File No: NA/654

December 1998

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME

FULL PUBLIC REPORT

Blue REN 535

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Telephone: (61) (02) 9577 9514
Facsimile: (61) (02) 9577 9465

Director
Chemicals Notification and Assessment

FULL PUBLIC REPORT

Blue REN 535

1. APPLICANT

Ciba Specialty Chemicals of 235 Settlement Rd., THOMASTOWN, VIC 3074 has submitted a standard notification statement in support of their application for an assessment certificate for Blue REN 535.

2. IDENTITY OF THE CHEMICAL

Chemical Name: 4,11-Triphenodioxazinedisulphonic acid,3,10-bis[(2-aminopropyl)amino]-6,13-dichloro-, reaction products with 2-amino-1,4-benzenedisulphonic acid, 2-[(4-amino phenyl)sulphonyl]ethyl hydrogen sulphate and 2,4,6-trifluoro-1,3,5-triazine, sodium salts

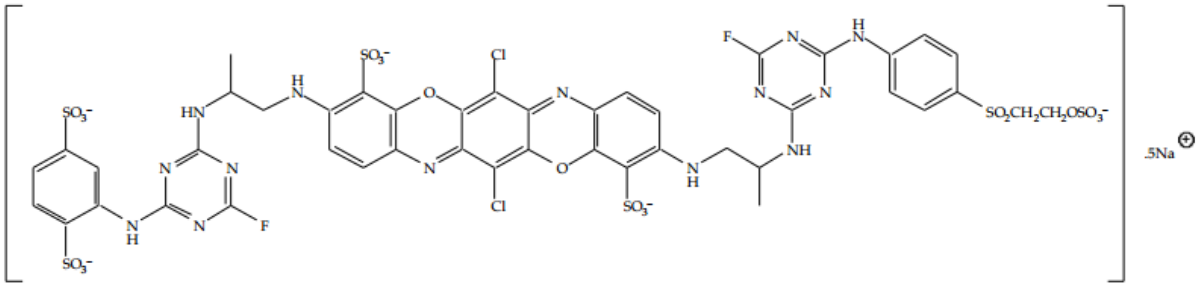
Chemical Abstracts Service (CAS) Registry No.: 191877-09-5

Other Names: FAT 40566/A

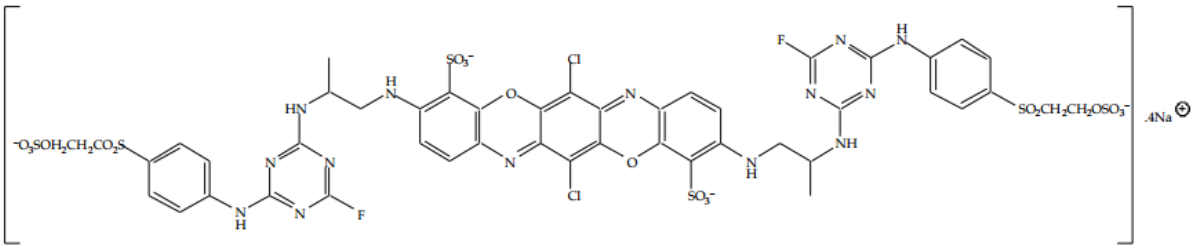
Trade Name: Blue REN 535
Cibacron Blue LS-G (contains 70 % notified chemical)

Molecular Formula: Main Component A: $C_{44}H_{33}N_{14}O_{20}S_6Cl_2F_2Na_5$
Main Component B: $C_{46}H_{38}N_{14}O_{20}S_6Cl_2F_2Na_4$
Main Component C: $C_{42}H_{28}N_{14}O_{20}S_6Cl_2F_2Na_5$

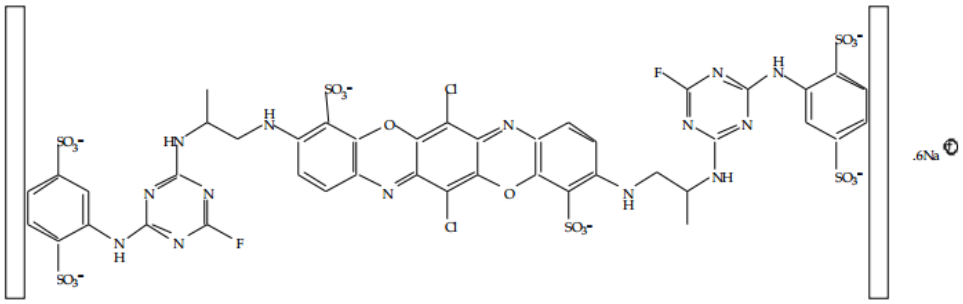
Structural Formula:



Main Component A (19.8 %)



Main component B (13.9 %)



Main Component C (8.7 %)

Molecular Weight:

Main Component A: 1494 g/mol
Main Component B: 1500 g/mol
Main Component C: 1488 g/mol

Method of Detection and Determination:

physical testing, IR spectroscopy, UV/Vis spectroscopy and NMR spectroscopy

Spectral Data:

uv/vis
neutral/basic solution
peaks at 264 nm, 630-635 nm

acid solution
peaks at 270 nm, 759 nm

IR
strong complex spectrum, major peaks 2850-3000 cm^{-1}, 1050-1650 cm^{-1}

nmr
complex spectrum, peaks at 10.544, 10.500, 10.400, 10.308, 10.169, 8.610, 8.397, 8.340, 8.054, 8.025, 7.949, 7.921, 7.898, 7.811, 7.781, 7.749, 7.720, 7.674, 7.658, 7.648, 7.631, 7.604, 7.457, 7.428, 7.291, 7.256, 7.225, 7.140, 7.115, 7.083, 7.061, 7.028, 6.750, 6.723, 6.696, 6.650, 6.621, 6.579, 6.549, 6.308, 6.253, 6.246, 6.156, 6.137, 6.123, 6.104, 4.326, 4.302, 4.152, 3.970, 3.948, 3.933, 3.911, 3.596, 3.574, 3.551, 3.245, 1.272, 1.249, 1.231, 1.212 ppm

