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NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME

FULL PUBLIC REPORT

Notified Chemical in Cibacron (Reactive) Blue TZ 3533

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Director
Chemicals Notification and Assessment

FULL PUBLIC REPORT

Notified Chemical in Cibacron (Reactive) Blue TZ 3533

1. APPLICANT

Ciba Specialty Chemicals Limited of 235 Settlement Road THOMASTOWN VIC 3074 has submitted a standard notification statement in support of their application for an assessment certificate for to 'Notified Chemical in Cibacron (Reactive) Blue TZ 3533'**.**

2. IDENTITY OF THE CHEMICAL

Notified Chemical in Cibacron (Reactive) Blue TZ 3533 is considered to be a hazardous substance based on the data provided.

The notifier applied the following information to be exempted from publication in the Full Public Report and the Summary Report.

The chemical name;
Molecular and structural formulae;
Molecular weight;
Spectral data;
Purity;
Details of the composition, and
Import volume.

Other Names:	Cibacron (Reactive) Blue TZ 3533 / FAT 41017/A
Trade Name:	Cibacron Blue 4R
Method of Detection and Determination:	UV/Vis, IR and NMR spectrum

Spectral Data:	3 UV/Vis spectra (in water, 0.1 N hydrochloric acid or 0.1 N sodium hydroxide), IR and NMR data were provided

3. PHYSICAL AND CHEMICAL PROPERTIES

Physical and chemical property data provided by the notifier were derived from Cibacron (Reactive) Blue TZ 3533 which contains more than 60% of the notified chemical.

Appearance at 20°C and 101.3 kPa:	odourless, dark blue to violet-black powder
Melting Point:	> 300°C
Density:	1 630 kg/m^3 (22°C)
Vapour Pressure:	not determined (see comments below)
Water Solubility:	> 154 g/L at 20°C
Partition Co-efficient (n-octanol/water):	log P_{ow} < -1.79 at pH 7.5 and 25°C
Hydrolysis as a Function of pH:	$T_{1/2}$ < 1 day at pH 4.0 and 25°C (unstable) $T_{1/2}$ > 1 year at pH 7.0 and 25°C $T_{1/2}$ < 1 year at pH 9.0 and 25°C $T_{1/2}$ < 1 day at pH 4.0 and 50°C
Adsorption/Desorption:	not determined (see comments below)
Dissociation Constant:	-SO_3: -2.5 > pKa > -3.0 Ph-NH-Ph: pKa ≈ 0.8 Ph-NH-Tr: pKa ≈ 0.8 Tr-NH-CH_2: pKa < 1.0 Anthraquinone, 2-NH_2: pKa < 1.0
Flash Point:	not determined
Flammability Limits:	self-ignition at 280°C
Autoignition Temperature:	280°C
Explosive Properties:	non-explosive

Reactivity/Stability:	not an oxidising agent
Surface Tension:	55.5-55.8 mN/m at 1 g/L, 20°C 47.2-48.7 mN/m at 10 g/L, 20°C
Fat Solubility:	< 0.07 mg/100 g at 37°C
Particle Size:	mean 129 µm, < 40 µm: 4% (by weight) < 63 µm: 11% < 100 µm: 32% < 200 µm: 95% < 400 µm: 99.9%

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. It is expected to be negligible, as similar dyestuffs previously submitted exhibited very low (calculated) vapour pressures. The notified chemical is a high molecular weight, organic tetrasodium salt.

Preliminary testing over 5 days revealed that at 50°C the hydrolysis of the notified chemical was more than 99.4% at pH 4. Hence, it has a half-life of less than one day at 25°C. At pH 7 the degree of hydrolysis was 6.4%. Therefore, the half-life was determined to be more than one year. At pH 9, the degree of hydrolysis was 70.4%. Therefore, the half-life was determined to be less than one year. The identity of the hydrolysis products is unclear from the hydrolysis test report.

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 unlikely to bind/adsorb strongly to soil and to move with the ground water. However, it is expected that the chemical will bind to positively charged substances such as clay particles (Weber, 1991).

The notified chemical contains sulfonic acid functionalities that will be expected to completely dissociate under environmental conditions. The important pKs are the strongly acidic sulfite groups that will render the molecule fourfold 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 surface active at a concentration of 1 g/L. However, at higher concentrations, surface activity is increased. By definition, a chemical has surface activity when the surface tension is less than 60 mN/m (EEC Directive 92/69).

The particle size of Cibacron Blue TZ 3533 is not in the respirable range, with less than 4% of particles (by weight) less than 40 µm in diameter.

