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September 2001

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME

FULL PUBLIC REPORT

FAT 40557/A

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Director
Chemicals Notification and Assessment

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1. APPLICANT

Ciba Specialty Chemicals of 235 Settlement Rd., Thomastown, VIC 3074 (ABN 97 005 061 469) has submitted a standard notification statement in support of their application for an assessment certificate for FAT 40557/A.

2. IDENTITY OF THE CHEMICAL

The chemical name, CAS number, molecular and structural formulae, molecular weight, spectral data, details of purity and import volume have been exempted from publication in the Full Public Report and the Summary Report.

Other Names:	FAT 40557/A Reactive Orange 135
Marketing Names:	Reactive Orange RUE 80 Cibacron Orange RUE 80

3. PHYSICAL AND CHEMICAL PROPERTIES

Appearance at 20°C & 101.3 kPa:	red-brown powder
Melting Point:	> 400°C
Specific Gravity:	1.76 at 20°C
Vapour Pressure:	$< 1 \times 10^{-9}$ kPa at 25°C
Water Solubility:	246 g/L at 20°C
Surface Tension:	65 mN/m (1 g/L) at 20°C
Particle Size:	mass median diameter 100 μm

Size Range (μm)	Mass %
> 200	0.88
100 – 200	43.04
63 – 100	36.57
24.26 – 63	13.27
11.83 – 24.26	3.81
6.09 – 11.83	1.58
3.04 – 6.09	0.53
1.53 – 3.04	0.18
0.74 – 1.53	0.09
0.35 – 0.74	0.03
< 0.35	0.01

Partition Co-efficient (n-octanol/water): log P_{ow} = -3.22 at 20°C

Hydrolysis as a Function of pH: $T_{1/2}$ at pH 4.0 and 25°C = 71.2 hr
$T_{1/2}$ at pH 7.0 and 25°C > 1 year
$T_{1/2}$ at pH 9.0 and 25°C = 124 days

Adsorption/Desorption: Koc = 690-939
log Koc = 2.83-2.97 (see comments below)

Dissociation Constant: not determined

Flash Point: not applicable

Flammability Limits: not flammable

Autoignition Temperature: > 400°C

Explosive Properties: not explosive

Reactivity/Stability: non-oxidising; expected to be stable under normal environmental conditions

3.1 Comments on Physico-Chemical Properties

The melting temperature was investigated using the capillary method based on OECD TG 102. No melting point was observed up to 400ºC, the limit of the test (Ciba-Geigy, 1996a).

The relative density was determined using a gas comparison pycnometer according to OECD TG 109 (RCC Umweltchemie, 1997a).

Vapour pressure was calculated according to OECD TG 104 using the Modified Watson Correlation and found to be very low indicating low volatility (RCC Umweltchemie, 1996a).

Water solubility was measured at pH 7.5 using the flask method of OECD TG 105 with

spectrophotometric determination. The results indicate that the notified chemical is readily water soluble (Ciba-Geigy, 1996b).

The partition coefficient could not be determined by analytical methods as the notified chemical dissociates in water. Therefore the log Pow calculated from the individual solubilities of the chemical in water and n-octanol according to OECD TG 117 and was found to be very low (RCC Umweltchemie, 1996b).

Hydrolysis as a function of pH was determined according to OECD TG 111. At pH 4 and 9 the half-lives were determined via extrapolation of the test results and estimated using the Arrhenius equation. For pH 7 the chemical was found to be stable at 50ºC (Ciba-Geigy, 1997).

Adsorption/desorption was measured according to OECD TG 106 and the following results were obtained (RCC Umweltchemie, 1997c).

Soil	Soil Type	Organic Carbon (g/100 g dry soil)	K mL/g	Koc mL/g	Kom mL/g
Speyer	Loamy Sand	2.29	21.50	939	545
Sisseln	Sandy Loam	1.57	11.73	747	433
Les Barges	Silt Loam	3.80	26.21	690	400

The adsorption step was performed in duplicate at a concentration of 4.88 mg chemical/L. The amount of chemical adsorbed to the soil was 80.00 % for soil Speyer, 68.28 % for soil Sisseln and 82.48 % for soil Les Barges. After the adsorption two desorption steps were performed. The amount of chemical desorbed was 18.33 % from soil Speyer, 22.03 % from soil Sisseln and 13.13 % from soil Les Barges. The notified chemical is therefore not likely to be mobile in soils.

