File No: NA/497

Date: June 1997

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME

FULL PUBLIC REPORT

Reactive Blue CAC10

This Assessment has been compiled in accordance with the provisions of *the Industrial Chemicals (Notification and Assessment) Act* 1989 (the Act), and Regulations. This legislation is an Act of the Commonwealth of Australia. The National Industrial Chemicals Notification and Assessment Scheme (NICNAS) is administered by Worksafe Australia which also conducts the occupational health & safety assessment. The assessment of environmental hazard is conducted by the Department of the Environment, Sport, and Territories and the assessment of public health is conducted by the Department of Health and Family Services.

For the purposes of subsection 78(1) of the Act, copies of this full public report may be inspected by the public at the Library, Worksafe Australia, 92-94 Parramatta Road, Camperdown NSW 2050, between the following hours:

Monday - Wednesday	8.30 am - 5.00 pm
Thursday	8.30 am - 8.00 pm
Friday	8.30 am - 5.00 pm

For Enquiries please contact the Administration Coordinator at:

Street Address: 92 Parramatta Rd Camperdown, NSW 2050, AUSTRALIA
Postal Address: GPO Box 58, Sydney 2001, AUSTRALIA
Telephone: (61) (02) 9577-9466 **FAX (61) (02) 9577-9465**

Director
Chemicals Notification and Assessment

FULL PUBLIC REPORT

Reactive Blue CAC10

1. APPLICANT

Ciba Specialty Chemicals Ltd of 235 Settlement Road THOMASTOWN VIC 3074 has applied for the following information relating to 'Reactive Blue CAC10' to be exempt from publication in the Full Public and Summary Reports.

2. IDENTITY OF THE CHEMICAL

Reactive Blue CAC10 is considered to be hazardous according to Worksafe Australia's *Approved Criteria for Classifying Hazardous Substances* (1) (Approved Criteria) on the basis of its skin sensitisation properties. However, for commercial reasons, the chemical identity and the nature of impurities have been exempted from publication in the Full Public Report and the Summary Report. The conditions of these exemptions being allowed are as follows:

- A descriptive generic name, dioxazine monochlortriazine trisulfonic acid, sodium salt, be used to identify the substance in public reports and the Material Safety Data Sheet (MSDS),
- The relevant employee unions shall be informed of the conditions of use of dioxazine monochlortriazine trisulfonic acid, sodium salt,
- The full chemical name shall be provided to any health professionals in the case of a legitimate need where exposure to the chemical may involve a health risk,
- The full chemical name shall be provided to those on site who are using the chemical and to those who are involved in planning for safe use, etc. in the case of a legitimate need,
- The Director of NICNAS will release the full chemical name etc in the case of a request from a medical practitioner,
- Confidentiality will expire after a 3 year period,
- The chemical be identified as a sensitiser in the Health Effects Section of the MSDS, and that reference to its assessment by NICNAS be made on the MSDS,
- These conditions shall be published in the Chemical Gazette

Other Names:	FAT 40'407/A oxazine dye
Generic Name:	dioxazine monochlortriazine trisulfonic acid, sodium salt
Trade Name:	Cibacron Blue GN-E (product containing approximately 66% of the notified chemical)
Molecular and Structural Formula:	unspecified (complex mixture)
Molecular Weight:	unspecified (reaction products consist of over 20 components)
Method of Detection and Determination:	infrared (IR), ultraviolet/visible (UV/Vis) and nuclear magnetic resonance (NMR) spectra; physical testing

3. PHYSICAL AND CHEMICAL PROPERTIES

Appearance at 20°C and 101.3 kPa:	the notified chemical is a dark blue powder
Melting Point:	> 300°C
Specific Gravity:	1.74 at 22°C
Vapour Pressure:	not determined
Water Solubility:	> 90 g/L at 20°C
Partition Co-efficient (n-octanol/water):	log P_{ow} < -3.34 at 25°C (pH 6.9)
Hydrolysis as a Function of pH:	$T_{1/2}$ at pH 4.0 < 1 year at 25°C $T_{1/2}$ at pH 7.0 and pH 9.0 > 1 year at 25°C
Adsorption/Desorption:	not determined
Dissociation Constant:	estimated dissociation constants for the main components of the notified chemical are: $-SO_3^-$: -2.5 > pK_a > -3.0 Tr–NH–Ph: pK_a approximately 0.8 Tr–NH–Alkyl: pK_a< 1.0 Ph–NH–Alk resp Ph NH_2: pK_a approximately 2.4
Fat Solubility:	< 0.1 mg/100 g fat at 37°C