4. PURITY OF THE CHEMICAL

Composition and Impurities of Notified Chemical:

Name	*CAS Number*	*% Weight*
main substance	143683-23-2	60-70%
coloured and uncoloured by-products	–	10-30%
inorganic salts	–	< 10%
water	7732-18-5	< 10%

Composition of Cibacron (Reactive) Blue TZ3533:

Name	*CAS Number*	*% Weight*
notified chemical	143683-23-2	>60%
inorganic salts	–	< 5%
dispersant	9084-60-4	< 20%
anti-dusting agent		1%
water	7732-18-5	< 10%

5. USE, VOLUME AND FORMULATION

The notified chemical is a reactive dye used for colouring cellulose textiles by the exhaust dyeing method. It is expected to replace other reactive dyestuffs in the market place. The dye is claimed to have a fixation performance of 80%.

The notified chemical will not be manufactured in Australia. It will be imported in a powder form as a component of the product Cibacron Reactive Blue TZ 3533 ready for use. It will be imported in 20 kg sealed containers with antistatic polyethylene lining. Import volumes for the notified chemical are less than 5 tonnes for each of the first 5 years.

End use

The dyestuff will be used in a limited number dyehouses (3, all located in city areas) in Australia.

The dyestuff is fixed to the substrate by the pad-batch method, steaming or with dry heat. It will be weighed, added into warm water in a blending vessel and the dye solution is pumped through a closed system to a pad trough for dyeing. The cloth is fed through the pad trough. Following fixation to the fabric, the dyed cloth is led to the wash-off baths where the fabric is washed free of un-fixed dye and dried.

Fixation and fastness reports were provided to indicated that 70-80% of the notified chemical is fixed to the cloth.

Repackaging
Some minimal re-packing will occur for the purpose of supplying samples or material for mill trials. Repackaging will be carried out at one warehouse.

6. OCCUPATIONAL EXPOSURE

The vapour pressure of the notified chemical is expected to be very low, so dermal contamination would be the main route of occupational exposure. In addition, an anti-dusting substance is added to the dye (at 1%) to prevent dusting. The particle size is above the respirable range, however, a proportion (32-95%) is in the inspirable range. Workers who will handle the notified chemical include transport workers, dyehouse workers and storemen.

Transport and storage
Transport workers and storemen are unlikely to be exposed to the notified chemical unless the package is breached.

Repacking
Most customers will receive full 20 kg containers of the notified chemical. If packs need to be broken, then repacking will occur at the Ciba Specialty Chemicals warehouse with facilities for safe handling of hazardous substances. In the down-flow booth in which dyes are repacked, the air flow is away from operators and the capture velocity for particulates is exceeded to minimise the exposure. The repack operators are trained in the handling of hazardous substances. There will be 2 repack operators. It is estimated that less than 100 kg will need to be repacked which takes 15-20 minutes per day and up to 10 days annually. During these operations, workers wear elbow-length PVC gloves, safety glasses, face shield and overalls.

End use

Briefly, the incorporation of the dyestuff in a dye-bath solution can be represented:

Weighing → Adding to blending vessel → Transfer to dyeing apparatus

It is expected that up to 40-50 workers will handle the notified chemical in dyehouses. This would include 6 weighing-operators, 12 wash-off operators, 30 drier operators and 6 laboratory technicians.

Occupational exposure during weighing and mixing procedures is possible. The product containing the notified chemical will be weighed in a dispensary equipped with local exhaust ventilation. The weighed powder is added to the blending vessel also under local exhaust ventilation. It has been observed that the personal protective equipment worn by weighing

operators includes half-face piece particulate filter respirator, long impervious neoprene or rubber gloves, overall, industrial footwear and safety spectacles with side shields.

Padding of the dye and fixation are carried out in a closed system so there is no occupational exposure during this process.

During wash-off and dry processes after fixation, workers will handle the dyed cloth for short time only. The dyestuff has a high fastness and becomes chemically bonded to the cellulose fibres. There is no evidence of loss of dye fixed to fibre subsequent to wash-off or during drying. Dyed cloth is taken up on beams or trucks so that little manual handling of the cloth will be involved. During these operations, workers will wear protective gloves.

Laboratory technicians will take and analysis samples containing the notified chemical. The exposure to the notified chemical for laboratory technicians is expected to be low.

Worker exposure to the notified chemical during weighing was calculated by the notifier. Assuming a quantity of 8.4 kg is weighed each day with a weighing duration of 15 minutes, the notifier calculated that the average daily lifetime exposure could be 0.0016 mg/kg/day. Inhalation exposure only was assumed, with the values used in the estimation taken from a US monitoring study. Using the same monitoring values but without correcting for life expectancy would result in an average daily exposure of 0.0043 mg/kg/day.