No data on dissociation constant was provided by the notifier but the chemical is a sodium salt of a strong acid and is expected to dissociate in the environmental pH range 4-9.

Surface tension was determined using the ring tensiometer method of OECD TG 115 on an aqueous solution (100 g/L) of the notified chemical (RCC Umweltchemie, 1997b). The results indicated that the chemical is not a surface active substance.

4. PURITY OF THE CHEMICAL

Degree of Purity: 100 % as reaction products mixture

Hazardous Impurities: none present at above the cutoffs for classification of the notified chemical as a hazardous substance

Additives/Adjuvants: none

The notified chemical is in a mixture of at least 23 reaction products (identified by HPLC) which are not individually isolated. The physico-chemical data and the toxicity testing refer to the mixture of reaction products, FAT 40557/A.

5. USE, VOLUME AND FORMULATION

The notified chemical will not be manufactured in Australia. It will be imported as a component at greater than 60 % of the formulated reactive dyestuff Cibacron Orange CR-N, for application to cellulosic textiles by the exhaust dyeing, continuous dyeing and pad-batch methods. The dyes will be used in dyehouses only.

The formulated dyestuffs will be imported in 30 kg cardboard containers with polyethylene lining. Most imported dye will be sold as received, although up to 100 kg per year may be repacked into smaller containers as samples or for use in mill trials. Repackaging will take place at the importer's facility. The dye will only be available to industrial users.

The estimated import volumes for the notified chemical over the next five years are in the range of up to 10 tonnes per year.

6. OCCUPATIONAL EXPOSURE

Routes of Exposure
The notified chemical will be imported in commercial form as a powdered solid which will be dissolved in warm water to produce the dye solutions. Workers may be exposed to the dust, although the notifier states that an antidusting additive is present, and that no significant workplace air contamination will occur. The most probable route of exposure to the aqueous solution will be dermal.

Transport and storage
No details of the numbers of workers exposed to the notified chemical during these activities were given. These workers could be exposed to the notified chemical in the case of an accident where the packaging was breached.

Repackaging
The notifier estimates that up to 100 kg of the notified chemical will be repackaged into smaller containers at the warehouse. Two workers will be exposed to the imported powder for approximately 15 to 20 minutes per day, ten days per year. The worker exposure during this process will be to the powder. The repackaging of dye is conducted in a down-flow weighing booth.

The notifier indicates that respiratory protection would normally be used during this process. The Material Safety Data Sheet (MSDS) indicates that a dust mask should be used in conjunction with local exhaust ventilation, and a half face mask in the absence of local exhaust ventilation. The MSDS also indicates that eye protection must be worn.

End Use
The following procedures are carried out at a small number of customer facilities. The

notifier estimates that between 240 and 300 workers throughout Australia will be exposed to the notified chemical during these activities. Of these workers, approximately 50 (weighing and mixing operators and laboratory staff) may have contact with the powdered dyestuff, while the remainder will control the dying process in open or enclosed vessels, or handle wet fabric from the dye bath.

Weighing and mixing
At the customer facilities, the powdered dye will be weighed, normally in intact 30 kg containers, but sometimes smaller amounts will be weighed. The dye is then mixed with approximately 500 L of water in an enclosed vat to prepare the dye solution. The weighing of dye and addition to the blending vat will be carried out under local exhaust ventilation. The dye containing the notified chemical will be used on approximately 50 days per year.

The MSDS indicates that workers involved in handling the powdered solid should wear protective gloves and glasses, and respiratory protection.

Dyeing
The dye solution will be transferred through an enclosed system to a tank then dispensed into an enclosed dyeing machine. There is the possibility of worker exposure if the dyeing machine has to be opened in the case of malfunction. The cloth is then washed to remove unfixed dye, and manually led to a dryer. The concentrations of free dye at this time are expected to be very low as the dye is fixed to the cloth and the excess is washed out during the dyeing process. It is estimated that between 190 and 250 workers throughout Australia may be exposed during these activities.

The notifier indicated that personal protective equipment including eye protection, gloves and overalls will be used where manual intervention or equipment maintenance occurs.

