribution width had increased 16%
Organ weights:	in high dose females, increased liver:body weight ratio (by 20%) and decreased autopsy body weights were noted after 4 weeks but were normal in the recovery group
Histopathology:	minimal grades of lymphoid cell foci, focal epidermal hyperplasia or focal epidermal necrosis were correlated with the alopecia observed in high dose females; similar alterations were noted in the recovery group
Test method:	EEC Directive 92/69/EEC, Test Method B.7 (European Economic Community, 1992a)
Result:	slight anaemia was evident in high dose females, indicated by decreased values for red blood cell count, haemoglobin and haematocrit after 28 days of treatment; these changes were restored during the 14-day recovery period; high dose females exhibited decreased body weight and food consumption, and recovered during the 14-day treatment-free period; increased cholesterol levels were observed in high dose males and females but the reason for this was unclear; an increase in liver:body weight ratio in high dose females may have been an adaptive response; the skin of high dose females showed alopecia accompanied by minimal histopathological changes which may have been treatment-related
	based on these changes at the high dose, the NOEL was judged to be 200 mg/kg/day

9.3 Genotoxicity

9.3.1 *Salmonella typhimurium* and *Escherichia coli* Reverse Mutation Assay (Verspeek-Rip, 1997b)

Strains:	TA 1535, TA 1537, TA 98, TA 100, *E. coli* WP2*uvrA*
Concentration range:	0 – 5 000 μg/plate
Test method:	OECD guideline TG471 (Organisation for Economic Co-operation and Development, 1995-1996)

Result: the notified chemical was mutagenic in TA 1537, TA 98 and TA 100 with the same potency in the absence as in the presence of metabolic activation provided by S9 fraction isolated from the livers of Arochlor1254-treated rats; no mutagenicity was detected in TA 1535 or *E. coli* WP2*uvrA*

the notified chemical precipitated above 33 μg/plate and this was reflected in a sub-proportional increase in induced mutation frequency with dose; no evidence of toxicity of the notified chemical was evident from inspection of the background lawn

maximum mutagenic potencies were as follows:

Strain	***S9***	***Estimated Mutagenic Potency (mutants/μg)***
TA 1537	-	10^{-2}
	+	10^{-2}
TA 98	-	2.5
	+	1.8
TA 100	-	0.53
	+	0.86
Positive Controls		
9-aminoacridine	-	4.7 (TA 1537)
daunomycin	-	129 (TA 98)
methylmethane sulphonate	-	0.8 (TA 100)
2-aminoanthracene	+	167 (TA 1537)
	+	1.25 (TA 98)
	+	1 037 (TA 100)

Conclusion: the notified chemical induced both frameshift and base substitution mutations in bacteria independent of the presence of metabolic activation

9.3.2 Mouse Lymphoma L5178Y TK+/- Mutation Assay (Verspeek-Rip, 1997a)

Cell line: mouse lymphoma L5178Y TK+/-

Doses: 0, 1, 3, 10, 33 μg/mL for 24 hours in the absence and 3 hours in the presence of rat liver microsomal enzymes (Arochlor1254-treated rats, liver S9 fraction)

Test method: OECD guideline TG476 (Organisation for Economic Co-operation and Development, 1995-1996)

Result:	positive controls ethylmethane sulphonate and dimethylnitrosamine demonstrated the sensitivity of the assay in the absence and presence of S9 fraction, respectively; the solvent control gave the expected response no increase in mutation frequency above background (1-3 x 10^{-5}); the notified chemical was not mutagenic in mouse cells

9.3.3 Micronucleus Test in Bone Marrow Cells of the Mouse (Bertens, 1997)

Species/strain:	mouse/Swiss
Doses/sampling times:	5 mice/sex received 0, 500, 1 000 or 2 000 mg/kg notified chemical in aqueous 1% carboxymethyl cellulose by gavage; bone marrow sampled 24 and 48 hours after dosing
Test method:	OECD guideline TG474 (Organisation for Economic Co-operation and Development, 1995-1996)
Result:	the notified chemical did not induce an increase in the frequency of micronucleated polychromatic erythrocytes; cyclophosphamide, the positive control, demonstrated the sensitivity of the test