7. PUBLIC EXPOSURE

Cibacron Blue TZ3533 will not be sold to the public. Public exposure to the notified chemical during storage, distribution and dyeing processes is expected to be negligible. In the case of accidents, spills will be collected and disposed of by secure landfill or incineration as indicated in the Material Safety Data Sheet (MSDS).

The dye has a high fastness (80%) on cellulose fibre substrate and about 20% would be discharged in the dyeing house effluent. Waste water from dyeing houses will be treated in the dye house biological effluent treatment works or the community Sewage Treatment Plant before being released to receiving waters. The concentration of the dye in receiving waters was estimated to be less than 0.002 ppb. Traces of the notified chemical remaining in empty packaging will be disposed of by secure landfill or incineration. Public exposure from disposal should be minimal.

The dyed cellulose fabrics will be used as domestic or industrial textiles. There will be extensive dermal contact with the dyed fabrics by the general public. However, the dye will be chemically bonded to the cellulose fibres, and the dye is unlikely to be dermally absorbed. Public exposure is expected to be low.

8. ENVIRONMENTAL EXPOSURE

Release

The bulk of the dye will become chemically fixed to the cellulose textiles, and in this state is not expected to impact on the environment. The result of fastness performance tests shows that a high order of fastness rating is achieved in all cases. After application to fabrics, the dye undergoes a chemical change involving 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 the traces remaining from repacking operations and clean-up of spills, and to trace residues in empty packaging (estimated by the Environment Australia at a maximum of 0.1% based on previous similar notifications by the notifier).

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

Fate

The dye normally released in water as effluent from the dyehouse is expected to constitute the major environmental exposure. The dye may either partition to sediment or stay in the aqueous compartment. Hobbs (1988) reports 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 fluoride. 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 1092 mg O_2/g. 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 (modified AFNOR) test for ready biodegradability. No inhibition of the activity of the bacteria was observed in this test. The dye's inherent biodegradability was 0% after 21 days according to the test procedure that followed OECD 302B guidelines (Zahn Wellens Test).

Although the dye is not biodegradable, the potential for bioaccumulation is low due to the low partition coefficient (log P_{OW} < -1.79), very high water solubility of the substance and low fat solubility (< 0.07 mg/100 g). Hydrophilic dyes with log P_{OW} < 3 have been shown not to bioaccumulate (Yen et al, 1991). Also, biological membranes are not permeable to chemicals of very large molecular size and therefore bioaccumulation of the notified polymer is not

expected (Anliker et al, 1988, Gobas et al, 1986).

Residues that persist after sewage treatment will enter marine 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 sulfonic 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.

9. EVALUATION OF TOXICOLOGICAL DATA

9.1 Acute Toxicity

Summary of the acute toxicity of Cibacron (Reactive) Blue TZ 3533

Test	*Species*	*Outcome*	*Reference*
acute oral toxicity	rat	LD_{50} > 2 000 mg/kg	(Hartmann, 1992a)
acute dermal toxicity	rat	LD_{50} > 2 000 mg/kg	(Hartmann, 1992b)
skin irritation	rabbit	slight irritant	(Hagemann, 1992a)
eye irritation	rabbit	slight irritant	(Hagemann, 1992b)
skin sensitisation	guinea pig	skin sensitiser	(Hagemann, 1993)

9.1.1 Oral Toxicity (Hartmann, 1992a)

Species/strain:	rat/RAI f (SPF)
Number/sex of animals:	5/sex
Observation period:	14 days
Method of administration/dose:	oral (gavage), 2 000 mg/kg in water
Clinical observations:	piloerection, hunched posture, dysphea, reduced locomotor activity and diarrhoea was observed in all animals, females displayed ataxia, surviving animals recovered within 6 days
Mortality:	2 females died within 24 hours after administration
Morphological findings:	nil
Test method:	limit test, OECD TG 401 (Organisation for

	Economic Co-operation and Development, 1995-1996)
LD_{50}:	> 2 000 mg/kg
Result:	the notified chemical was of very low acute oral toxicity in rats

9.1.2 Dermal Toxicity (Hartmann, 1992b)

Species/strain:	rat/RAI f (SPF)
Number/sex of animals:	5/sex
Observation period:	14 days
Method of administration/dose:	dermal application of 2 000 mg/kg under semi-occlusive dressing for 24 hours (vehicle: water)
Clinical observations:	piloerection observed, animals recovered within 1 day
Mortality:	nil
Morphological findings:	nil
Test method:	limit test, OECD TG 402 (Organisation for Economic Co-operation and Development, 1995-1996)
LD_{50}:	> 2 000 mg/kg
Result:	the notified chemical was of low dermal toxicity in rats

9.1.3 Inhalation Toxicity

No studies were available. The acute inhalation test is not practical to carry out because of the expected low vapour pressure, low oral toxicity, non-dust form of the commercial products and manner of use.