Laboratory
The notifier estimates that two laboratory technicians in each customer facility will be involved in quality control checks on the dye solution. Laboratory technicians will only be exposed to the dye in solution form during these activities. Standard laboratory protective equipment is expected to be used by these workers.

Exposure Estimates
The notifier provided an estimate of exposure of workers to notified chemical dust during weighing in the absence of exhaust ventilation and respiratory protection. The calculation assumed 2 weighings per day, each of 2.5 kg, and a duration of 15 minutes per weighing. All of the dust was assumed to be inspirable. The calculation indicated a lifetime inhalation exposure of 0.00064 mg/kgbw/day.

7. PUBLIC EXPOSURE

The product containing the notified chemical will only be available to industrial end users. Once the cloth is dyed it will be washed to remove unfixed dye. Products which may be dyed include domestic textiles used for apparel, sheeting and other uses. There is no evidence of bleeding of the dye from dyed cloth. Therefore public exposure to the dyed product is significant whereas exposure to the chemical is not likely to be significant. No public exposure to the notified chemical is expected during repackaging or disposal of industrial

waste water.

There is a chance of public exposure due to rupture of containers in an accident. The MSDS for the product advises that any spill should be contained and collected for later disposal in accordance with regulations. Public exposure through importation and transportation is therefore negligible.

8. ENVIRONMENTAL EXPOSURE

8.1 Release

The bulk of the dye will become chemically fixed to the cellulosic textiles, and in this state is not expected to impact on the environment. After application to fabrics, the dye undergoes a chemical reaction involving chemical bonding with hydroxy groups on the cellulose fibres.

The rinsate generated via fabric rinsing will contain up to 13 % of the applied dye. This will be combined with other waste water from the dyehouse (including equipment cleaning and spill clean-up) and will be disposed of to sewer as industrial waste water, sometimes after treatment in the dyehouse effluent system. This will represent the major route of environmental exposure (up to 1300 kg per annum of the notified chemical).

Any solid waste generated at the dyehouse is likely to be disposed of to a secure landfill with incineration being an alternative.

The notifier indicates that nearly all of the dye will be removed from the container liners by shaking. However, it has previously been estimated that up to 0.5 % of the containers contents will remain as residue. This equates to < 50 kg of waste notified chemical annually. This is likely to be disposed to landfill along with the container liners.

8.2 Fate

Since approximately 13 % of the imported notified chemical will end up in the waste water from the dyehouses the maximum amount of notified chemical reaching the sewer annually would be < 1300 kg. This is expected to be the major route of environmental exposure.

Hobbs (1988) reports that reactive dyes have been found not to adsorb to sludge in model systems. Any dye that binds to the sludge during the waste treatment process would be disposed of through incineration or landfill. Incineration is the preferred option because of the high water solubility and potential mobility of the material. Incineration of the dye will produce oxides of carbon, nitrogen and sulfur, together with sodium salts in the ash.

Dye released into landfill as residues would not be expected to be mobile and the notified chemical would be expected to degrade very slowly via biotic and abiotic processes. Disposal by landfill will be at a secured site and the risk of leaching to the water table is therefore significantly reduced.

Once the dye has fixed to the cloth it is not expected to be mobile. Therefore the fate of the dye bound to the cloth would be the same as the cloth. Ultimately this may mean that it would be disposed of to landfill where it would remain inert.

Ready Biodegradability of the notified chemical was tested according to OECD TG 301 F (RCC Umweltchemie, 1996c). Bacterial sludge from a domestic sewage treatment plant was used as the inoculum. Aniline (99.5 % pure) was used as the reference substance at a concentration of 100 mg/L. The reference had a degradation of 77.4 % within 14 days and an average biodegradation rate of 82.4 % after 28 days. After 28 days only 0.8 % of the theoretical oxygen consumption occurred in a test solution of the chemical at 152 mg/L, therefore the notified chemical cannot be considered as readily biodegradable.

Although the dye is not readily biodegradable, the potential for bioaccumulation is low due to the low partition coefficient and very high water solubility of the substance. Hydrophilic dyes with log P_{ow} < 3 have been shown not to bioaccumulate (Yen, 1991).