Surface Tension:	61.9 mN/m at 1 g/L at 20°C 45.4 mN/m at 10 g/L at 20°C
Flash Point:	not flammable
Flammability Limits:	not flammable
Autoignition Temperature:	not autoflammable
Explosive Properties:	not explosive
Particle Size:	median particle size: 231 μm
Reactivity/Stability:	dye is considered 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.

Vapour pressure was not determined, though the notifier expects that it will be negligible. This conclusion is supported by the fact that similar dyestuffs previously submitted by the notifier exhibited very low (calculated) vapour pressures and the notified chemical is a high molecular weight, organic trisodium salt.

Preliminary testing revealed that at 50°C the hydrolysis of the notified chemical was less than 10% at pH 7 and 9. Hence, it has a half-life period longer than one year at 25°C at pH 7 and 9. At pH 4 and 50°C, the degree of hydrolysis was 34.4%. Therefore, the half-life was determined to be less than one year. Testing was not undertaken to further refine the half-life. It is unclear from the hydrolysis test report what the hydrolysis products will be.

Adsorption/desorption data were not provided. High water solubility and a low partition coefficient would normally indicate low affinity for soil or sediment. The notifier has indicated the notified chemical is likely to bind/adsorb strongly to clay, and the free acid to bind to organic material in the soil. It is expected that the chemical will bind to positively charged substances such as clay particles. However, binding of the chemical to organic matter is unlikely, considering that such binding would occur only where cations are involved (2).

The notified chemical contains sulfate functionalities that will be expected to completely dissociate under environmental conditions. The important pK's are the strongly acidic $–SO_3^-$ groups that will render the main component molecule threefold negatively charged over the whole environmentally relevant pH region. The possible protonation of the weakly basic amino groups will have negligible effect in the lowest pH region (below pH 2).

The notified chemical is not expected to be surface active at a concentration of 1 g/L. However, at higher concentrations, surface activity is likely to increase. By definition, a chemical has surface activity when the surface tension is less than 60 mN/m (3).

The notified chemical is expected to be relatively insoluble in fat.

4. PURITY OF THE CHEMICAL

The notified chemical has a main component comprising 40.3% of the substance together with a variety of identified and unidentified related impurities (38.8%). Additional non-hazardous impurities at levels above 1% are sodium chloride (4.9%), disodium hydrogen phosphate (1.5%) and water (5.2%).

5. USE, VOLUME AND FORMULATION

The notified chemical will not be manufactured in Australia. It will be imported into Australia in powder form as a component of the product Cibacron Blue GN-E at a concentration of approximately 66%. The notified chemical will be used for the colouration of cellulose textiles by the exhaust dyeing method.

Import volumes are as follows:

		Year		
		1	***2-3***	***4-5***
Import Volume (tonnes)	***Product***	5 - 6	8 - 10	10 - 15
	Notified chemical	3.3 - 4	5.3 - 6.6	6.6 - 10

6. OCCUPATIONAL EXPOSURE

The notified chemical will be imported in 30 kg sealed containers with antistatic polyethylene lining. Exposure during transport and warehousing of the containers is likely to be limited to rare accidents and leaking packaging. Limited repackaging is likely to occur at the notifier's warehouses in which case ocular, dermal and inhalational exposure may occur.

Following transport to dyehouses, the dyestuff is scooped from the 30 kg drum into a weighing container. Dissolution in water at 90°C in a mixing tank follows, after which the dye solution is metered into an enclosed dyeing vessel over a specified period. The notifier states that dye weighers are potentially exposed to the dye via the three main routes of exposure.

During the exhaust dyeing procedure, operators handle mixed dye liquors and thread cloth. Dermal exposure is likely under these conditions, although the time of exposure is stated to be several minutes per hour and the dye concentration is a maximum of 3.0%.

At the completion of the dyeing process, the unfixed dye (approximately 33% of added dye) is removed from the textile to which it has been applied by boiling in a soapy bath, after which the wash water is drained to sewer.