9.1.4 Skin Irritation (Hagemann, 1992a)

Species/strain: rabbit/New Zealand White

Number/sex of animals: 3 females

Observation period: 10 days

Method of administration/dose: dermal application of 0.5 mg the notified chemical moistened with water to right flank under an occlusive dressing for 4 hours

Draize scores (Draize, 1959):

Animal #	***Time after treatment (days)***				
	1	***2***	***3***	***7***	***10***
Erythema					
1	[a]1	0	0	0	0
2	0	0	0	0	0
3	2	1	1	1	0
Oedema					
1	0	0	0	0	0
2	0	0	0	0	0
3	1	0	0	0	0

[a] see Attachment 1 for Draize scales

Test method: OECD TG 404 (Organisation for Economic Co-operation and Development, 1995-1996)

Result: the notified chemical was a slight irritant to the skin of rabbits

9.1.5 Eye Irritation (Hagemann, 1992b)

Species/strain: rabbit/New Zealand White

Number/sex of animals: 2 males and 1 female

Observation period: 17 days

Method of administration/dose: 55 mg of the notified chemical was applied into the conjunctival sac of the left eye of each animal

Draize scores (Draize, 1959):

	Time after instillation													
Animal	***1 day***		***2 days***		***3 days***		***7 days***		***10 days***		***14 day***		***17 day***	
Cornea[a]														
1	[1]1		1		1		1		1		0		0	
2	0		0		0		0		0					
3	1		1		1		0		0					
Iris														
1	1		1		0		0		0		0		0	
2	1		1		0		0		0					
3	1		1		1		0		0					
Conjunctiva	***r***	***c***	***r***	***c***	***r***	***c***	***r***	***c***	***r***	***c***	***r***	***c***	***r***	***c***
1	2	2	2	1	1	1	1	0	1	0	1	0	0	0
2	2	1	2	0	2	0	1	0	0	0				
3	2	3	2	1	2	1	1	0	0	0				

[1] see Attachment 1 for Draize scales
[a] opacity r redness c chemosis

Test method: OECD TG 405 (Organisation for Economic Co-operation and Development, 1995-1996)

Result: at 1 h after treatment, corneal effect in one animal could not be evaluated due to blue staining;

the notified chemical was a slight irritant to the eyes of rabbits

9.1.6 Skin Sensitisation (Hagemann, 1993)

Species/strain: guinea pigs/Pirbright White Strain (Tif:DHP)

Number of animals: 10/sex (test), 5/sex (control)

Induction procedure: day 1-intradermal induction: 3 pairs of injections (0.1 mL) were made on shaved neck of each animal

- saline:Freund's Complete Adjuvant (FCA) (1:1) (v/v)
- the notified chemical in saline (5%, w/v)
- the notified chemical in FCA/saline mixture

(5%, w/v)

day 8-topical induction: occluded application of the notified chemical in vaseline (50%) for 48 hours

Challenge procedure: day 28-challenge: occluded application of the notified chemical in vaseline (50%) for 24 hours

Challenge outcome:

	Test animals		***Control animals***	
Challenge concentration	***24 hours****	***48 hours****	***24 hours***	***48 hours***
50%	**17/20	18/20	0/10	0/10

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

Test method: Magnusson and Kligman Maximisation Test, similar to OECD TG 406 (Organisation for Economic Co-operation and Development, 1995-1996)

Result: the notified chemical was a strong sensitiser to the skin of guinea pigs

9.2 Repeated Dose Toxicity (Gerspach, 1993)

Species/strain: rats/Tif: RAIf (SPF)

Number/sex of animals: 5/sex per dose group

Method of administration: oral (gavage)

Dose/Study duration: the notified chemical in water was given to 4 dose groups daily for a period of 4 weeks:
group 1: 0 mg/kg/day (control)
group 2: 0 mg/kg/day (control, plus 4 week recovery)
group 3: 10 mg/kg/day
group 4: 50 mg/kg/day
group 5: 200 mg/kg/day
group 6: 1 000 mg/kg/day
group 7: 1 000 mg/kg/day (plus 4 week recovery)