9.4 Overall Assessment of Toxicological Data

The notified chemical was of very low acute oral toxicity (LD_{50} > 5 000 mg/kg) and low acute dermal toxicity (LD_{50} > 2 000 mg/kg) in rats, non-irritant to rabbit skin and a slight eye irritant in rabbits. It was a strong skin sensitiser in guinea pigs. All treated animals exhibited skin sensitisation when induced with injection of the notified chemical at a concentration of 2% followed by topical application at a concentration of 20%.

In a 28-day oral repeated dose study, a range of effects were seen in high dose animals including anaemia, alopecia, increased cholesterol levels and an increase in relative liver weight. The NOEL was 200 mg/kg/day.

The notified chemical was mutagenic in *S. typhimurium* (but not *E. coli*) in the absence of rat liver microsomal enzymes and induced both base-pair substitution and frameshift mutations. However, it did not induce mutations in mouse lymphoma L1578Y cells *in vitro* or micronuclei in mouse bone marrow polychromatic erythrocytes.

The notified chemical would be determined to be hazardous according to NOHSC *Approved Criteria for Classifying Hazardous Substances* (National Occupational Health and Safety Commission, 1994a) on the basis of skin sensitisation.

10. ASSESSMENT OF ENVIRONMENTAL EFFECTS

The following ecotoxicity studies have been supplied by the notifier. The tests were performed in compliance with OECD/EEC Test Methods and according to OECD Principles of Good Laboratory Practices.

Test	*Species*	*Results (Nominal)*
Acute Toxicity (semi static) (OECD TG 203)	Rainbow trout *Oncorhynchus mykiss*	96 h LC_{50} > 100 mg/L 96 h LC_0 > 100 mg/L
Acute Toxicity - Immobilisation Test (Static Test) (OECD TG 202)	Water Flea (*Daphnia magna*)	48 h EC_{50} > 100 mg/L 48 h NOEC > 100 mg/L
Growth Inhibition - Growth (μ) & Biomass (b) (Static Test) (OECD TG 201)†	Green Algae (*Scenedesmus subspicatus*)	Experiment A $E\mu C_{50}$ = 89.9 mg/L# (21.9-n.d. mg/L) E_bC_{50} = 10.9 mg/L (1.0-299 mg/L) LOEC = 3.2 mg/L Experiment B $E\mu C_{50}$ = 377 mg/L (164-1699 mg/L) E_bC_{50} = 21.9 mg/L (11.5-55.8 mg/L)
Respiration Inhibition (OECD TG 209)	Activated Sludge - Aerobic Waste Water Bacteria	3 h IC_{50} > 100 mg/L

95% confidence limits in brackets.

† The method of this test was modified to differentiate between a reduced growth of algae due to real toxic effects of the notified chemical on the algal cells (Experiment A) or a reduced algal growth due to the indirect effect of light absorption in coloured test solutions (Experiment B).

Fish

A limit test, performed in accordance with the test guidelines, demonstrated that the notified substance had no toxic effects on the test fish up to concentration of nominal 100 mg/L. As such, the only concentration tested in the definitive study was 100 mg/L.

The results are all related to nominal concentrations of the notified substance. The analytically determined test substance concentrations in the test media varied in the range of 89% to 94% of the nominal value at the start of the test period. During the 96 hours of the test the concentration of the test substance dropped to 59% of the nominal value. Assuming the reaction product may also have a toxic effect, the sum of the reaction product and the test substance was between 89-94% and as such all biological results are related to nominal concentrations.

In the control and the test concentration of nominal 100 mg/L, all fish survived until the end of the test and no signs of intoxication were observed. The report notes that the test medium was coloured by the test substance.