9. EVALUATION OF TOXICOLOGICAL DATA

9.1 Acute Toxicity

Summary of the acute toxicity of FAT 40557/A

Test	*Species*	*Outcome*	*Reference*
acute oral toxicity	rat	LD_{50} > 2000 mg/kg	(RCC, 1996b)
acute dermal toxicity	rat	LD_{50} > 2000 mg/kg	(RCC, 1996a)
skin irritation	rabbit	non-irritating	(RCC, 1996e)
eye irritation	rabbit	slight irritant; stains conjunctiva	(RCC, 1996d)
skin sensitisation	guinea pig	non-sensitiser	(RCC, 1996c)

9.1.1 Oral Toxicity (RCC, 1996b)

Species/strain:	rat/HanIbm:WIST (SPF)
Number/sex of animals:	5/sex
Observation period:	14 days
Method of administration:	gavage; 0.2 g/mL in polyethylene glycol; dose 2000 mg/kg
Test method:	OECD TG 401
Mortality:	no premature deaths occurred during the study
Clinical observations:	diarrhoea was observed in one male 5 hours after dosing; no other clinical signs of toxicity were observed

Morphological findings:	no macroscopic abnormalities were observed at necropsy
LD_{50}:	> 2000 mg/kg
Result:	the notified chemical was of very low acute oral toxicity in rats

9.1.2 Dermal Toxicity (RCC, 1996a)

Species/strain:	rat/HanIbm:WIST (SPF)
Number/sex of animals:	5/sex
Observation period:	14 days
Method of administration:	semi-occlusive patch; 0.5 g/mL in polyethylene glycol; dose 2000 mg/kg; 24 hr exposure
Test method:	OECD TG 402
Mortality:	no premature deaths occurred during the study
Clinical observations:	no clinical signs of systemic toxicity were observed
Dermal observations:	the application area showed orange discolouration in all animals, persisting for between 5 and 13 days; one male showed scales between days 6 and 10
Morphological findings:	no macroscopic abnormalities were observed at necropsy
LD_{50}:	> 2000 mg/kg
Result:	the notified chemical was of low dermal toxicity in rats

9.1.3 Inhalation Toxicity

The notifier did not submit test reports on the inhalation toxicity of the notified chemical.

9.1.4 Skin Irritation (RCC, 1996e)

Species/strain:	rabbit/New Zealand white
Number/sex of animals:	1 male, 2 female
Observation period:	3 days
Method of administration:	semi-occlusive patch; 0.5 g notified chemical moistened with water; 4 hr exposure

Test method: OECD TG 404

Dermal observations: all Draize scores at 1, 24, 48 and 72 hours for erythema and oedema were zero; orange staining of the skin at the treatment sites persisted through the observation period

Result: the notified chemical was non-irritating to the skin of rabbits

9.1.5 Eye Irritation (RCC, 1996d)

Species/strain: rabbit/New Zealand white

Number/sex of animals: 1 male, 2 female

Observation period: 21 days

Method of administration: 0.1 g powdered notified chemical was placed in the conjunctival sac of the left eye of each animal; the right eye served as control

Test method: OECD TG 405

Draize scores of unirrigated eyes:

	Time after instillation									
Animal	***1 hour***		***1 day***		***2 days***		***3 days***		***7 days***	
Cornea	All Draize scores were zero									
Iris	All Draize scores were zero									
Conjunctiva	***r***	***c***	***r***	***c***	***r***	***c***	***r***	***c***	***r***	***c***
1♂	2[1]	1	1	1	1	0	1	0	0	0
2♀	2	1	1	1	1	0	1	0	0	0
3♀	2	1	1	1	1	0	1	0	0	0

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

Comment: discharge was observed at 1 and 24 hours

orange staining of the conjunctiva persisted in all animals to the end of the observation time (21 days); staining of the sclera and lid hairs resolved by day 14

Result: the notified chemical was slightly irritating to the eyes of rabbits; it caused persistent staining of the conjunctiva

9.1.6 Skin Sensitisation (RCC, 1996c)

Species/strain: guinea pig/Ibm: GOHI SPF

Number/sex of animals: 20 females test group, 10 females control group

Induction procedure:

test group:

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

- 1:1 (v/v) mixture of Freund's Complete Adjuvant and physiological saline
- the test material diluted to 5 % in polyethylene glycol 400
- the test material diluted to 5 % by emulsion with 1:1 (v/v) mixture of Freund's Complete Adjuvant and physiological saline

day 7 — dermal irritation at the injection area was induced by application of 10 % sodium lauryl sulphate in paraffinum perliquidum

day 8 — a filter paper patch with 0.3 mL of test material 50 % (w/v) in polyethylene glycol 400 was placed over the injection area; covered with aluminium foil and secured with elastic plaster and impervious adhesive tape

control group: the induction procedure was identical to that for the test group, except that polyethylene glycol 400 was used in place of the solution of test material in both induction phases