7. PUBLIC EXPOSURE

No public exposure to the notified chemical is expected during storage, distribution, the dyeing procedure or disposal.

Fabrics treated with the notified chemical will be used in the manufacture of outer clothing garments, and on this basis prolonged dermal contact with such fabrics is not anticipated. If dermal contact were to occur the notified chemical is stated to be strongly fixed to the cellulose fibre, and therefore no significant migration of the notified chemical from the clothing to the skin is expected.

8. ENVIRONMENTAL EXPOSURE

Release

The bulk of the dye will become chemically fixed to the cellulosic 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 chemical bonding with hydroxy groups on the cellulose 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. It is reported (4) that reactive dyes have been found not to absorb 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 0 mg O_2/g. The chemical oxygen demand (COD) was determined to be 718 mg/g O_2. The dye was found to be not readily biodegradable

(measured as dissolved organic carbon (DOC) and expressed as percentage elimination, biodegradation amounted to 0% at the end of the 28-day exposure to micro-organisms from a domestic sewage treatment plant) in the OECD 301A Test for ready biodegradability (modified AFNOR Test). No inhibition on the activity of the bacteria was observed in this test. The dye's inherent biodegradability was not measured.

Although the dye is not readily biodegradable, the potential for bioaccumulation is low due to the low partition coefficient (log P_{OW} < -3.34), very high water solubility of the substance and low fat solubility (< 0.01 mg/100 g). Hydrophilic dyes with log P_{OW} less than 3 have been shown not to bioaccumulate (5). Also, biological membranes are not permeable to chemicals of very large molecular size and therefore bioaccumulation of the notified polymer is not expected (6, 7).

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 (2). 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 Reactive Blue CAC10

Test	*Species*	*Outcome*	*Reference*
acute oral toxicity	rat	LD_{50} > 2 000 mg/kg	(8)
acute dermal toxicity	rat	LD_{50} > 2 000 mg/kg	(9)
skin irritation	rabbit	non-irritant	(10)
eye irritation	rabbit	slight irritant	(11)
skin sensitisation	guinea pig	moderate sensitiser	(12)

9.1.1 Oral Toxicity (8)

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

Number/sex of animals: 5/sex

Observation period: 14 days

Method of administration: oral; vehicle was bi-distilled water

Clinical observations:	none
Mortality:	none
Morphological findings:	none
Test method:	according to OECD Guidelines (13)
LD_{50}:	> 2 000 mg/kg
Result:	the notified chemical was of low acute oral toxicity in a limit test in rats

9.1.2 Dermal Toxicity (9)

Species/strain:	rat/HanIbm:WIST(SPF)
Number/sex of animals:	5/sex
Observation period:	14 days
Method of administration:	test substance was dissolved in bi-distilled water; single dermal dose of 2 000 mg/kg applied to an intact skin site; site covered with semi occlusive dressing; dressing removed after 24 hours and site washed with lukewarm water
Clinical observations:	scales were noted on 1 male and 2 females during the study; the exposed skin of all animals was discoloured blue for the duration of the study
Mortality:	none
Morphological findings:	none
Test method:	according to OECD guidelines (13)
LD_{50}:	> 2 000 mg/kg
Result:	the notified chemical was of low acute dermal toxicity in a limit test in rats

9.1.3 Inhalation Toxicity

Not performed. The notifier states that the notified chemical will be imported in a product which contains an anti-dusting agent, which will reduce the potential for dust to form.

9.1.4 Skin Irritation (10)

Species/strain:	rabbit/ChbbIbm: NZW (SPF)
Number/sex of animals:	2 males/1 female
Observation period:	72 hours
Method of administration:	0.5 g of the test substance was moistened with bi-distilled water and applied to a 6 cm^2 intact dorsal skin site; skin was covered by gauze and semi-occlusive dressing for 4 hours; site washed with lukewarm water after dressing removed; observations were made at 1 hour, 2, 3 and 4 days after removal of dressing and scored according to the method of Draize (14)
Draize scores (14):	the female animal had very slight erythema at the 1 hour time point; all other skin irritation scores were zero; blue discolouration of the skin was noted throughout the study
Test method:	according to OECD guidelines (13)
Result:	the notified chemical was not a skin irritant in rabbits

