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NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME

FULL PUBLIC REPORT

Notified Chemical in Cibacron Red W-B 150%

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Director
Chemicals Notification and Assessment

FULL PUBLIC REPORT

Notified Chemical in Cibacron Red W-B 150%

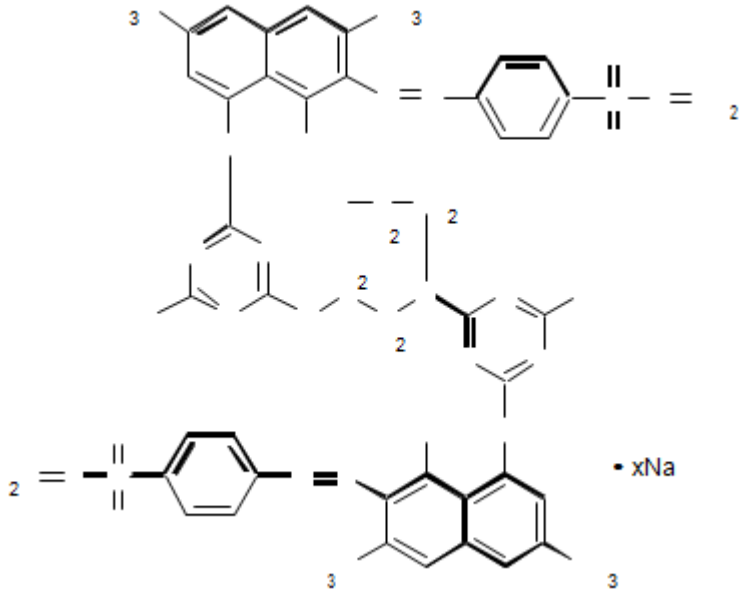
1. APPLICANT

Ciba Specialty Chemicals of 235 Settlement Road THOMASTOWN VIC 3074 has submitted a standard notification statement in support of its application for an assessment certificate for the Notified Chemical in Cibacron Red W-B 150%. Cibacron Red W-B 150% is the trade name of the product containing the notified chemical.

2. IDENTITY OF THE CHEMICAL

Chemical Name:	2,7-naphthalenedisulfonic acid, 5-[[4-chloro-6-[[2-[[4-chloro-[[7-[[4-(ethenylsulfonyl) phenyl] azo]-8-hydroxy-3,6-disulfo-1-naphthalenyl] amino]-1,3,5-triazin-2-yl] amino] ethyl] (2-hydroxyethyl) amino-1,3,5-triazin-2-yl] amino]-3-[[4-(ethenylsulfonyl) phenyl] azo]-4-hydroxy-, sodium salt
Chemical Abstracts Service (CAS) Registry No.:	171599-85-2
Other Names:	FAT 40548/A (laboratory designation) N,N'-bis-[6-chloro-4-[6-(4-vinylsulfonyl-phenlazo)-2,7-disulfo-5-hydroxy-naphth-4-ylamino]-[1,3,5] triazine-2-yl]-N-(2-hydroxyethyl)-ethane-1,2-diamine-, sodium salt
Trade Name:	Cibacron Red W-B 150%, Red Ren 363
Molecular Formula:	$C_{46}H_{38}C_{12}N_{14}O_{19}S_6 \cdot xNa$

Structural Formula:



Molecular Weight:	1 522
Method of Detection and Determination:	IR, UV/Vis and NMR spectroscopy
Spectral Data:	IR major peaks at 2 930, 2 860, 1 600, 1 550, 1 470, 1 380, 1 310, 1 205, 1 140, 1 050, 980 and 760 cm^{-1}

Comments on Chemical Identity

The molecular weight of 1 522 g/mol is calculated from the molecular formula and is based on the assumption that there are four sodium atoms present.

3. PHYSICAL AND CHEMICAL PROPERTIES

Appearance at 20°C and 101.3 kPa:	an odourless dark red powder
Melting Point:	> 400°C
Density:	1 200 kg/m^3
Vapour Pressure:	$8x10^{-45}$ kPa at 25°C (calculated)
Water Solubility:	> 400 g/L

Partition Co-efficient (n-octanol/water): $\log P_{ow} \leq -2$ at 20°C (estimated)

Hydrolysis as a Function of pH: $T_{1/2} = 1.2 \times 10^4$ hours (pH 4.0, 7.0 and 9.0, 25°C), stable at pH 7, less stable at pH 4 and 9 (see comment below)

Adsorption/Desorption: not determined (see comments below)