Clinical observations:	bluish discolouration of the whole body occurred in group 6 and 7 and began to disappear during the recovery period, bluish faeces were observed in the animals in groups 5 and 6; the mean bodyweight gain of group 6 males decreased throughout the treatment period with only minimal recovery in group 7; there was a tendency to lower bodyweight gain for males in group 4 and 5; food consumption was slightly decreased in male group 4 and 5 and markedly in male group 6, slightly decreased mean food consumption persisted in group 7 during the recovery period
Clinical chemistry:	in group 6, changes in clinical chemistry included increases in plasma urea, creatinine, cholesterol, alanine aminotransferase, and inorganic phophorus; a disturbed electrolyte balance was noted including decreased sodium and chloride and increased potassium in males, decreased chloride and increased calcium in females, reversibility was demonstrated for some effects in group 7 within the recovery period; males at this dose also had increased aspartate aminotransferase and decreased globulin fraction. in group 5, increased plasma creatinine and cholesterol were observed in males.
Haematology:	in group 6, a slight anaemia in males and females was associated with an increased number of reticulocytes; also leukocytosis with neutrophilia and relative lymphopenia and higher platelet counts were observed; these changes in haematological parameters were reversible.
Pathology:	various organs and tissue presented with bluish discolouration in groups 5 and 6 at the end of treatment and in group 7 after the recovery period; enlarged kidneys were found in group 6 at the end of treatment and in group 7 males after the recovery period at this dose. the mean carcass weight of males in group 6 was markedly decreased at the treatment end and still slightly low in group 7 after the recovery period;

	liver weights in groups at 1 000 mg/kg/day were increased at treatment end and after the recovery period; a dose-related increase of kidney weights was found in males in groups 5 and 6 and in females in groups 4, 5 and 6; the kidney weights were still markedly high in group 7 after the recovery period.
Histopathology:	kidney: cytoplasmic vacuoles frequently containing pale bluish material were found in renal tubular epithelium in male group 4 (minimal severity), and in both sexes in groups 5 and 6 at the end of the treatment and in group 7 after the recovery period; the change was associated with inflammation and fibrosis of the interstitial cortical tissue in group 7 males. liver: bluish pigment was found in Kupffer cells in group 6; necrosis of single hepatocytes was present in male groups 5 and 6; increased mitotic activity of hepatocytes was seen in male at 1 000 mg/kg/d; the changes were detected at the end of the treatment and after the recovery period. lymph node and spleen: phagocytic cells containing pale bluish material were found at minimal to moderate severity in the mesenteric lymph node in groups 5 and 6 at the end of the treatment and in group 7 after the recovery period; similar cells were seen in spleen in group 6 at the end of the treatment. adrenal: cytoplasmic fatty vacuoles occasionally containing pale bluish material were found in increased number and size in adrenal cortical cells in group 6 at the end of the treatment and in group 7 after recovery period.
Test method:	OECD TG 407 (Organisation for Economic Co-operation and Development, 1995-1996)
Result:	based on the kidney weight increase in females at 50 mg/kg/day and the hepatocyte necrosis and adverse kidney effects in general at 200 mg/kg/day, the NOEL for the notified chemical was considered to be 10 mg/kg/day.

9.3 Genotoxicity

9.3.1 *Salmonella typhimurium* and *Escherichia coli* Reverse Mutation Assay (Ogorek, 1992)

Strains:	*S. typhimurium* TA100, TA1535, TA98 and TA1537, and *E.coli* WP2uvrA
Concentration range:	original experiment: 61.7, 185.2, 555.6, 1 666.7 and 5 000 µg/plate in the presence or absence of S9 metabolic activation confirmatory experiment: 102.9, 308.6, 925.9, 2 777.7 and 8 333 µg/plate in the presence or absence of S9 metabolic activation
Test method:	OECD TG 471 (Organisation for Economic Co-operation and Development, 1995-1996)
Comment:	no increase in the incidence of mutants was observed for the notified chemical in comparison with the negative control (solvent). Mutations were observed with the positive controls.
Result:	the notified chemical did not induce gene mutations in the strains of bacteria tested in the study with and without metabolic activation

9.3.2 Induction of Chromosome Aberrations in Chinese Hamster ovary (CHO) Cells In Vitro (Ogorek, 1993a)

Species/strain:	cultured Chinese hamster ovary (CHO) cells
Doses:	experiments without metabolic activation 18 hours incubation time - original and confirmatory studies: 39.06, 78.13 and 156.25 µg/mL, (312.5 µg/mL), supplementary study: 60, 80, 120 and 160 µg/mL, (300 µg/mL). 42 hours incubation time - 39.06, 78.13 and 156.25 µg/mL, (312.5 µg/mL). experiments with S9 metabolic activation 3 hours incubation followed by 15 hours recovery period -

	original and confirmatory studies: 312.5, 625 and 1 250 µg/mL. 3 hours incubation followed by 39 hours recovery period - 312.5, 625 and 1 250 µg/mL.
Test method:	OECD TG 473 (Organisation for Economic Co-operation and Development, 1995-1996)
Comment:	a first series of experiments without and with metabolic activation with concentrations up to 5 000 µg/mL produced cytotoxicity at the upper concentrations. no biologically relevant increase in the number of specific chromosome aberrations was observed with and without metabolic activation for the notified chemical, in comparison with the negative control. A high incidence of specific chromosomal aberrations was observed in the positive controls.
Result:	the notified chemical was not clastogenic in CHO cells *in vitro* with and without metabolic activation