Aquatic Invertebrates
Nominal concentrations of 4.6, 10, 21, 46 and 100 mg/L and a control were tested in parallel. The results are all related to nominal concentrations of the notified substance. The analytically determined test substance concentrations in the test media varied in the range of 92% to 96% of the nominal value at the start of the test period. During the 48 hours of the test the concentration of the test substance dropped to between 55 and 62% of the nominal value. Assuming the reaction product may also have a toxic effect the sum of the reaction product and the test substance was in all treatments above 90%.

The 24 h and 48 h LC_{50} and NOEC were determined to be greater than 100 mg/L as no daphnids were noted to be immobilised at the highest concentration tested. One daphnid in each of the control and 46 mg/L treatment was observed to be immobile at the 24 h and 48 h observation. This appears to have been an occurrence unrelated to the chemical concentration and has no bearing on the final results and as such all biological results are related to nominal concentrations.

A *Daphnia sp.* reproduction test was not supplied. However, based on the low acute toxicity to both fish and daphnids, reproduction effects on daphnids are not expected.

Algae
Nominal concentrations of 1.0, 3.2, 10, 32 and 100 mg/L and a control were tested. The analytically determined concentrations in the analysed test media varied in the range from 99% to 103% on the nominal values. During the 72 hours of the test the concentration of the test substance declined to between 55 and 62% of the nominal value. Assuming the reaction product may also have a toxic effect, the sum of the reaction product and the test substance was all above 90% and as such all biological results are related to nominal concentrations.
In experiment part A, where the algae grew in test media with dissolved test substance, a statistically significant inhibitory effect on the growth of algae occurred after 72 hours at the concentration of 3.2 mg/L. As such, the 72 h NOEC was determined to be 0.9 mg/L. The EC-values (indicated in the above table) were calculated for the algal biomass (b) and the growth rate (μ) after 72 hours. There was no observed difference in the shape of algal cells when compared to those growing in the control.

In experiment part B, where the algae grew in test water without the test substance, but under the reduced light intensities due to the filter effect of the coloured test media, the algal growth was significantly reduced compared to the control after 72 hours at the test concentration of 1.0 mg/L. The EC_{50} values and the percentage inhibition of the algal growth rate μ after 72 hours of exposure in this experiment were of a slightly lesser magnitude than in experiment part A.

The modified growth inhibition test showed that there were differences between the growth of *Scenedesmus subspicatus* under the two different test regimes. Growth inhibition when the algae grew in test water without the test substance, but under the reduced light intensities of the coloured test media, compared to when the algae grew in directly in the test media with the dissolved test substance, was greater at some concentrations than the 10 % allowed under

the test protocol. Therefore, the notifier claims that the real toxic effect of the notified chemical cannot be excluded at the test concentrations of 1.0, 10 and 32 mg/L.

In conditions where release to the environment occurs, algistatic effects from reduced light incidence as well as the algitoxic effects of the chemical may still lead to an undesirable environmental impact if exposure is continuous. Therefore, the combined effects on the test algae should be taken into account. Thus, the notified chemical can be considered as slightly toxic to algae.

Microorganisms
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 practically no toxic effects, with the respiration rate not inhibited when exposed to nominal test concentrations in the range 3.2 to 100 mg/L over the exposure period of 30 minutes, with a final 3 hour IC_{50} greater than 100 mg/L.

Conclusion
The ecotoxicity data for the notified substance indicate that it is practically non-toxic to fish, aquatic invertebrates and microorganisms, and slightly toxic to algae (with effects on biomass and growth rate). Reproductive effects on aquatic invertebrates are not expected.

11. ASSESSMENT OF ENVIRONMENTAL HAZARD

The environmental hazard from the dye, when fixed to the cellulose fibre, is rated as negligible.