Dissociation Constant:

Reaction Centre	*Nature of the Reaction Centre*	*Acid Dissociation Constant*	
1	AR-SO_3H	pK_a (1)	-6.4
2	AR-SO_3H	pK_a (2)	-5.7
3	AR-SO_3H	pK_a (3)	-5.1
4	AR-SO_3H	pK_a (4)	-4.3
5	AR-OH	pK_a (5)	7.2
6	AR-OH	pK_a (6)	7.8
7	R-CH_2OH	pK_a (7)	14.9

Flash Point: not flammable

Flammability Limits: not flammable

Autoignition Temperature: not auto-flammable

Explosive Properties: not explosive

Reactivity/Stability: stable

Surface Activity: 59.0 mN/m at 1 g/L and 20°C

Particle Size:

Range (µm)	Mass (%)
< 0.36	0.06
0.36-0.76	0.20
0.76-1.56	0.40
1.56-3.11	0.71
3.11-6.24	1.74
6.24-12.12	4.56
12.12-24.85	12.25
24.85-63	29.50
63-100	30.22
100-200	20.21
>200	0.15
mean particle size	approximately 60-65 µm

Comments on Physico-Chemical Properties

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

No melting point was detected up to 400°C using the capillary method (Pighetti E, 1996). The density was determined by means of a gas comparison pycnometer at 20.0°C (Grothe, 1996a). The vapour pressure was estimated based on the boiling point using the Modified Watson Correlation (Grothe, 1996b). The boiling point was calculated to be 1 040°C using Meissner's method.

The water solubility was determined using the flask shake method (Del Vaglio, 1996). The notified chemical (1.00 g) was added to 2.0 mL distilled water and shaken. In the resulting mixture, a dark red and highly viscous solution with no undissolved particles was observed. Further analysis at higher concentrations could not be undertaken because the highly viscous solution could not be exactly measured by pipetting.

The percentage hydrolysis in the test solutions after an incubation time of 2.4 hours and 5 days is given in the following table (Grothe, 1996c):

	% Hydrolysis at 2.4 h and 50°C	***% Hydrolysis at 5 days and 50°C***
pH 4.0	< 5	12.5
pH 7.0	< 5	< 5
pH 9.0	< 5	21.5

The hydrolysis half-life times ($t_{1/2}$) for pH 4.0 and pH 9.0 at 50°C were calculated to be 39 days and 16 days, respectively. According to the guideline, the $t_{1/2}$ at pH 4.0 and pH 9.0 is estimated to be shorter than one year and longer than one day at 25°C. Additional testing involving the calculation of the activation energies and k_{25} values estimated the $t_{1/2}$ for pH 4.0 and pH 9.0 to be about 1.3 years and 2.1 years at 25°C, respectively.

The partition coefficient was estimated to be log P_{OW} < -5.3 by calculation using the saturation concentration of the notified chemical in the pure solvents, i.e. 3.0 mg/L in n-octanol and > 550 g/L in double distilled water (Grothe, 1996d). However, the results obtained in the preliminary partitioning experiment indicated that the expected log P_{OW} lies outside the range accessible by the flask shaking method. In conclusion, the partition coefficient was estimated to be log $P_{OW} \leq -2$ at 20°C.

The notifier has not provided adsorption/desorption data but anticipates that the notified chemical will bind/adsorb strongly to clay as indicated by the low partition coefficient. The free acid form may also bind to organic material in the soil. The notifier provided a reference that supports their claim that the notified chemical may bind to positively charged substances such as clay particles and silicates (Dragun, 1988).

The molecular structure of the free acid was used for the estimation of the dissociation behaviour (Grothe, 1996e). The dissociation constant for each of the seven acidic protons (reaction centres) found in the molecular structure were predicted. The pKa values predicted for the notified chemical show that reaction centres 1 to 4 will be completely dissociated. The presence of sulphonic acid functionalities (reaction centres 1 to 4) are expected to be completely dissociated under environmental conditions.