9.3.3 Mouse Micronucleus Assay In Vivo (Ogorek, 1993b)

Species/strain:	mouse/Tif: MAGf (SPF)
Number and sex of animals:	5/sex
Doses:	750 mg/kg (1 group), 1 500 mg/kg (1 group) and 3 000 mg/kg (3 groups)
Method of administration:	oral (gavage)
Test method:	OECD TG 474 (Organisation for Economic Co-operation and Development, 1995-1996)
Comment:	in all groups assessed after the different treatment periods, no significant increase in the number of micronucleated polychromatic erythrocytes was observed when compared with the negative control group
Result:	the notified chemical tested negative in this assay

9.4 Overall Assessment of Toxicological Data

The notified chemical was of very low acute oral toxicity (LD_{50} > 2 000 mg/kg) and low acute dermal toxicity (LD_{50} > 2 000 mg/kg) in rats. It was a slight skin and eye irritant in rabbits. It was a strong skin sensitiser in guinea pigs.

In a 28-day repeat dose study in rats, the animals were administered the notified chemical by gavage at 10, 50 200 or 1 000 mg/kg/day. Liver and kidney effects were observed. These included intracellular deposition of bluish material in the liver and kidneys, the spleen, mesenteric lymph nodes and adrenals. Phagocytic cells were observed in the mesenteric lymph node at 200 mg/kg/day and 1 000 mg/kg/day, and the spleen at 1 000 mg/kg/day. Cytoplasmic vacuoles were found in adrenal cells. At the highest dose, slight anaemia and a number of changes in clinical chemistry parameters were observed indicating the hepatic and renal effects of the notified chemical. Based on the kidney weight increase in females and cytoplasmic vacuoles containing bluish material in males at 50 mg/kg/day, and hepatocyte necrosis and adverse kidney effects in general at 200 mg/kg/day, the NOEL for the notified chemical is established at 10 mg/kg/day.

The notified chemical was not mutagenic in a reverse mutation assay and was not clastogenic in CHO cells *in vitro*. The notified chemical tested negative in an *in vivo* micronucleus test in the mouse.

According to the NOHSC *Approved Criteria for Classifying Hazardous Substances* , the notified chemical is classified as a hazardous substance 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 Practices.

Ecotoxicity Test Results

Test	*Species*	*Results (Nominal)*
Acute Toxicity (static test) (OECD 203)	Zebra fish *Brachydanio rerio*	96 h LC_{50} = 67.0 mg/L 96 h LC_0 = 32.5 mg/L
Acute Toxicity -Immobilisation Test (Static Test) (OECD TG 202)	Water Flea *Daphnia magna*	48 h EC_{50} = 33.5 mg/L (calculated) 48 h NOEC ≥ 5.8 mg/L
Growth Inhibition -Growth (μ) & Biomass (b) (Static Test) (OECD TG 201)	Green Algae *Scenedesmus subspicatus*	72 h E_bC_{50} = 26.4 mg/L 72 h NOEC(μ)< 3.7 mg/L
Respiration Inhibition (OECD TG 209)	Activated Sludge - Aerobic Waste Water Bacteria	3 h IC_{50} > 320 mg/L
Toxicity to earthworms (OECD TG 207)	Earthworm *Eisenia foetida foetida*	14 d EC_{50} < 1 000 mg/kg (calculated)

Comments on Ecotoxicity testing

Fish

A static test was performed in accordance with the test guidelines. Nominal concentrations of 10, 17.8, 32, 56 and 100 mg/L and a control were tested in parallel. All reported results are related to nominal concentrations as the test substance was sufficiently stable during the test period. The analytically determined concentrations varied from 97% to 110% of the nominal values. Results demonstrated that the notified substance had slightly toxic effects on the test fish with a 96 h LC_{50} = 67.0 mg/L. No deaths occurred at 32 mg/L, two fish died at 56 mg/L and all ten fish in the 100 mg/L treatment died. The results of the test did not allow a probit analysis to check the calculations of the LC_{50} as there was only one point between 0 and 100% mortality. Where deaths are measured, this type of analysis requires at least two points.

All control fish survived until the end of the test and no abnormal responses of the fish were observed during testing.

Aquatic Invertebrates

Nominal concentrations of 5.8, 10, 18, 32, 58 and 100 mg/L and a control were tested in parallel. All reported results are related to nominal concentrations as the test substance was sufficiently stable during the test period. The analytically determined concentrations varied from 97% to 100% of the nominal values.