The notifier has specified that a limited number of dyehouses (approximately 6) in city and country areas will be using the notified dye. The environmental hazard has been determined for two dyehouses located in two general locations, one metropolitan based dyehouse and the other country based. The Predicted Environmental Concentration (PEC) is estimated below.

Calculation Factor	***Country Dyehouse high use***	***City Dyehouse***
Typical use of dyestuff expected per day	25 kg	25 kg
Volume of notified chemical	7.5 kg	7.5 kg
Quantity in wastewater (at fixation rate)	0.1875 kg	0.1875 kg
Quantity of water used incl. wash-off water (at 75 L/kg)	14 000 L	42 000 L
Effluent conc. in dye-specific wash-water; fixation 97.5%	13.39 mg/L	4.46 mg/L
Dilution factor in dyehouse by other wash-waters	119	95
Influent concentration	0.11 mg/L	0.05 mg/L
Dilution factor in sewage treatment plant	2	250
Conc. balance in effluent from sewage treatment plant	0.06 mg/L	0.19 mg/L
Dilution factor in receiving waters	3	10
PEC in receiving waters	0.06 mg/L	0.19 µg/L

Safety factor (EC_{50}/PEC) for exposure to most sensitive aquatic organism, Algae[1] (72 h $E_{\mu}C_{50}$ ≈4.7 mg/L)	251	250 040

[1] The growth of Green algae was inhibited by 50% after 72 hr at a test concentration of 4.7 mg/L. (see *Environmental Effects* section).

These calculations assume that no dye is removed in treatment of the different waste effluents and represent the worst case scenario for dyehouses. The "typical use of dye expected per day" amount was supplied by the notifier, and is expected to be a representation of maximum use.

The calculations show that the exposure to fish, daphnia, algae and waste water treatment bacteria is at levels unlikely to cause any significant effect. At higher release rates, there is still unlikely to be any significant effect on these species. Once in the aquatic environment, the chemical is expected to swiftly dilute to undetectable concentrations, and undergo biotic and abiotic degradation. A lessened but adequate safety factor exists if the notified chemical is to be used in country locations.

The only other source of environmental contamination is from accidental spills and disposal of packaging. The MSDS is adequate to limit the environmental exposure and therefore limit the environmental effects.

12. ASSESSMENT OF PUBLIC AND OCCUPATIONAL HEALTH AND SAFETY EFFECTS

From the toxicological data supplied, the notified chemical is not likely to be acutely toxic nor to cause systemic toxic effects on repeated or prolonged exposure. It is not likely to be a skin irritant but may be a slight eye irritant. The notified chemical is likely to be a strong skin sensitiser. The notified chemical induced both base-pair substitution and frameshift mutations in *S. typhimurium* but did not induce base-pair substitution mutations in *E. coli*. It was negative in a mouse lymphoma mutagenicity assay and also negative in the mouse bone marrow micronucleus assay for clastogenicity. One of the metabolites of the notified chemical, 2-chloro-4-nitroaniline has been shown to be mutagenic in *S. typhimurium* but negative in a rat hepatocyte unscheduled DNA synthesis assay. This suggests the notified chemical can interact with DNA but is less likely to do so in mammalian cells than in bacteria. The notified chemical would be determined to be hazardous according to NOHSC *Approved Criteria for Classifying Hazardous Substances* (National Occupational Health and Safety Commission, 1994a) on the basis of skin sensitisation. It would warrant the risk phrase R43, "may cause sensitisation by skin contact".

Transport and storage
Waterside, transport and storage workers should only be exposed to the notified chemical in the event of accidental spillage.

Repacking
Repacking of less than 100 kg per year is undertaken at the notifier's warehouse using a ventilated weighing booth to minimise exposure. Thus the risk of adverse health effects in these workers should be low.