The notified chemical is expected to be slightly surface active (Grothe, 1996f). 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

Degree of Purity: 57.8%

Impurities, including isomers and by-products:

Name	*CAS Number*	*% Weight*
known coloured by-products	—	24.8
unknown coloured by-products	—	3.1
sodium chloride	7647-14-5	0.5
sodium phosphate	7601-54-9	1.2
sodium tripolyphosphate	7758-29-4	2.4
sodium sulphate	7757-82-6	4.2
water	7732-18-5	3.7
unsulfonated primary aromatic amines	—	< 10 mg/kg

Identity of Additives/Adjuvants:

The composition of the imported commercial product, Cibacron Red W-B 150% containing the notified substance, is as follows:

Name	*CAS Number*	*% Weight*
substance containing 57.8% notified chemical		77
dispersing agent (napthalenesulfonic acid, polymer with formaldehyde, sodium salt)	9084-06-4	11.8
sodium tripolyphosphate	7758-29-4	2.9
anti-dusting additive (paraffin oils)	8012-95-1	0.7
water	7732-18-5	7.6

Comments on Purity

Both known and unknown impurities have been reported. None of the impurities of known CAS number is on the *NOHSC List of Designated Hazardous Substances*. The hazardous

nature of the remaining substances has not been determined individually. Health effect classifications were not requested because the toxicity studies were performed on samples that included these impurities.

Additives and adjuvants are included only in the commercial version of the dyestuff, not in the notified chemical itself.

5. USE, VOLUME AND FORMULATION

Use/Import Volume

The notified chemical is the primary component of the imported dye product Cibacron Red W-B 150%. The dye preparation will be used for the colouration of cellulose textiles by the exhaust dyeing method. The dye has a reported fixation performance of 71%.

The notified chemical will not be manufactured in Australia. It will be imported into Australia in powder form in the formulated dyestuff product in robust 30 kg antistatic polyethylene lined cardboard containers. Import volumes for the notified substance are as follows:

Import Volume (tonnes)	***Year***				
	1	**2**	**3**	**4**	**5**
Cibacron Red W-B 150%	5-6	8-10	8-10	10-15	10-15
notified chemical	3.9-4.6	6.2-7.7	6.2-7.7	7.7-11.6	7.7-11.6

End use

Ciba Specialty Chemicals will supply the dyestuff directly to approximately 15 dyehouses in Australia.

The notified chemical is intended for use as a colourant in textile dyes by the exhaust dyeing method at a concentration of < 10%. It will be weighed, added into warm water in a blending vessel and the dye solution is pumped through a closed system for dyeing. The cloth is fed through a winch system into a series rollers designed for continuously cycling through the enclosed dyeing machine. Following fixation to the fabric, the dyed cloth is then led to the wash-off baths where the fabric is washed free of un-fixed dye and then dried.

Repacking

A small amount of Cibacron Red W-B 150% will be repacked by Ciba Specialty Chemicals into 5 and 10 kg containers for the purpose of mill trials, etc. Repacking will be carried out at one warehouse.

6. OCCUPATIONAL EXPOSURE

The vapour pressure of the notified chemical is very low, so dermal contamination would be the main route of occupational exposure. The notified chemical is in a non-dusting

formulation and the particle size is mostly above the respirable range. The effectiveness of the anti-dusting additive is not provided. Workers who will handle the notified chemical include transport workers, repacking workers, dyehouse workers and storemen.

Transport and storage

There will be 10 to 15 transport and storage workers handling the notified chemical. Transport workers and storemen are unlikely to be exposed to the notified chemical unless the package is breached.

Repacking

Most customers will receive the 30 kg containers of notified chemical. However, the notifier estimates that approximately 4% of imported containers will need to be repacked into smaller containers. If packs need to be broken, then repacking will occur in the warehouse with facilities for safe handling of hazardous substances. The repack operators are trained in the handling of hazardous substances. There will be 2 repack operators. It is estimated that less than 240 to 600 kg per year will need to be repacked resulting in a potential exposure time of 4 to 10 hours annually. During these operations, weighing will be carried out in a ventilated (downdraft) booth, and workers will wear safety glasses, long impervious neoprene or rubber gloves, overalls and industrial footwear. These items are specified in the Material Safety Data Sheet (MSDS). The notifier has indicated that respiratory protection is also provided to operators when repacking.

Weighing and mixing

It is expected that up to 500 workers will handle the notified chemical in dyehouses. This would include approximately 60 weighing operators, 240 dyeing operators, 120 curing/drying operators and 35 laboratory technicians.