3. PHYSICAL AND CHEMICAL PROPERTIES

Appearance at 20°C and 101.3 kPa: dark blue powder

Particle Size:

Range (μm)	Mass (%)
< 5	0.00
5 – 8	0.03
8 – 15	0.37
15 – 32	5.96
32 – 40	0.97
40 – 63	8.99
63 – 90	13.8
90 – 125	34.4
125 – 160	30.9
160 – 200	3.83
> 200	0.82

< 7 – 10 μm respirable < 180 μm inspirable

Melting Point: >400°C

Specific Gravity: 1.75 g/cm^3

Vapour Pressure: < 1 × 10^{-8} kPa at 25°C

Water Solubility: > 222 g/L at 25°C

Partition Co-efficient (n-octanol/water): log P_{ow} ≤ -4 (calculated)

Surface Tension 73 mN/m at 20.8°C

Hydrolysis as a Function of pH:

Main Component A			Main Component C		
pH	T (°C)	$t_{1/2}$ (h)	pH	T (°C)	$t_{1/2}$ (h)
4	25[a]	39.0	4	25[a]	39.9
	25[b]	39.1		25[b]	39.9
	50	4.6		50	4.0
7	25[a]	933.5	7	25[a]	12676.3
	40	106.0		40	1656.1
	50	27.8		50	473.8
9	25	<1	9	25	<1

[a]Extrapolated [b]Measured

Adsorption/Desorption: According to OECD TG106 (Organisation for Economic Cooperation and Development, 1993a)

Soil	Soil Type (USDA Classification)	Organic Carbon (g/100 g dry soil)	K_{OC} (ml/g)
Speyer	loamy sand	2.29	1045
Sisseln	sandy loam	1.57	556
Les Barges	silt loam	3.80	1499

Dissociation Constant: The dissociation constant for the acidic and basic functionalities of the three main components were estimated as follows.

Component	Acid/Base	pK_a	Method
A, C	arenesulfonic acid 1	-7.1	1
A, C	arenesulfonic acid 2	-6.9	1
A, B, C	arenesulfonic acid 3	<-7.1	1
A, B, C	diazo group	<0	2
A, C	Secondary amine 1	~1	2
A, B, C	Secondary amine 2	3.1	3
A, B, C	Secondary amine 3	not determined	
A, B	Secondary amine 4	~1	2
A, B	alkyl sulfuric acid	<<-7	2

Method 1 Hammett 2 General estimation 3 Taft

Flash Point: not flammable

Flammability Limits: not flammable

Autoignition Temperature: not autoflammable

Explosive Properties: not explosive

Reactivity/Stability: stable under conditions of intended use

Comments on Physico-Chemical Properties

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

No melting point was detected up to 400°C using the capillary method (Pighetti, 1997a). The density was determined by means of a gas comparison pycnometer at 20.0°C (Schmiedel, 1997a). The vapour pressure was estimated based on the boiling point using the Modified

Watson Correlation (Schmiedel, 1997b). The boiling point was calculated to be 1040°C using Meissner's method.

Water solubility was determined using the flask shake method (Pighetti, 1997b). 2.50 g of the notified substance was added to 10.0 mL distilled water and agitated at 30°C for up to 72 h. After centrifugation aliquots of the solutions were diluted and the concentration of the chemical determined spectrophotometrically.

Hydrolysis studies for two of the main components of the notified substance indicate that they are relatively stable at neutral pH and room temperature with half-lives of greater than 40 days (Petschel, 1997). At pH 4, hydrolysis was more rapid with half-lives of approximately 40 h at room temperature. At pH 9, hydrolysis of both components was very rapid at room temperature (half-lives < 1 h).

The partition coefficient was estimated to be log P_{OW} < -4 by calculation using the saturation concentration of the notified substance in the pure solvents, i.e. 5 mg/L in n-octanol and 90,000 mg/L in double distilled water (Schmiedel, 1997c).

The adsorption/desorption data provided by the notifier, obtained by the standard batch equilibrium method, indicates that the notified chemical is not expected to be mobile in soils (Völkel, 1997).

The molecular structure of the free acid was used for the estimation of the dissociation behaviour (Schmiedel, 1997d). The dissociation constant for each of the four acidic protons [three arenesulfonic acids and one alkyl sulfuric acid] and four secondary amines found in the molecular structure were predicted. The calculated pK_a values predicted for the acid functionalities indicate that these functional groups will be completely dissociated. The pK_a values determined for three of the four secondary amine functionalities indicate that these functionalities are not likely to be protonated in the environmental pH range. The dissociation constant for secondary amine 3 was not determined because a suitable equation could not be found. The report indicates that it is not possible to exclude the possibility of this amine functionality being protonated in the environment. The sulfonic acid and sulfuric acid functionalities are expected to be completely dissociated under environmental conditions, so the main components of the notified chemical will be present in anionic form over the entire environmental pH range.

The notified chemical is not expected to be surface active. By definition, a chemical has surface activity when the surface tension is less than 60 mN/m (European Economic Community, 1992).

4. PURITY OF THE CHEMICAL

The notifier has provided extensive detail on the formulation of the commercial product. Details of the purity of the chemical as imported which relate to non-hazardous impurities and additives have been exempted from publication in the Full Public Report and the Summary Report.

The data in this section refer variously to the notified chemical, Blue REN 535, and the commercial product, Cibacron Blue LS-G (containing 70 % (w/w) Blue REN 535). The formulation to which each item refers is specified.

The notified chemical substance, Blue REN 535, consists of a mixture of chemicals of variable composition, i.e. a UVCB substance. It has three main components which make up 42.4 % by weight of the notified substance. The remaining identified material consists of oligomers of the main components, hydrolysed reactants and rearrangement products.

Degree of Purity: Blue REN 535
42.4 % (w/w) main components A, B and C; comprised of 96 % (w/w) identified components. The components are listed below.

Chemical Name	*CAS No.*	*Weight %*
main components A, B and C	none	42.4 %
known coloured by-products	none	34.5 %
known uncoloured byproducts	none	7.1 %
unknown coloured byproducts	none	3.1 %
unknown uncoloured byproducts	none	0.7 %
sodium fluoride	7601-54-9	0.4 %
inorganic salts (non-hazardous)	none	< 10 %
water	7732-18-5	< 10 %

Toxic or Hazardous Impurities: The notified chemical is a mixture of at least 42 reaction products (identified by HPLC) which are not individually isolated. The physico-chemical data and the toxicity testing refer to the notified chemical.