The 48 h LC_0 and NOEC were determined to be 5.8 mg/L. At the next highest concentration tested, 5% of daphnids (1 of 20) were immobilised after 48 hours, compared with 15% at 18 mg/L, 20% at 32 mg/L and 95% at 58 mg/L. After 48 hours at 100 mg/L, 100% of daphnids were immobilised. The calculated LC_{50} was 33.5 mg/L with 95% confidence limits of 26.8-44.4 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 for the notified chemical.

Algae
Nominal concentrations of 1.23, 3.7, 11, 33 and 100 mg/L and a control were tested. The analytically determined concentrations in the analysed test media varied from 93% to 98% of the nominal values, and as such all biological results are related to nominal concentrations.

The notifier has not performed a modified growth inhibition test to differentiate between reduced algal growth caused by real toxic effects of the notified chemical on the algal cells or reduced algal growth caused indirectly 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 a calculated E_bC_{50} of 26.4 mg/L (95% confidence limits: 26.2-41.2 mg/L), 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 10 to 320 mg/L over the exposure period of 30 minutes.

Earthworms
The toxic effect of the notified substance on earthworms was investigated in a 14 day exposure test. The notified substance showed slightly toxic effects when exposed to nominal test concentrations in the range 12.3 to 1 000 mg/kg. A 20% death rate was recorded in the highest concentration after 14 days, against 3% death rate in the untreated control. The observed NOEC and LC_0 were 12.3 mg/kg. The LC_{50} was calculated to be >1 000 mg/kg. The notified substance may be classified as very slightly toxic to earthworms.

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

11. ASSESSMENT OF ENVIRONMENTAL HAZARD

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

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

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 represent maximum use.

Predicted Environmental Concentration (PEC)

Calculation Factor	*Country Dyehouse*	*City Dyehouse*
Typical use of product expected per day (700 kg cloth)	8.4 kg	8.4 kg
Amount of notified chemical	5.8 kg	5.8 kg
Conc. in wastewater (fixation rate 80%)	1.16 kg	1.16 kg
Quantity of water used incl. wash-off water (50 L/kg)	35 000 L	35 000 L
Effluent conc. in product-specific wash-water	33 mg/L	33 mg/L
Dilution factor in dyehouse by other wash-waters	1:70 (2.5 ML/day effluent)	1:100 (4 ML/day effluent)
Influent concentration	0.47 mg/L	0.33 mg/L
Dilution factor in sewage treatment plant[1]	1:10	1:100
Conc. balance in effluent from sewage treatment plant	47 µg/L	3.3 µg/L
Dilution factor in receiving waters	1:2 (river)	1:10 (ocean)
PEC in receiving waters	23.5 µg/L (23.5 ppb)	0.33 µg/L (0.33 ppb)
Safety factor for exposure to most sensitive aquatic organism, algae[2] (NOEC of 3.7 mg/L)	>157	>11 000

[1] The dilution at a rural town could reasonably be expected to be about 5-6 ML/day, while for a major city, say Sydney, it would be between 150-500 ML/day.
[2] The growth of Green algae was not inhibited up to a test concentration of 3.7 mg/L. The 72 hr E_bC_{50} was 26.4 mg/L (see *Environmental Effects* section).

These calculations show that the exposure to fish, daphnia, algae and waste water treatment bacteria is at levels unlikely to cause any significant effect. At higher release rates, there is

still unlikely to be any significant effect on these species. Once in the aquatic environment, the chemical is expected to swiftly dilute to undetectable concentrations, and undergo biotic and abiotic degradation. The notifier also claims that the dye will only be used in city dyehouses but an adequate safety factor exists for use in country locations.

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

12. ASSESSMENT OF PUBLIC AND OCCUPATIONAL HEALTH AND SAFETY EFFECTS

The notified chemical was of very low acute oral and dermal toxicity in rats. It was a slight skin and eye irritant in rabbits. Strong skin sensitisation was observed in a guinea pig maximisation test (GPMT) challenged with 50% of the notified chemical. In a 28-day oral repeat dose study in rats, the NOEL was established l0 mg/kg/day, based on increased kidney weights and cytoplasmic vacuoles containing blue dye at the next highest dose (50 mg/kg/day). There was evidence that the notified chemical was taken up and stored in a number of tissues, and only gradually removed or released. The target organs of toxicity were the liver (hepatocellular necrosis) and kidneys (increased weight, inflammation and fibrosis). The notified chemical was not mutagenic in a reverse mutation assay and was not clastogenic in CHO cells *in vitro*. The notified chemical tested negative in an *in vivo* micronucleus test in the mouse. The notified chemical is determined to be a hazardous substance based on the its skin sensitising effects according to the NOHSC *Approved Criteria for Classifying Hazardous Substances* (National Occupational Health and Safety Commission, 1994a).