9.1.5 Eye Irritation (11)

Species/strain:	rabbit/ChbbIbm: NZW (SPF)
Number/sex of animals:	2 males/1 female
Observation period:	72 hours
Method of administration:	0.1 g of the test material was placed in the conjunctival sac of the left eye of each animal; right eye served as control
Draize scores (14) of unirrigated eyes:	slight corneal effects were noted in all animals at the 1 hour time point; these had resolved by the 48 hour reading; no iridial effects were

noted; slight conjunctival redness was present in all animals at 24 hours; this persisted in one animal through to the 48 hour time point; slight chemosis present in all animals at 1 hour had cleared in 2 of 3 animals by the 24 hour time point

blue discolouration of the conjunctivae and lid hairs was noted throughout the study

Test method: according to OECD guidelines (13)

Result: the notified chemical was a slight eye irritant in rabbits

9.1.6 Skin Sensitisation (12)

Species/strain: guinea pig/Himalayan spotted

Number of animals: 30 males; 20 test, 10 control

Induction procedure:

Day 1: 3 pairs of intradermal injections:
- 0.1 mL Freund's complete adjuvant (FCA): physiological saline (1:1(v/v))
- 0.1 mL of 5% concentration of test material in physiological saline
- 0.1 mL of 5% concentration of test material in FCA: physiological saline(1:1 (v/v))

Day 7: test area treated with 10% (w/w) sodium lauryl sulfate in *petrolatum*

Day 8: occluded application of filter paper soaked in test material (25% in vaselinum album) for 48 hours

Challenge procedure: Day 22: occluded application of filter paper soaked in test material (25% in *vaselinum album*) for 24 hours

Challenge outcome:

	Test animals		Control animals	
Challenge concentration	**24 hours***	**48 hours***	**24 hours**	**48 hours**
25%	9/20**	7/20	0/10	0/10

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

Test method: according to OECD guidelines (13)

Result: the notified chemical was a moderate skin sensitiser in guinea pigs under the conditions of the study

9.2 Repeated Dose Toxicity (15)

Species/strain: rat/HanIbm: WIST/SPF

Number/sex of animals: 30/sex; control and high dose groups: 10/sex
low and mid dose groups: 5/sex

Method of administration: gavage; vehicle was bi-distilled water

Dose/Study duration: dose levels were based on the results of an oral repeat dose 5 day range finding study in rats (16)

test material administered daily for a total of 28 days:

control:	0 mg/kg/day
low dose:	50 mg/kg/day
mid dose:	200 mg/kg/day
high dose:	1 000 mg/kg/day

All animals were sacrificed at the end of the treatment period, with the exception of 5 animals from control and high dose groups, which were maintained for an additional 2 week recovery period before sacrifice

Clinical observations: increased relative food consumption was noted in males from the high dose group; alopecia was noted in one female from the low dose group; these effects were thought not to be related to the test article

blue discolouration of the faeces was noted in

	all animals treated with the test substance from day 7 onward
Clinical chemistry/Haematology	no toxicologically significant changes in haematological, clinical biochemical and urinalysis parameters at the end of the treatment period, however a yellowish-green discolouration of the urine in some animals from mid and high dose groups was noted at termination of treatment; this had reversed at the end of the treatment-free period
Histopathology:	absolute and relative pituitary weights of females in the mid-dose group and the absolute pituitary weight and pituitary/brain weight ratio were decreased in low-dose females; there were no treatment related pituitary effects in males, no effects in the high dose group and no macroscopic or microscopic findings to support these weight changes, the effect was considered to be unrelated to treatment with the test substance bluish discolouration of a number of organs (kidneys, stomach, duodenum, jejunum, ileum, caecum, colon, rectum, tongue, lymph nodes, testes, lungs) was noted in some animals of both sexes of all treatment groups; some of these organs showed storage of a blue foreign pigment; there was no evidence of tissue damage; these findings were thought to indicate substance distribution only
Test method:	according to OECD guidelines (13)
Result:	no significant treatment-related findings noted at necropsy or during histopathology examination for both male and female rats when the notified chemical was administered by gavage for a period of 28 days

9.3 Genotoxicity

9.3.1 *Salmonella typhimurium* Reverse Mutation Assay (17)