Non-hazardous Impurities (> 1 % by weight): see above

Additives/Adjuvants: In Cibacron Blue LS-G

Name: inorganic salts (non-hazardous)
Weight percentage: 10 - 20 %
CAS No.: none

Name: dispersing agent (non-hazardous)
Weight percentage: < 10 %

CAS No.:	9084-06-4
Chemical name:	paraffin oils
Synonyms:	antidusting additive
Weight percentage:	1 %
CAS No.:	8012-95-1
Chemical name:	water
Weight percentage:	< 10 %
CAS No.:	7732-18-5

5. USE, VOLUME AND FORMULATION

The notified chemical will not be manufactured in Australia. It will be imported as a component of the dye product Cibacron Blue LS-G which will be used in dyehouses for the colouration of cellulose textiles by the exhaust dying method only. The expected import volume is 1-2 tonnes per annum initially rising to 5-6 tonnes after 5 years.

Cibacron Blue LS-G will be imported by ship in 30 kg sealed containers with antistatic polyethylene lining. The dye will then either be sold as received (the majority) or it will be repacked into 5 and 10 kg containers at the importer's facility (approximately 4 % of the imported material). The dye will only be available to industrial users. The notifier indicates that approximately 15 dyehouses throughout Australia would use this product. At the dyehouses, the material will be weighed manually and dissolved in water at 80-90 °C to a concentration of less than 1 % (w/v). The notifier estimates that Cibacron Blue LS-G will comprise a maximum of 10 % of the various dyes used in any dyehouse, and that about 2 kg of the dye will be used approximately ten times daily.

The dye is then applied to fabrics, with 75 % of the dye being fixed to the fabric. The residual dye solution is disposed of in the aqueous waste stream, while the fixed dye is considered to be completely immobilised.

6. OCCUPATIONAL EXPOSURE

Routes of Exposure
The notified chemical will be imported as a powdered solid (0.4 % respirable; the majority of the remainder inspirable) which will be dissolved in water to produce the dye solutions. Exposure of workers to the dust from the solid will be by inhalation, and by dermal and ocular contact. The most probable route of exposure to the aqueous solution will be dermal.

Transport and storage
The notified chemical will be imported by ship in 30 kg sealed containers with polyethylene antistatic lining. The containers will be transferred by waterside workers for road transport to

a warehouse facility, where (except where repackaging is required) they will be stored for transport to the customer facilities. It is estimated that 10-15 workers will be involved in transport and storage. These workers could only be exposed to the notified chemical in the case of an accident where the packaging was breached.

Repackaging
The notifier estimates that up to 4 % of the imported volume will be repackaged into 5 or 10 kg containers at the warehouse. This will involve 2 workers with an exposure time of approximately 1.5 hours in the first year of import, rising to 4.5 hours in the fifth year. The worker exposure during this process will be to the powder. The repackaging of dye is conducted in a downdraught weighing booth.

End Use
The following procedures are carried out at the customer facilities, of which there will be about 15 throughout Australia.

Weighing and mixing
At the customer facilities, the powdered dye will be weighed out from the 5, 10 or 30 kg containers in approximately 2 kg lots, and mixed with water to prepare the dye solution. This is expected to be done about ten times daily. The process will involve 2 operators per shift in two shifts, manually scooping dye from the drums into the weighing container, then adding the dye to water heated to 80-90 °C in a mixing tank. The containers for the solid dye will then be resealed. Empty polyethylene liner bags will be shaken thoroughly into the newly opened containers to recover remnant powder during the repackaging and weighing and mixing operations. The exposure is estimated at 45 minutes per operator per shift. The exposure to the notified chemical will be to the powdered solid, as well as to a 1 % (w/v) aqueous solution.

The notifier states that the workers involved in the weighing and mixing procedures will follow existing procedures in the dyehouses that require workers to wear overalls, protective gloves and glasses, and use respiratory protection (not defined). Mechanical ventilation of the weighing area will also reduce the buildup of dye dust.

The notifier has provided an exposure estimate for the workers involved in the weighing of the dye powder. The notifier states that an occupational exposure survey carried out in 24 dyehouses in the United States indicated that the mean concentration of airborne dye dust was approximately 0.18 mg/m^3. Based on an inhalation rate of 10 m^3 of air per person per shift, a typical body weight of 70 kg and a respirable fraction of 0.4 %, the maximum total respired dose of dye dust is 0.1 µg/kg/day. As it is expected that Cibacron Blue LS-G will comprise a maximum of 10 % of the dyes used in a dyehouse, the maximum dose of the notified chemical is less than 0.01 µg/kg/day.

Dyeing
The dye solution (maximum concentration 1 % (w/v)) will be manually transferred to a feed tank then automatically sprayed onto the cloth on a continuous roller in an enclosed dyeing machine. Skin contamination may occur during handling of the dye solution. There is further possibility of worker exposure if the cloth becomes tangled and the machine has to be opened to realign it on the rollers. The machine is also opened on a regular basis to clean loose fibres out of a filter with a hose. It is estimated that about 8 workers per shift (two shifts per day)

will be exposed during these activities. Exposure episodes would be frequent, given the frequency of use of the dye. Individual exposure times may be short as Cibacron Blue LS-G is expected to comprise a maximum of 10 % of the dyes used at any dyehouse, and the dye application occurs for 20 minutes of the 3 hour dyeing cycle.

The used dye solution will then go into the waste stream. The dyed cloth is washed in warm soapy water to remove any free dye at the end of the enclosed dyeing cycle.

Operators of the dyeing machines wear gloves and glasses when handling dye solutions and for threading the cloth.

Drying/curing
In each dyehouse, 4 operators per shift (two shifts a day) are normally involved in drying the cloth. This involves manually loading the dyed cloth into dryers. The concentrations of free dye at this time are expected to be very low as the dye is fixed to the cloth and the excess is washed out during the dyeing process. The exposure time for each worker to this particular dye is expected to be 45 minutes per shift.