The particle sizes are above the respirable range, however, a large percentage of the particles (32-95%) are within the inspirable size range. These may be deposited in the nose, pharynx and larynx. They may be transported to the gastrointestinal tract by secondary ingestion, hence absorbed into the body. Inhalation exposure is expected to constitute a minor source of exposure because the notified chemical is taken as having low vapour pressure, and it will be imported as a component in a non-dusting solid product, Cibacron (Reactive) Blue TZ 3533. Skin contamination is expected to be the main route for occupational exposure. However, due to the high molecular weight of the notified chemical, dermal absorption is unlikely.

Transport and storage

The health risk for transport workers and storemen is expected to be negligible unless the package is breached.

Repacking

Exposure to the notified chemical during repacking is estimated to be infrequent and of short duration. It is essential that the exposure control measures identified by the notifier are in place, namely exhaust ventilation, plus the wearing of personal protective equipment, because the chemical is a strong skin sensitiser.

End use

The weighing operators have potentially the highest exposure to the notified chemical. Local exhaust ventilation is provided in the dispensary and blending vessel areas, and as the operators wear respirator, overalls and gloves to minimise exposure, the risk of adverse systemic health effects during this operation is low. Based on data reported to be from a US air monitoring study, the notifier estimated that the average daily lifetime exposure of a worker during weighing would be 0.0016 mg/kg/day. This estimate assumes only one weighing operation per shift for a total duration of 15 minutes per day. Using the same data but not correcting for lifetime expectancy, the average daily exposure is 0.0043 mg/kg/day. Based on a NOEL of 10 mg/kg/day for kidney effects, a margin of exposure (MOE) for this estimate is 2326. If an additional safety factor of 10 is applied to take into account a longer weighing operation and/or a greater amount of dye in the Australian work situation, then an MOE of 200 would still indicate that the risk of adverse health effects resulting from exposure during weighing is of low concern. Note that this estimation of exposure excludes any contamination via the dermal route. As the notified chemical is a strong skin sensitiser and exposure to small amounts may be harmful, it is very important that a high level of exposure control is implemented.

Operators in charge of padding and fixing processes will have low exposure to the unfixed notified chemical as these stages are enclosed. Workers in charge of wash off and drying processes may need to handle waste chemical as well as the cloth containing the chemical bound to the cellulose cloth fibres. Workers involved in handling the dried cloth will wear protective gloves. Therefore exposure and subsequent health risk to these workers is low.

As the notified chemical is a skin sensitiser, workers who maintain the dye solutions or handle the wash-off solutions should wear overalls, goggles and gloves. Any workers who have become sensitised to the notified chemical should not continue to work with it.

Public health

There will be extensive dermal contact with dyed fabrics by the general public. However, since the notified chemical has a low partition coefficient, a relatively high molecular weight and poor fat solubility, and is chemically bonded to the cellulose fibres with a high level of fastness, the notified chemical is unlikely to be dermally absorbed. The proposed use of the notified chemical is not expected to pose a significant hazard to public health.

13. MATERIAL SAFETY DATA SHEET

The MSDS for the notified chemical was prepared 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.

14. RECOMMENDATIONS

To minimise occupational exposure to the notified chemical in Cibacron (Reactive) Blue TZ3533 the following guidelines and precautions should be observed:

- Respirator should be selected and fitted in accordance with Australian/New Zealand Standard (AS/NZS) 1715 (Standards Australia/Standards New Zealand, 1994a);
- Industrial clothing should conform to the specifications detailed in AS 2919 (Standards Australia, 1987) and AS 3765.1 (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, 1994b);
- 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.
- Any workers who have become sensitised to the notified chemical should not continue to handle it in the workplace.

15. REQUIREMENTS FOR SECONDARY NOTIFICATION

Under the Act, secondary notification of the notified chemical shall be required if any of the circumstances stipulated under subsection 64(2) of the Act arise. No other specific conditions are prescribed.

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Attachment 1

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

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

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

CORNEA

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

CONJUNCTIVAE

Redness	*Rating*	*Chemosis*	*Rating*	*Discharge*	*Rating*
Vessels normal	0 none	No swelling	0 none	No discharge	0 none
Vessels definitely injected above normal	1 slight	Any swelling above normal	1 slight	Any amount different from normal	1 slight
More diffuse, deeper crimson red with individual vessels not easily discernible	2 mod.	Obvious swelling with partial eversion of lids	2 mild	Discharge with moistening of lids and adjacent hairs	2 mod.
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