Strains:	*Salmonella typhimurium* TA 1535, TA 1537, TA 1538, TA 98, TA 100
Concentration range:	experiment 1: 10, 100, 333.3, 1 000 and

	5 000 μg/plate experiment 2: 10, 100, 1 000, 2 500 and 5 000 μg/plate vehicle was water; assays were carried out in the presence and absence of rat liver S9 fraction
Test method:	according to OECD guidelines (13)
Result:	a significant, reproducible and dose-dependent increase in revertant colonies was found in strains TA 1535, TA 1537 (both with and without S9 fraction) and TA 1538 (without S9 fraction); a significant increase was also noted in TA 1538 in the presence of S9 fraction at the highest dose level in both experiments; the notified chemical was found to be mutagenic in *Salmonella typhimurium* strains TA 1535, TA 1537 and TA 1538 under the conditions of this assay both in the presence and absence of rat liver S9 fraction.

9.3.2 Chromosome Aberration Assay in Chinese Hamster V79 Cells (18)

Dosing schedule:	a pre-test determined that the substance showed toxic effects at concentrations higher than 10 μg/mL without S9 fraction, and 300 μg/mL with S9 fraction present 18 hour fixation time: without S9 fraction: 1 - 300 μg/mL with S9 fraction: 30 - 2 000 μg/mL 28 hour fixation time: without S9 fraction: 10 - 300 μg/mL with S9 fraction: 300 - 2 000 μg/mL for all groups, the treatment interval was 4 hours; cells were fixed at 18 and 28 hour timepoints and scored for structural chromosomal aberrations
Test method:	according to OECD guidelines (13)
Result:	the notified chemical did not induce structural chromosomal aberrations in Chinese hamster V79 cells, in either the presence or absence of metabolic activation

9.3.3 Micronucleus Assay in the Bone Marrow Cells of the Mouse (19)

Species/strain:	mouse/NMRI
Number and sex of animals:	42/sex
Doses:	5 000 mg/kg; vehicle was distilled water; animals were sacrificed 24, 48 or 72 hours after treatment
Method of administration:	gavage
Test method:	according to OECD guidelines (13)
Result:	the notified chemical did not induce micronuclei in mouse bone marrow cells when orally administered at a dose which induced slight toxic effects

9.4 Overall Assessment of Toxicological Data

The notified chemical exhibited low oral and dermal toxicity in rats (LD_{50} > 2 000 mg/kg for both studies). It was not a skin irritant in rabbits, but was a slight eye irritant in this species. The notified chemical was a moderate skin sensitiser when tested in guinea pigs. Results of a repeat dose oral toxicity study indicated that there were no significant treatment-related effects in male or female rats following 28 day repeated oral administration.

The notified chemical was found to be mutagenic in a bacterial reverse mutation assay, although no clastogenicity was observed in Chinese hamster cells *in vitro*, and it did not cause chromosome damage in mouse bone marrow cells *in vivo*.

Based on the information provided by the notifier, Reactive Blue CAC10 would be classified as hazardous according to the Approved Criteria, based on its skin sensitising effects.

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 Practice.

Ecotoxicity Test Results

Test	*Species*	*Results (Nominal)*
Acute Toxicity (Static Test) (OECD TG 203)	Zebra Fish (*Brachydanio rerio*)	96 h $LC_0 \geq$ 1 000 mg/L 96 h LC_{50} > 1 000 mg/L
Acute Toxicity - Immobilisation Test (Static Test) (OECD TG 202)	Water Flea (*Daphnia magna*)	48 h NOEC $\geq$ 125 mg/L 48 h $EC_{50} \geq$ 342 mg/L
Growth Inhibition - Growth (μ) & Biomass (b) (Static Test) (OECD TG 201)	Green Algae (*Scenedesmus subspicatus*)	72 h E_bC_{50} = 7.7 mg/L 72 h $E_\mu C50$ = 98.3 mg/L 72 h NOEC(μ) < 0.5 mg/L
Respiration Inhibition (OECD 209)	Activated Sludge - Aerobic Waste Water Bacteria	3 h IC_{50} > 100 mg/L

It is claimed in the water flea acute toxicity test report that all test concentrations were intensively coloured, down to the lowest test concentration (nominal 63 mg/L). There is no remark made in the algae growth inhibition test report concerning the colouration of test media. However, it is suspected that all test media down to the lowest concentration tested (nominally 0.5 mg/L) were probably slightly to intensely coloured. Tests determined that the test media concentrations were all sufficiently stable.