Laboratory
The notifier estimates that 2 laboratory technicians in each customer facility and also 5 technicians at the importer facility will be involved in weighing and mixing small samples of the dye for colour matching purposes. The exposure time is estimated to be several minutes per day. Laboratory technicians may be exposed to the dye in either powder or solution form during these activities.

7. PUBLIC EXPOSURE

Cibacron Blue LS-G will be available to industrial users only, and will not be sold to the public. There is very low potential for public exposure arising from transport, handling and dyeing operations. Dilution of the wash-water effluent in the waste-water treatment plant and receiving waters will ensure that the environmental concentrations are at undetectable levels. The dye is strongly bound to the fibre, and so public exposure during washing and dry cleaning of the fabric is expected to be negligible.

8. ENVIRONMENTAL EXPOSURE

The dye preparation will be used only for the colouration of cellulose textiles for the exhaust dyeing method. Repackaging will be carried out at the notifier's Thomastown warehouse only, where facilities for the safe handling of hazardous substances are used.

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. A fixation plot indicates that fixation of up to 75 % of the dyestuff can be expected. After application to fabrics, the dye undergoes a chemical change involving chemical bonding with hydroxy groups on the cellulose fibres. The dyestuff is strongly fixed to the fibre with the notifier claiming that negligible residues

will result due to the fabric being washed.

The major environmental exposure to dye will come from effluent discharge from dyehouses and waste water treatment systems. This release will also consist of the hydrolysed derivative due to the alkaline nature of these systems (Ecological and Toxicological Association of the Dyestuffs Manufacturing Industry, 1991). Other releases will be limited to traces remaining from repackaging operations and clean-up of any spills, and from trace residues in empty packaging (5 g per 30 kg bag).

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

Fate

The substance (including the hydrolysed derivative) normally released in water as effluent from the dyehouse is expected to be the major environmental exposure. The substance may either partition to sediment or stay in the aqueous compartment. While it has been reported (Hobbs, 1988) that reactive dyes have been found not to absorb to sludge in model systems, adsorption/desorption results suggest this could be significant. Any substance that binds to the sludge will be disposed of through incineration or landfill.

Residues that persist after sewage treatment will enter marine environments in solution (from city waste water treatment systems). 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 (Weber, 1991). 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.

Incineration is one of the preferred options for disposal 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 with a small amount of hydrogen chloride. Disposal of the substance to landfill at a secured site is also recommended by the notifier. Empty product packaging should contain minimal residues of the notified chemical and will be disposed of as waste to an approved secure landfill site or by incineration.

The biochemical oxygen demand (BOD) of the dye was tested and the five day study showed the BOD_5 was 0 mg O_2/g (Schnalke, 1997a). The chemical oxygen demand (COD) was determined to be 720 mg/g O_2 (Schnalke, 1997b). The dye was found to be not readily biodegradable in the OECD 301A Test for ready biodegradability (Organisation for Economic Cooperation and Development, 1993c) (modified AFNOR Test). Measured as dissolved organic carbon (DOC) and expressed as percentage elimination, biodegradation amounted to 9 % at the end of the 28 day exposure to microorganisms from a domestic sewage treatment plant. No inhibition on the activity of the bacteria was observed in this test.

The notified substance was found not to biodegrade over a 28 day exposure period when exposed to microorganisms from a domestic waste water treatment plant, according to the OECD Test Guideline 302B (Organisation for Economic Cooperation and Development, 1993b) Zahn-Wellens/ EMPA Test (Schnalke, 1997c). Throughout the entire exposure period,

the mean dissolved organic carbon (DOC) concentrations remained practically unchanged in comparison to the initial mean DOC concentrations measured on day 0 after 3 hours of exposure. Expressed as a percentage removal, average biodegradation ranged from 0 % to 6 % over the exposure period.

Although not readily or inherently biodegradable, the potential for bioaccumulation is low due to the low estimated partition coefficient (log $P_{OW} \leq -4$) and very high water solubility of the substance (>222 g/L). Hydrophilic dyes with log $P_{OW} < 3$ have also been shown not to bioaccumulate (Yen, 1991).

9. EVALUATION OF TOXICOLOGICAL DATA

9.1 Acute Toxicity

Summary of the acute toxicity of Blue REN 535

Test	*Species*	*Outcome*	*Reference*
acute oral toxicity	rat	LD_{50} > 2000 mg/kg	(Allard, 1997a)
acute dermal toxicity	rat	LD_{50} > 2000 mg/kg	(Arcelin, 1997a)
skin irritation	rabbit	not irritating	(Braun, 1997b)
eye irritation	rabbit	serious eye damage	(Braun, 1997a)
skin sensitisation	guinea pig	non-sensitising	(Arcelin, 1997b)

9.1.1 Oral Toxicity (Allard, 1997a)

Species/strain:	rat/HanIbm: WIST (SPF)
Number/sex of animals:	5/sex
Observation period:	15 days
Method of administration/dose:	gavage, 20 % (w/v) aqueous solution; dose 2000 mg/kg
Mortality:	no deaths occurred during the study
Clinical observations:	no clinical signs of toxicity were observed during the study
Morphological findings:	no macroscopic findings were observed at necropsy
Test method:	limit test, OECD TG 401 (Organisation for

	Economic Cooperation and Development, 1987c)
LD_{50}:	greater than 2000 mg/kg
Result:	the notified chemical was of very low acute oral toxicity in rats

9.1.2 Dermal Toxicity (Arcelin, 1997a)

Species/strain:	rat/HanIbm: WIST (SPF)
Number/sex of animals:	5/sex
Observation period:	15 days
Method of administration/dose:	semi-occluded patch; 24 hour exposure dose 2000 mg/kg; test material applied as a 33.33 % (w/v) aqueous solution
Mortality:	no deaths occurred during the study
Clinical observations:	no clinical signs of toxicity were observed during the study; a blue discolouration was observed on all animals as a local effect of the test substance
Morphological findings:	no macroscopic findings were observed at necropsy
Test method:	limit test, OECD TG 402 (Organisation for Economic Cooperation and Development, 1987a)
LD_{50}:	greater than 2000 mg/kg
Result:	the notified chemical was of low dermal toxicity in rats

9.1.3 Inhalation Toxicity

The notifier indicated that the notified chemical is in the form of a powder with 0.4 % of particles below 15 µm diameter and thus the respirable fraction is sufficiently low for inhalation to not be considered as a major route of exposure.