End use

The main groups of workers likely to be exposed to the notified chemical on a regular basis are those involved in dye weighing and those handling the dyed fabric after the exhaust dyeing is completed when the cloth is lead to wash off baths. The notifier states that the dyestuff, containing the notified chemical at a concentration of up to 40%, is non-dusting. However, the extent of non-dustiness was not provided. In addition, a large proportion of the chemical powder (83.2%) is of respirable size. It is therefore very important that adequate controls are in place to strictly control the extent of respiratory exposure to the notified chemical, to reduce the chance of workers developing respiratory sensitisation. In this situation respiratory exposure is controlled by the use of the non-dusting formulation and the existence of local exhaust ventilation while weighing. The dye weighers are stated to wear elbow-length PVC gloves, safety glasses, face-shield and protective overalls. Therefore, the risk of skin sensitisation should also be controlled. The non-dusting form of the chemical and the use of personal protective equipment should also reduce contamination and the risk of genotoxic effects. Workers handling the wet dyed cloth will also need to use personal protective equipment although at this time 2.5% of the added dye remains unfixed. After washing the fabric, the risk of adverse health effects to dyehouse workers handling the fabric should be negligible.

In NA/642, the same notifier has provided some information from a US air monitoring study in a dye weighing area. The estimated average daily lifetime exposure of a worker during weighing was calculated to be 0.0016 mg/kg/day assuming one weighing operation per day for 15 minutes. In the current submission the notifier calculated that the absorbed dose to dye weighers as no more than 0.0008 mg/kg/day, the difference to the previous submission being due to the concentration of dye in the dyestuff and the frequency of weighing. Using the same data but not correcting for life expectancy would yield an exposure of approximately 0.002 mg/kg/day. Based on a NOEL of 200 mg/kg/day from the subacute oral toxicity study the margin of exposure would be 100 000. On this basis the risk of systemic effects would be negligible. However, this finding needs to be qualified because the estimate of respiratory exposure is for weighing times only and does not take into account any other times of airborne exposure. Dermal contamination and absorption is not included and the NOEL is derived from a short term study, when the genotoxic data indicate that longer term health effects cannot be excluded, at undetermined doses.

Dye weighers are stated by the notifier to be the group of workers receiving the highest exposure. Given that the chemical is a sensitiser and mutagenic effects were observed in an Ames test, it is important that strict exposure controls are in operation to minimise exposure and the risk of adverse health effects. Any workers who have become sensitised to the notified chemical should not continue to work with it.

The notified chemical is for industrial use only and is unlikely to come in contact with the general public. According to the notifier, the notified chemical is strongly fixed to the fibre and public exposure to the chemical in treated items during washing or wearing will be negligible. In addition, with the industrial controls to minimise environmental release, public exposure is likely to be low.

13. MATERIAL SAFETY DATA SHEET

The MSDS for the imported dyestuff containing the notified chemical was provided in accordance with the *National Code of Practice for the Preparation of Material Safety Data Sheets* (National Occupational Health and Safety Commission, 1994b).

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.

14. RECOMMENDATIONS

To minimise occupational exposure to the notified chemical 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);
- Impermeable gloves should conform to AS/NZS 2161.2 (Standards Australia/Standards New Zealand, 1998);
- All occupational footwear should conform to AS/NZS 2210 (Standards Australia/Standards New Zealand, 1994c);
- If adequate ventilation is not available to control exposure to dust, respiratory protection conforming to AS/NZS 1715 and 1716 (Standards Australia/Standards New Zealand, 1994a; Standards Australia/Standards New Zealand, 1994b) should be worn;
- Spillage of the notified chemical should be avoided. Spillage should be cleaned up promptly with absorbents which should then be put into containers for disposal;
- Good personal hygiene should be practised to minimise the potential for ingestion;
- A copy of the MSDS should be easily accessible to employees.
- Any workers who have become sensitised to the notified chemical should not continue to work with it.

15. REQUIREMENTS FOR SECONDARY NOTIFICATION

Under the Act, secondary notification of the notified chemical shall be required if 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.
		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