The ecotoxicity data for the substance shows that the dye is practically non-toxic to the zebra fish and water flea. No abnormal responses of the fish were observed during testing.

The notifier has not performed a modified growth inhibition test to differentiate between a reduced growth of algae due to real toxic effects of the notified chemical on the algal cells, or due to an indirect effect, a reduced algal growth by light absorption in coloured test solutions. Since the test solution is intensely coloured deleterious effects can be caused by the interception of light (shading effect) necessary for algal growth. 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, with an E_bC_{50} of 7.7 mg/L, the notified chemical can be considered as moderately toxic to algae.

The notified chemical showed practically no toxic effects to the respiration rate of aerobic waste water bacteria in the respiration test, with a 3 hour IC_{50} greater than 100 mg/L.

11. ASSESSMENT OF ENVIRONMENTAL HAZARD

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

The notifier has specified that a limited number of dyehouses (approx. 8) in city and country areas will be using the notified chemical. The environmental hazard has

been determined for three dyehouses located in two general locations, one metropolitan based dyehouse and the other country based. Two examples of dye usage are given for country dyehouses. The Predicted Environmental Concentration (PEC) is estimated below.

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.

Predicted Environmental Concentration (PEC)

Calculation Factor	*City Dyehouse*	*Country Dyehouse - High Dye Use*	*Country Dyehouse - Low Dye Use*
Typical use of dye expected per day	30.0 kg	60.0 kg	10.0 kg
Amount of Active (notified chemical) (@ 66% Active)	19.8 kg	39.6 kg	6.6 kg
Conc. in wastewater (fixation rate 67%)	6.5 kg	13.1 kg	2.2 kg
Quantity of water used incl. wash-off water (@ 75 L/kg)	150 000 L	150 000 L	75 000 L
Effluent conc. in dye-specific wash-water	43.6 mg/L	87.1 mg/L	29.0 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	3.35 mg/L	6.7 mg/L	1.1 mg/L
Dilution factor in sewage treatment plant	1:100	1:3	1:2
Conc. balance in effluent from sewage treatment plant	33.5 μg/L	2.23 mg/L	0.56 mg/L
Dilution factor in receiving waters	1:10 (ocean)	1:2 (river)	1:2 (river)
(PEC) in receiving waters	3.35 μg/L (3.35 ppb)	1.12 mg/L (1.12 ppm)	0.28 mg/L (0.28 ppm)
Safety factor for exposure to most sensitive aquatic organism, Algae (E_bC_{50} = 7.7 mg/L)	2300	7	27

It has been assumed in the 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 dye to the organic sludge and possible complexation of the dye (2). Therefore, the actual concentration in receiving waters is likely to be lower than that calculated. (The PEC in receiving waters for a country dyehouse with a high usage using 6% depth of shade (extreme maximum) is 1.12 mg/L, with a safety factor of 7.)

These calculations show that the exposure to fish and daphnia is at levels unlikely to cause any significant effect, although levels are near those where significant inhibition of algal biomass by the dye did occur. It was not determined whether this was a function of decreased light intensity or change in light quality reaching the algae in the coloured media, or a direct chemical toxicity to algal cells. However, dye concentrations greater than 1 ppm can give rise to intensely coloured effluent that is unacceptable to waste water authorities (4, 20). In any event, the dye's high solubility suggests that once released to the waterways, dilution would be expected to reduce the environmental concentration. Therefore, there is also unlikely to be any significant effect on algae.

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

It can be predicted from the toxicological data provided that the notified chemical is not likely to be acutely toxic nor to exhibit toxic effects on repeated or prolonged exposure. It is not likely to be a skin irritant but may be a slight eye irritant, may be weakly genotoxic and is likely to be a respiratory and a skin sensitiser. The chemical would be classified as hazardous according to the Approved Criteria on the basis of sensitisation potential. In the sensitisation study, the maximum concentration used for induction was considerably less than 100% which resulted in moderate sensitisation. The chemical to be imported is, therefore, potentially a strong sensitiser.