Occupational exposure during weighing and mixing procedures is possible. Two operators are normally involved in the weighing and mixing during each shift (2 shifts per day) at each dyehouse. The notifier has estimated that a maximum exposure time of approximately 45 minutes per day could be expected for each operator per day. The dye containers are opened and the dyestuff is manually scooped from the drums into a weighing container. The package container is designed so that the polyethylene lining and container lid can both be resealed after opening. Mechanical ventilation of the weighing area will prevent a build up of dye dust. The weighed powder is dissolved in warm water in a mixing tank fitted with a stirrer. Personal protective equipment typically worn by weighing operators handling other dyes includes half-face piece particulate filter respirator, long impervious neoprene or rubber gloves, overalls, industrial footwear and safety spectacles with side shields. Feed tanks on the dye machines are filled with mixed dye liquor manually by the operators. Details of this manual process were not provided. During handling of the mixed dye solutions, the operators wear overalls, gloves and safety glasses.

Dyeing

Dyeing and fixation are carried out in a closed system so there is little opportunity for occupational exposure during this process. Cibacron Red W-B 150% is expected to comprise a maximum of 10% of the various dyes used at the dyehouse. As dye application occurs for

approximately 20 minutes during each dyeing cycle (approximately 3 hours), potential exposure to the notified chemical for each operator will be limited to several minutes per day. There is potential for short term exposure if the cloth becomes tangled and the operators are required to open the machine to realign it on the rollers. Workers wear overalls, gloves and goggles when opening the machine to untangle the cloth.

The dyeing machines are predominantly self-cleaning as a result of the washing cycle of the dyeing process. The operators clean the filters on the dyeing machines on a regular basis using a hose to remove the loose fibres.

Drying/curing

During wash-off and dry processes after fixation, workers load the wet washed cloth into the dryer. As the dyestuff becomes chemically bonded to the cellulose fibres during fixation, there should be little exposure to the notified chemical in an available form. A maximum exposure time of approximately 45 minutes per day can be estimated for each operator. During these operations, workers will wear protective gloves.

Laboratory

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

7. PUBLIC EXPOSURE

Exposure of the public to the notified chemical is expected to be low due to the high MW and low vapour pressure of the notified chemical. In the event of a transport accident, the notifier states that spills should be dampened down first with water and taken up mechanically. The final residue is washed with water and detergent, followed by absorption in inert material and landfilling or incineration in accordance with local regulations.

The notified chemical will be used for industrial processes and will not be sold to the public. It is anticipated that the public will come into contact with fabrics dyed with the notified chemical, however, it is assessed by the notifier as being of low concern since the dyestuff is strongly fixed to the fibre and no significant transfer to the skin would result on exposure. The fastness with respect to washing and dry cleaning is high and hence negligible residues would find their way into cleansing liquors (domestic washing). Furthermore, due to its colour value, fabrics containing the notified chemical will be used mainly for outwear, reducing the possibility for continuous contact with the skin.

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. A fixation plot indicates that fixation of up to 71% 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 repacking 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 MSDS.

Fate

The notified chemical 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. Hobbs (1988) reports that reactive dyes have been found not to absorb to sludge in model systems. However, the notifier claims that the notified substance will bind/adsorb strongly to clay and organic material, indicating that it may be (partially) removed through adsorption to sludge in the waste water treatment process. Any substance that binds to the sludge will be disposed of through incineration or landfill.

Residues that persist after sewage treatment will enter aquatic environments in solution (from both city and country waste water treatment systems), rapidly diluting to undetectable levels. While azo dyes are generally stable under aerobic conditions, they are susceptible to reductive degradation under anaerobic conditions characteristic of sediment (Hobbs, 1988). Also, highly sulfonated azo dyes have been shown to sorb to sediment through an anion-adsorption mechanism (Weber, 1991). Another 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.

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.

No biochemical oxygen demand (0 mg O_2/L) was measured for the substance in the two flasks, when exposed to micro-organisms from a domestic waste water treatment plant over a period of 28 days (Grützner, 1996a). As such, the notified chemical was determined to be non-biodegradable (0%) according to the OECD Test Guideline 301F (Manometric Respirometry Test). No inhibition on the activity of the bacteria was observed in this test, which is consistent with the findings of the Activated Sludge - Respiration Inhibition Test.

The notified chemical was found to be practically non-(inherently) biodegradable over a 28 day exposure period when exposed to micro-organisms from a domestic waste water treatment plant, according to the OECD Test Guideline 302B Zahn-Wellens/ EMPA Test (Grützner, 1996b). 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 5% over the exposure period.

Although not readily or inherently biodegradable, the potential for bioaccumulation is low due to the low estimated partition coefficient (log $P_{OW} \leq -2$) and very high water solubility of the substance (>400 g/L). Hydrophilic dyes with log $P_{OW} < 3$ have also been shown not to bioaccumulate (Yen, 1991).