9.1.4 Skin Irritation (Braun, 1997b)

Species/strain:	rabbit/New Zealand White
Number/sex of animals:	1 male, 2 female
Observation period:	3 days

Method of administration:	0.5 g of test material, moistened with bi-distilled water, was applied to a clipped intact region of the dorsal skin and secured under a gauze patch with a semi-occlusive dressing for 4 hours; at the end of this time residual material was removed with lukewarm water; animals were examined for skin reaction 1, 24, 48 and 72 hours following application of the test substance
Test method:	OECD TG 404 (Organisation for Economic Cooperation and Development, 1992a)
Observations:	no erythema or oedema observed at any time interval; blue staining of the treated skin
Result:	the notified chemical was not irritating to the skin of rabbits

9.1.5 Eye Irritation (Braun, 1997a)

Species/strain:	rabbit/New Zealand White
Number/sex of animals:	1 male, 2 female
Observation period:	3 days
Method of administration:	0.1 g of test material applied as supplied into conjunctival sac of the left eye of each animal; the contralateral eye served as the control; animals were examined for eye lesions 1, 24, 48 and 72 hours after test substance application; further observations were made after 7, 14 and 21 days
Observations	chemosis and discharge was observed in all of the animals 1 hour after application of the test material; hyperaemia of the scleral blood vessels was observed in one animal after 1 hour; no signs of irritation were seen in any of the animals after 24 hours or for the remainder of the study; persistent blue staining of the conjunctivae and sclera of the treated eyes was observed in all animals even after 21 days
Test method:	OECD TG 405 (Organisation for Economic Cooperation and Development, 1987b)

Result: the notified chemical was slightly irritating to the eyes of rabbits but must be considered to produce serious eye damage based on the persistence of the blue staining

9.1.6 Skin Sensitisation (Maximisation Test) (Arcelin, 1997b)

Species/strain: guinea pig/Dunkin-Hartley

Number of animals: 10 test, 5 control

Induction procedure: day 1: to a clipped area of the scapular dorsal skin, each animal received 3 pairs of 0.1 mL injections as follows –

test group:
- 1:1 (v/v) mixture of Freund's Complete Adjuvant and physiological saline
- the test material diluted to 5 % in bi-distilled water
- the test material diluted to 5 % by emulsion with 1:1 (v/v) mixture of Freund's Complete Adjuvant and physiological saline

control group:
- 1:1 (v/v) mixture of Freund's Complete Adjuvant and physiological saline
- bi-distilled water
- 1:1 (w/w) mixture of bi-distilled water and 1:1 (v/v) mixture of Freund's Complete Adjuvant and physiological saline

day 8

test group
a filter paper patch with 0.3 g of test material (25 % (w/v) in bi-distilled water) was placed over the injection area; covered with aluminium foil and secured with elastic plaster and impervious adhesive tape

control group
as above except using only bi-distilled water

Challenge procedure: day 22

two patches of filter paper, one saturated with 10 % (w/v) test material in bi-distilled water, the other

with bi-distilled water only were applied respectively to the left and right flanks of both the test and control groups under occlusive conditions as described above, for a period of 24 hours

Challenge outcome:

	Test animals		***Control animals***	
Challenge concentration	***24 hours****	***48 hours****	***24 hours***	***48 hours***
0 %	**0	0	0	0
10 %	0	0	0	0

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

Test method: OECD TG 406 (Organisation for Economic Cooperation and Development, 1992c)

Result: the notified chemical was not sensitising to the skin of guinea pigs

9.2 Repeated Dose Toxicity (Allard, 1997b)

Species/strain: rat/HanIbm: WIST (SPF)

Number/sex of animals:
group 1: 10/sex
group 2: 5/sex
group 3: 5/sex
group 4: 10/sex

Method of administration: gavage

Dose/Study duration::
group 1: 0 mg/kg/day
group 2: 50 mg/kg/day
group 3: 200 mg/kg/day
group 4: 1000 mg/kg/day

the study duration was 28 days; 5 animals per sex in groups 1 and 4 were then allowed to recover for 14 treatment free days

Clinical observations: no clinical signs of toxicity in any of the animals, except blue discolouration of faeces persisting until day 5 of the recovery period; one high dose female showed localised alopecia of the head

Clinical chemistry/Haematology methaemoglobin concentrations were moderately increased in both males and females of group 4;

	total bilirubin level moderately increased for males and females of group 4; total protein and globulin levels slightly decreased and albumin to globulin ratio slightly increased in males of group 4; light blue to blue plasma discolouration in all animals of groups 3 and 4
	urinalysis showed a slight increase in the score for ketone and blood for males of group 4; animals in groups 3 and 4; also one animal in group 2, showed urine discolouration ranging from light green to deep blue
	most findings indicated reversibility at the end of the recovery period although there was still a slight but statistically significant increase in total bilirubin for males; light green to green urine discolouration persisted throughout
Histopathology:	there were no significant differences in organ weights between test animals and the controls; many organs showed a bluish discolouration, particularly in the 200 and 1000 mg/kg groups, both before and after recovery; microscopic blue/black pigment particles, unassociated with any pathological alteration, could also be found in many organs
	treatment related lesions were confined to the stomach; hyperplasia of the foveolar epithelium was noted in 9/10 group 4 animals with minimal to moderate severity and in 1/5 group 3 females at the termination of the main study; this was still present at minimal severity in 2/5 group 4 females after recovery
	all animals of group 4 also showed other stomach lesions; an increase in incidence and severity of apoptotic bodies (minimal to moderate), limiting ridge epithelium vacuolation (minimal to moderate) and inflammatory cell infiltrates (minimal to moderate); apoptotic bodies remained slightly increased in group 4 animals after recovery; one group 4 female had a severe degree of mucosal erosion at the end of the main study
Test method:	OECD TG 407 (Organisation for Economic Cooperation and Development, 1995)
Result:	considering only the presence of lesions of the

	stomach, a NOAEL of 50 mg/kg could be established for the study; as discolouration of the faeces and urine were found at all doses, a NOEL cannot be established