It can be concluded that the main health risk associated with use of the notified chemical may be dermal or respiratory sensitisation to dye weighers in dye houses. The notifier states that the imported dyestuff is formulated to be “non-dusting”, in which case the risk of respiratory sensitisation is reduced, although it is not known to what extent the level of dust in the workplace is reduced. It is essential, therefore, that good general and local exhaust ventilation is employed in areas where the notified chemical is weighed out. This also applies to the notifier’s warehouses where a small amount of dyestuff is expected to be repackaged

Following dissolution of the dye in water at a maximum concentration of 3%, the risk of dermal sensitisation is still significant for workers handling dye liquors and threading cloth. Thus, personal protective equipment as described below should be employed by these workers. After fixation, excess dye is removed by boiling and waste water goes to sewer. Although the waste water still contains a significant dye concentration, exposure of workers should not occur.

The risk of adverse health effects to workers involved in transport and storage of the sturdy containers in which the notified chemical is imported is considered to be low, since exposure should only occur in the event of accident or damaged packaging.

The risk of public health effects arising from transport, storage, use or disposal of the notified chemical is expected to be minimal. The notified chemical was found to be weakly mutagenic in bacteria at the highest concentration tested and was a moderate skin sensitiser in guinea pigs. However, given it will be used at low levels in the manufacture of outer clothing garments and will be tightly bound to clothing fibres, no significant migration of the notified chemical from clothing to the skin is expected to occur, and therefore such use is unlikely to present a significant risk to public health.

13. RECOMMENDATIONS

To minimise occupational exposure to the notified chemical the following guidelines and precautions should be observed:

- Good general and local exhaust ventilation should be employed in areas where the notified chemical is weighed out;
- The following personal protective equipment should be employed when weighing the notified chemical and when contact with dye liquors or cloth containing unfixed dye is possible:
  - Safety goggles selected and fitted in accordance with Australian Standard (AS) 1336 (21) to comply with Australian/New Zealand Standard (AS/NZS) 1337 (22);
  - Industrial clothing should conform to the specifications detailed in AS 2919 (23);
  - Impermeable gloves or mittens should conform to AS 2161 (24);
  - All occupational footwear should conform to AS/NZS 2210 (25);
  - Respiratory protection conforming to AS/NZS 1715 (26) and 1716 (27) (where exposure to dust or aerosols is likely).
- Spillage of the notified chemical should be avoided, spillages 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.

14. MATERIAL SAFETY DATA SHEET

The MSDS for Cibacron Blue GN-E was provided in accordance with the *National Code of Practice for the Preparation of Material Safety Data Sheets* (28).

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.

16. REFERENCES

1. National Occupational Health and Safety Commission 1994, *Approved Criteria for Classifying Hazardous Substances [NOHSC:1008(1994)]*, Australian Government Publishing Service, Canberra.

2. Weber, E.J. 1991, 'Studies of benzidine-based dyes in sediment-water systems', *Environmental Toxicology & Chemistry*, vol. 10, pp. 609-618.

3. Community, E.E. 1992, *EEC Directive 92/69, Annex V, Part A, Methods for the determination of physico-chemical properties, A.5 "Surface Tension"*, EEC Publication No. L383.

4. Hobbs, S.J. 1988, *Industry Category Document: UK dye production and use in the textile industry*.

5. Yen, C.P., Perenich, T.A. & Baughman, G.L. 1991, 'Fate of Commercial Disperse Dyes in Sediments', *Environmental Toxicology and Chemistry*, vol. 10, pp. 1009-1017.

6. Anliker, R., Moser, P. & Poppinger, D. 1988, 'Bioaccumulation of dyestuffs and organic pigments in fish. Relationships to hydrophobicity and steric factors', *Chemosphere*, vol. 17, no. 8, pp. 1631-1644.

7. Gobas, F.A.P.C., Opperhuizen, A. & Hutzinger, O. 1986, 'Bioconcentration of hydrophobic chemicals in fish: relationship with membrane permeation', *Environmental Toxicology and Chemistry*, vol. 5, pp. 637-646.

8. Hoff, N., Ullmann, L. & Althaus, P. 1991, *Acute Oral Toxicity Study with FAT 40'407/A in Rats*, Project no., 298563, Research and Consulting Co AG, Itingen, Germany.