9. EVALUATION OF TOXICOLOGICAL DATA

All toxicological studies have been conducted on FAT 40548/A, which is the laboratory designation for the substance containing 57.8% of the notified chemical.

9.1 Acute Toxicity

Summary of the acute toxicity of FAT 40548/A

Test	*Species*	*Outcome*	*Reference*
acute oral toxicity	rat	$LD_{50} > 2\,000$ mg/kg	(Arcelin, 1996a)
acute dermal toxicity	rat	$LD_{50} > 2\,000$ mg/kg	(Arcelin, 1996b)
skin irritation	rabbit	not a skin irritant	(Braun, 1996a)
eye irritation	rabbit	a slight eye irritant, but caused damage to eyes	(Braun, 1996b)
skin sensitisation	guinea pig	a skin sensitiser	(Arcelin, 1996c)

9.1.1 Oral Toxicity (Arcelin, 1996a)

Species/strain:	rat/hanIbm: WIST (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:	diarrhoea was observed in 2 meals and 3 females between 2 and 5 hours after treatment
Mortality:	nil
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:	FAT 40548/A was of very low acute oral toxicity in rats

9.1.2 Dermal Toxicity (Arcelin, 1996b)

Species/strain:	rat/hanIbm: WIST (SPF)
Number/sex of animals:	5/sex
Observation period:	14 days
Method of administration/dose:	a dermal application of 2 000 mg/kg in water on intact skin and covered with semi-occlusive dressing for 24 hours
Clinical observations:	scales were seen in one male animal between day 6 and 8; purple discolouration of the skin at the application site was evident in all animals after removal of bandage and persisted until study termination in most of the animals

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:	FAT 40548/A was of very low dermal toxicity in rats

9.1.3 Inhalation Toxicity

An acute inhalation study was not performed as less than 7.7% of the dye powder is in the respirable range. In addition, the product contains an antidusting agent, which reduces the potential for dust to form and consequently lowers the risk to human health.

9.1.4 Skin Irritation (Braun, 1996a)

Species/strain:	rabbit/New Zealand White Chbb:NZW(SPF)
Number/sex of animals:	1 male, 2 females
Observation period:	72 hours
Method of administration:	0.5 g the test substance was applied on the clipped skin with a semi-occlusive dressing for 4 hours
Test method:	OECD TG 404 (Organisation for Economic Co-operation and Development, 1995-1996)
Result:	Draize scores (Draize, 1959) were zero for erythema and oedema in animals up to 72 hours, FAT 40548/A was not irritating to the skin of rabbits

9.1.5 Eye Irritation (Braun, 1996b)

Species/strain:	rabbit/New Zealand White Chbb:NZW(SPF)
Number/sex of animals:	1 male, 2 females
Observation period:	21 days
Method of administration:	0.1 g the test substance was installed into one eye of the rabbits
Draize scores:	conjunctiva: chemosis observed in all animals up to 24 hours, all other readings zero; cornea and iris: all scores zero.
Other relevant observations:	staining in conjunctivae (including nictitating membrane) and sclera was noted in all animals up to day 21, light violet staining of the cornea was noted in 2 of the 3 animals but was reversible by day 7.
Test method:	OECD TG 405 (Organisation for Economic Co-operation and Development, 1995-1996)
Result:	FAT 40548/A caused damage to the eyes of rabbits based on its persistent colouration effects. The substance was a slight irritant to rabbit eyes.

9.1.6 Skin Sensitisation (Arcelin, 1996c)

Species/strain:	guinea pig/Ibm: GOHI; SPF
Number of animals:	20 (test), 10 (control)
Induction procedure:	day 1-intradermal induction: 3 pairs of injections (0.1 mL) were made: • saline:Freund's Complete Adjuvant (FCA) (1:1) (v/v) • the notified chemical in water (5%, w/v) • the notified chemical (5%, w/v) in FCA/saline mixture (1:1)

	day 8-topical induction: occluded application of the test substance in water (50%) for 48 hours
Challenge procedure:	day 22-challenge: occluded application of the test substance in water (25%) for 24 hours

Challenge outcome:

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

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

Test method:	Magnusson and Kligman maximisation test, OECD TG 406 (Organisation for Economic Co-operation and Development, 1995-1996)
Comment:	the concentrations of test substance used for the induction and challenge procedures (50% and 25% respectively) were determined from a preliminary study where 10%, 15%, 25% and 50% were topically applied to the clipped and shaven skin of 4 guinea-pigs
Result:	FAT 40548/A was a moderate sensitiser to the skin of guinea pigs