9.3 Genotoxicity

9.3.1 *Salmonella typhimurium* and *Escherichia coli* Reverse Mutation Assay (Wollny, 1997)

Strains:	*Salmonella typhimurium*: TA98, TA100, TA1535, TA1537 *Escherichia coli*: WP2, WP2 *uvrA*
Concentration range:	33, 100, 333, 1000, 2500 and 5000 µg/plate
Metabolic Activation System:	rat liver S9 fraction from animals pretreated with phenobarbital and β-napthoflavone
Test method:	OECD TG 471 and TG 472 (Organisation for Economic Cooperation and Development, 1983a; Organisation for Economic Cooperation and Development, 1983c)
Positive controls	*Salmonella typhimurium*: 2-aminoanthracene (2AA) 2.5 µg/plate – all strains with metabolic activation sodium azide 10 µg/plate – TA1535, TA100, without metabolic activation 4-nitro-o-phenylenediamine (4-NOPD) 10 µg/plate – TA 98; 50 µg/plate –TA 1537, without metabolic activation *Escherichia coli*: 2-aminoanthracene (2AA) 10 µg/plate – all strains with metabolic activation methyl methane sulphonate 5 µL/plate – all strains without metabolic activation
Comment:	no toxic effects, either in the presence or absence of metabolic activation, occurred at the dose levels used; no substantial increase in the number of revertant colonies or indication of clear dose response the positive controls produced clear positive results indicating that the test system responded

	appropriately
Result:	the notified chemical was not mutagenic in the bacterial strains tested in the absence or presence of metabolic activation provided by rat liver S9 fraction

9.3.2 Chromosomal Aberrations in Chinese Hamster V79 Cells *In Vitro* (Czich, 1997)

Cells:	Chinese Hamster V79
Doses:	test material 25-800 µg/mL, 25-1600 µg/mL (without metabolic activation) 10-500 µg/mL, 5-150 µg/mL (with metabolic activation) positive controls ethylmethane sulphonate (EMS) 600 µg/mL (without metabolic activation) cyclophosphamide (CPA) 0.71 µg/mL (with metabolic activation)
Metabolic Activation System:	rat liver S9 fraction from animals pretreated with phenobarbital and β-napthoflavone
Test method:	OECD TG 473 (Organisation for Economic Cooperation and Development, 1983b)
Treatment Regime:	with metabolic activation: test material or positive control added to cell cultures in serum free medium, with 50 µL/mL S9 mix, for 4 hours; the cells were then washed and cultured in fresh complete medium to a total time of 18 or 28 hours without metabolic activation: test material or positive control added to cell cultures in complete medium for a total time of 18 or 28 hours without a change of medium colcemid was added to all cultures 2.5 hours before harvest to arrest cells in metaphase
Observations:	precipitation was observed 4 hours after the start of treatment for concentrations of 200 µg/mL and above (in the absence of S9) and 50 µg/mL and

	above (in the presence of S9); cytotoxic effects after treatment with 800 µg/mL and above (in the absence of S9) and 500 µg/mL and above (in the presence of S9)
	reproducible increases in the frequency of structural chromosome aberrations were observed in the absence of metabolic activation, but only at concentration of 800 µg/mL, where test material precipitation and cytotoxicity were observed; at the 28 hour interval an increased number of aberrant cells carrying exchanges were observed in both experiments; no significant increase in the frequency of chromosomal aberrations were observed in the presence of metabolic activation
	statistically significant increases in cells showing structural chromosome aberrations occurred for the positive control substances, indicating that the test system responded appropriately
Results:	the notified substance induced structural chromosome aberrations in the absence of metabolic activation at concentrations where precipitation and cytotoxicity occurred; the study authors suggested that an indirect mechanism for DNA damage may be involved

9.4 Overall Assessment of Toxicological Data

The notified chemical is very water soluble (>222 g/L) but fixes strongly to biological tissues. The major findings of most of the toxicological studies involved persistent staining of tissues and discolouration of fluids. This was most importantly observed throughout the 21 days of observation in the eye irritation study and in the treated recovery group in the 28 day repeat dose oral toxicity study. Ongoing gradual release of tissue bound chemical could be a mechanism for chronic exposure.

The acute oral toxicity in rats is very low (LD_{50}>2000 mg/kg) and the acute dermal toxicity in rats is low (LD_{50}>2000 mg/kg).

The notified chemical is not irritating to rabbit skin.

The notified chemical did not elicit corneal or iridal effects in rabbit eyes, though conjunctival effects were present at the one hour observation time. Persistent staining of the conjunctiva and sclera were observed, with staining present 21 days after the application of the test material. The mean scores for conjunctival effects were below the threshold for classification as irritating to eyes according to the 1994 NOHSC Approved Criteria (National Occupational Health and Safety Commission, 1994a) for eye contact. According to the EEC criteria (European Economic Community, 1993), and also to the draft updated NOHSC

criteria (consistent with 1996 amendments to EC directive 96/54/EEC), the persistent staining of eye tissue places the notified chemical under the risk phrase R41, 'Risk of serious damage to eyes'.

The notified chemical was not found to be a skin sensitiser in guinea pigs in an adjuvant skin sensitisation study. Caution is required as reactive dyes have been linked with cases of skin and respiratory sensitisation.

In a 28 day repeat dose oral toxicity study in rats, the animals were administered the notified chemical by gavage at 0, 50, 200 or 1000 mg/kg/day. The main findings were lesions of the stomach at the highest dose. Changes in a number of clinical biochemistry parameters were also observed at the highest dose. The faeces of all animals were discoloured and urine discolouration was observed at all doses. Considering only the incidence of stomach lesions, a NOAEL of 50 mg/kg/day could be established. As discolouration of faeces and urine were observed at all doses no NOEL can be established. No repeat dose inhalation study has been performed.