9. Hoff, N. & Althaus, P. 1991, *Acute Dermal Toxicity Study with FAT 40'407/A in Rats*, Project no., 298574, Research and Consulting Co AG, Itingen, Germany.

10. Ulmann, P., Lucini, P. & Hoff, N. 1991, *Primary Skin Irritation Study with FAT 40'407/A in Rabbits (4-hour Semi-occlusive Application)*, Project no., 298585, Research and Consulting Co AG, Itingen, Germany.

11. Hoff, N. & Lucini, P. 1991, *Primary Eye Irritation Study with FAT 40'407/A in Rabbits*, Project no., 298596, Research and Consulting Co AG, Itingen, Germany.

12. Ulmann, P., Kroling, C., Boni, C. & Hoff, N. 1991, *Contact Hypersensitivity to 40'407/A in Albino Guinea Pigs Maximisation Test*, Project no., 298607, Research and Consulting Co AG, Itingen, Germany.

13. Organisation for Economic Co-operation and Development 1995-1996, *OECD Guidelines for the Testing of Chemicals on CD-Rom*, OECD, Paris.

14. Draize, J.H. 1959, 'Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics', *Association of Food and Drug Officials of the US*, vol. 49, pp. 2-56.

15. Hoff, N. 1992, *Subacute 28-Day Oral Toxicity (Gavage) Study with FAT 40'407/A in the Rat*, Project no., 298620, Research and Consulting Co AG, Itingen, Germany.

16. Ulmann, P., Althaus, P., Hoff, N. & Vogel, O. 1991, *5-Day Oral Toxicity (Range-Finding) Study with FAT 40'407/A in Rats*, Project no., 298618, Research and Consulting Co AG, Itingen, Germany.

17. Poth, A. 1991, *Salmonella typhimurium Reverse Mutation Assay with FAT 40'407/A*, Project no., 229375, Cytotest Cell Research GmbH & Co KG, Rossdorf, Germany.

18. Heidemann, A. 1991, *Chromosome Aberration Assay in Chinese Hamster V79 Cells in vitro with FAT 40'407/A*, Project no., 229397, Cytotest Cell Research GmbH & Co KG, Rossdorf, Germany.

19. Volkner, W. 1991, *Micronucleus Assay in Bone Marrow Cells of the Mouse with FAT 40'407/A*, Project no., 229386, Cytotest Cell Research GmbH & Co KG, Rossdorf, Germany.

20. Shu, H.-Y. & Huang, C.-R. 1995, 'Degradation of Commercial Azo Dyes using Ozonisation and UV Enhanced Ozonisation Process', *Chemosphere*, vol. 31, no. 8, pp. 3813-3825.

21. Standards Australia 1994, *Australian Standard 1336-1994, Eye protection in the Industrial Environment*, Standards Association of Australia, Sydney.

22. Standards Australia/Standards New Zealand 1992, *Australian/New Zealand Standard 1337-1992, Eye Protectors for Industrial Applications*, Standards Association of Australia/Standards Association of New Zealand, Sydney/Wellington.

23. Standards Australia 1987, *Australian Standard 2919-1987, Industrial Clothing*, Standards Association of Australia, Sydney.

24. Standards Australia 1978, *Australian Standard 2161-1978, Industrial Safety Gloves and Mittens (excluding electrical and medical gloves)*, Standards Association of Australia, Sydney.

25. Standards Australia/Standards New Zealand 1994, *Australian/New Zealand Standard 2210-1994, Occupational Protective Footwear*, Standards Association of Australia/Standards Association of New Zealand, Sydney/Wellington.

26. Standards Australia/Standards New Zealand 1994, *Australian/New Zealand Standard 1715-1994, Selection, Use and Maintenance of Respiratory Protective Devices*, Standards Association of Australia/Standards Association of New Zealand, Sydney/Wellington.

27. Standards Australia/Standards New Zealand 1994, *Australian/New Zealand Standard 1716-1994, Respiratory Protective Devices*, Standards Association of Australia/Standards Association of New Zealand, Sydney/Wellington.

28. National Occupational Health and Safety Commission 1994, *National Code of Practice for the Preparation of Material Safety Data Sheets [NOHSC:2011(1994)]*, Australian Government Publishing Service, Canberra.

Attachment 1

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

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

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

CORNEA

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

CONJUNCTIVAE

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

IRIS

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