9.2 Repeated Dose Toxicity (Schmid et al., 1996)

Species/strain:	rat/HanIbm:WIST(SPF)
Number/sex of animals:	5/sex/group plus 5/sex for groups 1 and 4 (recovery group)
Method of administration:	oral (gavage)
Dose/Study duration:	group 1: 0 mg/kg/day group 2: 50 mg/kg/day group 3: 200 mg/kg/day group 4: 1 000 mg/kg/day (vehicle: water, recovery time: 2 weeks)

Clinical observations:	no clinical signs, no effect on body weight gain or food consumption
Clinical chemistry/Haematology	all treated animals had discolouration in their plasma and urine. females in group 4 had increases in reticulocyte count, proportional shift in the reticulocyte fluorescence ratios, increase in methaemoglobin concentration, slight increase in total bilirubin and triglyceride, and decrease in plasma glucose. males in groups 4 had increases in methaemoglobin concentration, creatinine, uric acid and total bilirubin in plasma and a higher incidence of blood cell counts in urine. after the recovery period, the above findings were reversible except a deep yellow pigmentation was noted in the urine of both sexes of group 4.
Pathology:	the absolute liver weight was marginally higher in group 4 animals, but the liver to body weight ratio in males was statistically significant higher when compared with the controls; pathologic examination of liver tissue revealed no treatment-related changes and therefore these marginal changes are considered to be part of the natural variation seen in rats of this strain and age. discolouration was noted in kidneys in group 3, and in gastrointestinal tract, kidneys, testes, mesenteric lymph node and skin in group 4 at the end of the treatment and in kidneys and mesenteric lymph nodes after the recovery period. there was an increase in the severity of renal tubular hyaline droplets in group 3 and 4 males and group 4 females; these droplets were morphologically similar to those containing the normal constituent of male rat urine $\alpha 2$ microglobulin and were not associated with tubular degeneration; these were also present in the kidneys of female rats of group 4 where they were accompanied in several cases by lipofuscin pigment.

	following the recovery phase, hyaline droplets remained increased in group 4 and the incidence and severity of lipofuscin in groups females had increased compared with rats of the same group killed at the end of the treatment a reversible increase in the degree of splenic haemopoiesis was noted in group 4 females
Test method:	OECD TG 407 (Organisation for Economic Co-operation and Development, 1995-1996)
Comments:	the full toxicological report was not provided for assessment; the methodology and summaries of test results were provided, but no detailed animal data
Result:	based on discolouration of urine and plasma at the lowest dose (50 mg/kg/day), no NOEL could be established from the study; based on the renal tubular hyaline droplets effects in males and tubular lipofuscin pigment in the kidneys of one female, the study authors determined the NOAEL to be 50 mg/kg/day.

9.3 Genotoxicity

9.3.1 *Salmonella typhimurium* and *Escherichia coli* Reverse Mutation Assay (Wollny, 1996)

Strains:	*Salmonella typhimurium* TA1535, TA1537, TA98 and TA100 and *Escherichia coli* WP2 and WP2uvrA
Concentration range:	33.3, 100.0, 333.3, 1 000, 2 500 or 5 000 µg/plate with or without S9 metabolic activation
Test method:	OECD TG 471 and 472 (Organisation for Economic Co-operation and Development, 1995-1996)
Comments:	no increase in the incidence of mutants was observed for the test substance in comparison with the negative control, mutations were observed with the positive controls

Result:	FAT 40548/A was considered to be non-mutagenic in the bacteria tested in the study

9.3.2 Chromosome aberration assay in Chinese hamster V79 cells *in vitro* (Czich, 1996)

Species/strain:	Chinese hamster V79 cells
Doses:	without S-9 activation 18 hours fixation interval: 50, 100, 300 and 500 µg/mL; 28 hours fixation interval: 300 and 500 µg/mL. with S-9 activation 18 hours fixation interval: 3, 10, 30, 50 and 100 µg/mL; 28 hours fixation interval: 30 and 50 µg/mL
Test method:	OECD TG 473 (Organisation for Economic Co-operation and Development, 1995-1996)
Comments:	in the absence of S9, in both experiments the mitotic index was reduced after treatment, with the highest evaluated concentration at fixation interval 28 hours; in the presence of S9, in both experiments the mitotic index and the cell numbers were reduced after treatment, with the highest evaluated concentration at fixation interval 18 hours; in both independent experiments, in the absence and presence of S9, there were no biologically relevant increases in cells carrying chromosome aberration after treatment with the test substance
Result:	FAT 40548/A was considered to be non-clastogenic in this chromosome aberration test

9.4 Overall Assessment of Toxicological Data

The substance containing 57.8% of the notified chemical is of very low acute oral toxicity and low dermal toxicity (both LD_{50} > 2 000 mg/kg) in rats. It is not a skin irritant but is an eye irritant with persistent colouration effects in rabbits. In a guinea pig maximisation study, the notified chemical was a moderate skin sensitiser.