The notified chemical was not mutagenic in bacterial test systems. The notified chemical induced chromosomal aberrations at the highest concentrations used in the absence of metabolic activation in an *in vitro* Chinese Hamster V79 cell cytogenetic assay. Precipitation and cytotoxic effects also occurred at these doses, suggesting that an indirect mechanism for DNA damage may be involved.

10. ASSESSMENT OF ENVIRONMENTAL EFFECTS

The following ecotoxicity studies have been supplied by the notifier. The tests were carried out according to OECD Test Methods.

Test	*Species*	*Results (Nominal)*[#]	*References*
Acute Toxicity Static Through 96 hour OECD TG 203 (Organisation for Economic Cooperation and Development, 1992b)	Zebra fish (*Branchydanio rerio*)	LC_{50} > 100 mg/L NOEC* = 100 mg/L	Böttcher, 1997
Acute Immobilisation Static 48 hour OECD TG 202 (Organisation for Economic Cooperation and Development, 1984c)	Water Flea (*Daphnia magna*)	EC_{50} > 100 mg/L NOEC* ≥ 100 mg/L	Maetzler, 1997
Growth Inhibition Static 72 hour OECD TG 201† biomass b growth rate μ (Organisation for Economic Cooperation and Development, 1984b)	Algae (*Scenedesmus subspicatus*)	Experiment A $E\mu C_{50}$ = 109.1 mg/L (79-168 mg/L) E_bC_{50} = 29.6 mg/L (22.8-39.7 mg/L) NOEC* = 3.2 mg/L Experiment B $E\mu C_{50}$ = 118.9 mg/L (73.2-260.9 mg/L) E_bC_{50} = 28.1 mg/L (22.1-36.7 mg/L)	Hertl, 1997
Respiration Inhibition 30 minute OECD TG 209 (Organisation for Economic Cooperation and Development, 1984a)	Aerobic Waste Water Bacteria	EC_{50} > 1000 mg/L	Schnalke, 1997d

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 due to an indirect effect, a reduced algal growth by light absorption in coloured test solutions (Experiment B).

* NOEC - no observable effect concentration

Fish

The fish study conducted at two nominal concentrations 45 and 100 mg/L, 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 101 % to 103 % of the nominal value during the test period.

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

A limit test, performed in accordance with the test guidelines, demonstrated that the notified substance had no toxic effects on the test Daphnids up to concentration of nominal 100 mg/L. The analytically determined concentrations varied from 97.0 to 97.3 % of the nominal values. The report notes that the test medium was coloured by the test substance.

The 24 h and 48 h LC_0 and NOEC were determined to be ≥100 mg/L.

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, 10.0, 32.0 and 100.0 mg/L and a control were tested. The analytically determined concentrations in the analysed test media varied in the range from 94 % to 106 % on the nominal values, and as such all biological results are related to nominal concentrations. The report notes that all test media down to the lowest test concentration were slightly to strongly coloured by the test substance.

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 10 mg/L. As such, the 72 h NOEC was determined to be 3.2 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 32 mg/L. The EC_{50} values and the percentage inhibition of the algal growth rate (μ) after 72 hours of exposure in this experiment part were in the same magnitude as in experiment part A.

The modified growth inhibition test showed that there was the same growth inhibition of *Scenedesmus subspicatus* when the algae grew in test water without the test substance, but under reduced light intensities by the filter effect of the coloured test media, to when the algae grew in directly in the test media with the dissolved test substance. Since the test solution is intensely coloured, deleterious effects can be caused by the interception of light (shading effect) necessary for algal growth. Therefore, the notifier claims that the real toxic effect of the notified chemical can be excluded up to the highest tested concentration of 100 mg/L.
However, it should be noted that for environmental purposes, growth inhibition, whether due to chemical or physical factors, is still of relevance. Algistatic effects may still lead to an undesirable environmental impact if exposure is continuous. Therefore, the calculated and determined EC_{50} values for algae should not be disregarded. Thus, the notified chemical can be considered as slightly toxic to algae.

Microorganisms

The inhibitory effect of the notified substance on aerobic waste water bacteria (activated sludge from a domestic waste water 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 25.6 to 1000 mg/L over the exposure period of 30 minutes.

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 (due to the effects on biomass). Reproductive effects on aquatic invertebrates are not expected.

11. ASSESSMENT OF ENVIRONMENTAL HAZARD

The notifier has specified that approximately 15 dyehouses in both city and country locations will be using the dyestuff containing the notified substance. The Predicted Environmental Concentration (PEC) has thus been estimated for three typical dyehouses (city, county high use and country low use) and represents “worst case”. The “typical use of product expected per day” amount was supplied by the notifier.

Calculation Factor	City Dyehouse	Country Dyehouse - High Dye Use	Country Dyehouse - Low Dye Use
Typical use of dyestuff expected per day	30 kg	60 kg	10 kg
Volume of notified substance (at 70 % product)	21 kg	42 kg	7 kg
Quantity in wastewater (fixation rate 75 %)	5.25 kg	10.5 kg	1.75 kg
Quantity of water used incl. wash-off water (at 75 $L.kg^{-1}$)	150 000 L	150 000 L	75 000 L
Effluent concentration in dye-specific wash-water	35 mg/L	70 mg/L	23 mg/L
Dilution factor in dyehouse by other wash-waters	1:13 (2 ML/day effluent)	1:13 (2 ML/day effluent)	1:26 (2 ML/day effluent)
Influent concentration	2.69 mg/L	5.38 mg/L	0.90 mg/L
Dilution factor in sewage treatment plant	1:100	1:2	1:2
Conc. balance in effluent from sewage plant	26.9 μg/L	2.69 mg/L	0.45 mg/L
Dilution factor in receiving waters	1:10 (to ocean outfall)	1:2 (to river outfall)	1:2 (to river outfall)
PEC in receiving waters	2.69 μg/L	1.35 mg/L	0.22 mg/L
Safety factor*	10,994	12	132

*For exposure to most sensitive aquatic organism, Algae (72h E_bC_{50} = 29.6 mg/L)

It has been assumed in the PEC calculations that no removal of the dye would take place during the wastewater treatment process. However, some of the dye would probably be removed due to the adsorption of the dyestuff to the organic sludge and possible complexation of the dye (Weber, 1991). As such, the actual concentration in receiving waters is likely to be lower than that calculated.