In the 28-day repeated dose oral study in rats, changes in haematology, plasma and urine parameters were observed at the top dose of 1 000 mg/kg/day. Increased severity of hyaline droplets in the kidney were seen in group 3 and 4 males, but this was not associated with tubular degeneration. In males these hyaline droplets are a normal constituent of male rat urine, α2 microglobulin. In this study, droplets were also present in the kidney in group 4 females and in one group 3 female, where they were accompanied in several cases by lipofuscin pigment. These morphologic alterations were considered to represent an increased metabolic load in the kidneys, and were not reversed during the recovery period. In group 3 animals (treated with 200 mg/kg/day), renal morphological changes were considered to be treatment related. Discolouration of urine was observed in all treated animals and remained at the end of the recovery period in group 4 animals. Based on this observation, a NOEL cannot be established for the study.

The notified substance did not induce gene mutation in the strains of *S. typhimurium* and *E. coli* and had no clastogenic effects in Chinese hamster V79 cells in vitro in a chromosomal aberration assay.

According to the NOHSC *Approved Criteria for Classifying Hazardous Substances (National Occupational Health and Safety Commission, 1994a),* the notified chemical is classified as a hazardous substance based on its skin sensitisation effect (R43 - May cause sensitisation by skin contact), and its persistent colouration effects in rabbit eyes (R41 - Risk of serious damage to eyes).

10. ASSESSMENT OF ENVIRONMENTAL EFFECTS

The following ecotoxicity studies have been supplied by the notifier. The tests were carried out to OECD Test Methods at facilities complying with OECD Principles of Good Laboratory Practice.

Test	*Species*	*Results (Nominal)*[#]	*References*
Acute Toxicity Static, 96 hour OECD TG 203	Rainbow Trout (*Oncorhynchus mykiss*)	LC_{50} > 100 mg/L NOEC = 100 mg/L	Hertl & Schreitmüller, 1996a
Acute Immobilisation Static, 48 hour OECD TG 202	Water Flea (*Daphnia magna*)	EC_{50} > 100 mg/L NOEC = 21 mg/L	Hertl & Schreitmüller, 1996b
Reproduction 14 days OECD TG 202-Part II	Water Flea (*Daphnia magna*)	—	
Growth Inhibition Static, 72 hour OECD TG 201† biomass b growth rate μ	Algae (*Scenedesmus subspicatus*)	Experiment A $E\mu C_{50}$ = 139 mg/L (61-2 725 mg/L) E_bC_{50} = 20.5 mg/L (10.7-41.1 mg/L) NOEC = 3.2 mg/L Experiment B $E\mu C_{50}$ = 132 mg/L (87-274 mg/L) E_bC_{50} = 32.1 mg/L (22.7-47.5 mg/L)	Hertl & Schreitmüller, 1996c
Respiration Inhibition 30 minute OECD TG 209	Aerobic Waste Water Bacteria	EC_{50} > 100 mg/L	Grützner I, 1996c

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).

Fish

A limit test, performed in accordance with the test guidelines, demonstrated that the notified chemical had no toxic effects on the test fish up to a nominal concentration of 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 chemical. The analytically determined test substance concentrations in the test media varied in the range of 92% to 95% 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
Nominal concentrations of 4.6, 10, 21, 46 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 95% to 110% 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 21 mg/L. At the next highest concentration tested, 15% of daphnids (3 of 20) were immobilised after 48 hours. After 24 hours at 100 mg/L, 5% of daphnids were immobilised increasing to 20% of daphnids at 48 hours.

A *Daphnia sp.* reproduction test was not supplied. However, based on the low acute toxicity to both fish and daphnids, the notified chemical is not expected to affect the reproduction of daphnids.

Algae
Nominal concentrations of 0.32, 1.0, 3.2, 10, 32 and 100 mg/L and a control were tested. The analytically determined concentrations in the analysed test media varied from 99% to 103% of 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 medium.

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 experiment part B 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}s 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 3.2 to 100 mg/L over the exposure period of 30 minutes.

Conclusion

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

The notifier has specified that approximately 15 dyehouses in both city and country locations will be using the dyestuff containing the notified chemical. 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 77% product)	23.1	46.2	7.7
Conc. in wastewater (fixation rate 71%)	6.7	13.4	2.2
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	44.7 mg/L	89.3 mg/L	29.8 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	3.44 mg/L	6.87 mg/L	1.15 mg/L
Dilution factor in sewage treatment plant	1:100	1:2	1:2
Conc. balance in effluent from sewage plant	34.4 μg/L	3.44 mg/L[1]	0.57 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	3.44 μg/L (3.44 ppb)	1.72 mg/L (1.72 ppm)	0.29 mg/L (0.29 ppm)
Safety factor for exposure to most sensitive aquatic organism, Algae (72h E_bC_{50} = 20.5 mg/L)	5970	12	70

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 (Dragun, 1988; Weber, 1991). As such, the actual concentration in receiving waters is likely to be (significantly) 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, Perenich, and Baughman, 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.

It is noted that the notified chemical is surface active. However, significant effects are not expected in the environment due to the predicted low concentrations in the aquatic compartment.

12. ASSESSMENT OF PUBLIC AND OCCUPATIONAL HEALTH AND SAFETY EFFECTS

The notified substance is of very low acute oral and low dermal toxicity in rats (LD_{50} > 2 000 mg/kg for both), is an eye irritant with persistent colouration effects but not a skin irritant in rabbits, and is a moderate sensitiser in a guinea-pig maximisation test. In the 28-day repeat dose study in rats, no NOEL was established due to discoloured urine in all treated animals and other kidney effects at mid and high doses. The notified substance was not mutagenic in *S. typhimurium* or *E. coli* with or without metabolic activation and had no clastogenic effects in Chinese hamster V79 cells *in vitro* with or without metabolic activation. The notified chemical is classified as a hazardous substance based on the its skin sensitising effects and persistent eye colouration effects according to the NOHSC *Approved Criteria for Classifying Hazardous Substances* (National Occupational Health and Safety Commission, 1994a).

The notified chemical will be imported as a component in a non-dusting solid product, Cibacron Red W-B 150%. The chemical has very low vapour pressure and the formulation presented has a high percentage (>90%) of particles above the respirable size range. Consequently, inhalation exposure is expected to be low and 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 for workers directly contaminated with the chemical before it has been added to the textiles. After dyeing, the chemical is fixed to the textile fibres and essentially unavailable for separately contaminating and being absorbed by the skin.

No injuries or diseases related to exposure to the chemical are known by the notifier. However, cases of skin and respiratory sensitisation have been observed with reactive dyes and care should be taken to avoid skin contact and inhalation.

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

Repacking
Given that the chemical is a skin sensitiser and eye irritant, it is important that workers repacking the content of 30 kg containers into 5 kg and 10 kg containers are adequately protected against topical exposure. Repacking workers are estimated to work infrequently (4-10 hours annually) on this task. The exposure controls itemised of ventilation and extensive personal protective clothing, including additional respiratory protection, should be adequate to protect them during this task.

End use
The weighing operators have potentially the highest exposure to the notified chemical. Given that the chemical is a skin sensitiser and may cause eye damage, it is important that topical exposure does not occur. The procedures described, namely local exhaust ventilation and the use of respirator, overalls and gloves to minimise the exposure, are necessary to maintain a low risk of adverse health effects.

Wash-off and drier operators will have low exposure to the unfixed notified chemical as the padding and fixation processes are enclosed and the dye is strongly fixed to the cloth. In addition, gloves are worn. Therefore, the 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.

The Material Safety Data Sheet recommends that sensitised workers should cease working with sensitising dyes such as this one.

Public health
There is negligible potential for public exposure to the notified chemical arising from its use as a colourant in textile dyes. There will be public contact with the notified chemical when incorporated into fabrics, but since the dyestuff is strongly fixed to the fibre, no significant transfer to the skin should result on exposure and the pattern of exposure would be intermittent. Based on the anticipated low toxic hazard and use pattern of the notified chemical, it is considered that the notified chemical will not pose a significant hazard to public health.

13. 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.

14. RECOMMENDATIONS

To minimise occupational exposure to the notified chemical in Cibacron Red W-B 150% 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.
- Workers who 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