The PEC calculations show that the exposure to fish, aquatic invertebrates and waste water treatment microorganisms is at levels unlikely to cause any significant effects, although levels are near those where a reduction in algal biomass occurred. This was shown to be due to a function of decreased light intensity or change in light quality reaching the algae in the coloured media. However, release of coloured effluent (concentrations greater than 1 ppm) would generally be of concern to textile and dye manufacturing industries and waste water authorities (Hobbs, 1988, Yen, 1991). In any event, once in the aquatic environment the substance is expected to be swiftly diluted to undetectable concentrations and be removed through a combination of sorption to particulates and possible reductive degradation.

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.

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

The notified chemical is not likely to present a hazard to the environment when it is stored, transported and used in the proposed manner.

12. ASSESSMENT OF PUBLIC AND OCCUPATIONAL HEALTH AND SAFETY EFFECTS

The acute toxicity of Blue REN 535 is low, and it is not an irritant to the skin or eyes of rabbits. The notified chemical is a hazardous substance based on the persistent staining of eye tissues, which warrants the classification R41 "Risk of serious damage to eyes". The notified chemical was negative in a Buehler skin sensitisation study in guinea pigs, however, as a general rule reactive dyes of this type should be handled carefully because of their potential skin and respiratory sensitisation effects.

For longer-term systemic effects, the NOAEL is 50 mg/kg/day, based on stomach lesions observed in a 28 day oral rat study. As discolouration of faeces and urine were observed at all doses no NOEL can be established. No long term toxicological studies such as a worker health study were provided.

A small proportion of the powdered solid is within the respirable range (< 0.4 %); however a large proportion is within the inspirable range and may be deposited in the respiratory tract. The low acute dermal toxicity and high molecular weight of the main components of Blue REN 535 would suggest that absorption via the skin is unlikely.

Occupational exposure to the notified chemical can be divided into exposure to the powdered solid, the 1 % dye solution, and to the dyed cloth. Of these, the former is likely to provide the greatest hazard, as the powdered solid contains particles of size as low as 5 μm diameter and thus atmospheric dust may be present. The formulated commercial product is stated to contain an antidusting additive, which would be expected to reduce the amount of airborne dust. The amount of free dye in the washed, dyed cloth is expected to be small. The dust will be a potential hazard by inhalation and by dermal and ocular exposure. Contact with the solution will be more easily avoided, but dermal and ocular exposure to drips and splashes will be possible. After fixation to the textile, the potential hazard should be negligible. In all cases, contact of solid or dissolved dye with the eyes should be avoided.

Transport and Storage
The health risk for transport and storage workers is expected to be negligible unless the packaging is breached.

Repackaging
Workers involved in repackaging the dye powder are likely to be exposed at infrequent intervals for short times. The exposure will be to the powdered solid, with the possibility of exposure to atmospheric dust. The notifier states that the repackaging is conducted using a downdraught weighing booth, which would be expected to substantially reduce dust exposure.

End Use
The workers involved in weighing and mixing the dye will be exposed to the powdered solid, and also to the dye solution. Based on a United States atmospheric monitoring study, the notifier has provided an estimate of the exposure of weighing and mixing workers of less than 0.01 μg/kg/day. This may be compared with the NOAEL established in the 28 day oral repeat dose study of 50 mg/kg/day (based on the presence of stomach lesions). A margin of exposure (MOE) of 5 000 000 may be estimated from these figures. It must be noted that the

NOAEL is drawn from an oral study, and treatment related effects were seen at low doses, while the exposure calculation is based on inhalation of respirable sized dust. Even taking a large additional safety factor to correct for this, the likelihood of systemic effects based on this comparison appears to be small. It must also be noted that, while the exposure estimate is based only on the respirable dust fraction of 0.4 %, the much larger proportion of inspirable dust (approximately 65 – 95 %) could be absorbed following secondary ingestion. The MOE does not include ocular or dermal exposure, for which all atmospheric dust must be considered.

The notifier indicates that existing dyehouse procedures require the wearing of overalls, protective gloves, glasses and respiratory protection while weighing and mixing the dye. Mechanical ventilation of the weighing area is also provided. Also, an antidusting additive is one component of Cibacron Blue LS-G. The personal protective equipment is required for protection against a variety of dyes, and should provide dermal, ocular and respiratory protection.

The dyeing machine operators will be exposed to the dye solution while manually filling the dye feed tank, and while loading and realigning the fabric. The exposure time for the operators is expected to be short. Gloves and safety glasses will be worn by these workers while handling the dye. Therefore the exposure and subsequent health risk for these workers will be low.

The workers involved in drying the dyed and washed cloth will have very low exposure as the excess dye will be removed from the cloth prior to this stage.

Laboratory

Laboratory workers will be exposed to small quantities of the notified chemical for short periods. The exposure could be in a variety of ways. Exhaust ventilation and personal protective equipment should be available as required.

The notifier recommends an exposure limit of 1 mg/m^3. This is an internal company limit applied to reactive dyes in general. The basis for selecting the value of 1 mg/m^3 as the exposure limit was not provided.

While the skin sensitisation study for the notified chemical was negative, caution should be exercised as reactive dyes have been linked with cases of skin and respiratory sensitisation. Individuals who become sensitised should not continue to handle the notified chemical.

Public Health

The notified chemical is for industrial use only and is unlikely to come in contact with the general public, except via dyed fabric. According to the notifier, the dye is strongly bound to the fibre, and public exposure during wearing, washing and dry cleaning of the fabric is expected to be negligible. In addition, with industrial controls to minimise environmental release, public exposure from this source is likely to be very low.

13. RECOMMENDATIONS

To minimise occupational exposure to Blue REN 535 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 3765.2 (Standards Australia, 1990);
- 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, 1994);
- 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;
- A copy of the MSDS should be easily accessible to employees.
- Workers who become sensitised to the notified chemical should not continue to handle it in the workplace.

14. MATERIAL SAFETY DATA SHEET

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

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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