Challenge procedure:

day 22 — a 24 hour occluded application of a 50 % solution of the test material in polyethylene glycol 400 was applied to the shaved flank of both test and control animals

Test method: OECD TG 406

Challenge outcome:

	Test animals		***Control animals***	
Challenge concentration	***24 hours****	***48 hours****	***24 hours***	***48 hours***
50%	0/19**	0/19	0/10	0/10

* time after patch removal

** number of animals exhibiting positive response

Comment: one animal in the test group died of non-treatment related causes on the day following challenge

	depilation by use of a cream was performed 3 hours prior to the first challenge observation to remove orange staining
Result:	the notified chemical was non-sensitising to the skin of guinea pigs

9.2 Repeated Dose Toxicity (RCC, 1997)

Species/strain:	rat/HanIbm:WIST (SPF)
Number/sex of animals:	10/sex (control and 1000 mg/kg/day) 5/sex (50 and 200 mg/kg/day)
Method of administration:	gavage; vehicle water
Dose/Study duration:	Group 1: vehicle only Group 2: 50 mg/kg/day Group 3: 200 mg/kg/day Group 4: 1000 mg/kg/day treatment daily for 28 days with an additional 14 day recovery period (no treatment) for Groups 1 and 4
Test method:	OECD TG 407

Mortality:
All animals survived the scheduled treatment period.

Clinical observations:
No clinical signs of toxicity were observed.

Body weights/Food consumption:
No effect of treatment on body weight was observed. Females at 1000 mg/kg/day showed slightly higher food consumption throughout, commencing prior to treatment.

Clinical chemistry/Haematology
With few exceptions, statistically significant changes in haematology, clinical biochemistry and urinalysis parameters were only observed for animals in the 1000 mg/kg/day dose group. Males at 50 mg/kg/day showed a slight increase in potassium levels, and a decrease in uric acid was observed for females at 50 mg/kg/day. Deep yellow urine discolouration was observed in 3 males and 3 females at 200 mg/kg/day.

For the 1000 mg/kg/day group, at the end of the treatment time, both sexes showed slightly shorter thromboplastin times. The males showed slightly lower mean corpuscular volume and mean corpuscular haemoglobin and slightly increased platelet and reticulocyte count. The females showed slightly increased total leukocyte count and a slight increase in lymphocytes. After the recovery period, the only statistically significant haematological change was slightly shorter thromboplastin time in the females.

Clinical biochemistry changes in the 1000 mg/kg/day group at the end of the treatment time included orange plasma discolouration, increased total bilirubin, slightly increased total cholesterol, triglyceride and phospholipid levels, and slightly decreased glucose levels in both sexes. In the males, slightly increased uric acid, total protein and globulin levels and decreased albumin to globulin ratio were observed. In the females, a slightly decreased potassium level was observed. After the recovery period, the only statistically significant clinical biochemistry changes were slightly decreased glucose and bilirubin in the males and increased triglyceride level in the females.

Urine discolouration (light orange to orange) was seen in all 1000 mg/kg/day animals at the end of the treatment time. Increased scores for blood in urine were seen for both sexes. These differences persisted through the recovery period.

Gross pathology:
Reddish discolouration of various organs including tongue, gastrointestinal tract, kidneys, urinary bladder, testes, skin, mammary gland and lymph nodes was observed, particularly in 1000 mg/kg/day animals including following the recovery period.

Absolute kidney weights were increased in both sexes at 1000 mg/kg/day (males +8 %, females +17 %) and females at 200 mg/kg/day (+11 %). Following the recovery period, the absolute kidney weights for 1000 mg/kg/day animals were still increased by 6 %, although the change was not statistically significant.

Histopathology:
Major histopathological changes were observed in the stomach and kidneys of the 1000 mg/kg/day group at the end of the treatment period. These organs were also examined in detail for the 50 mg/kg/day and 200 mg/kg/day groups. Microscopic examination was performed for these groups for few or none of the other organs where small or no changes were observed on comparing the 1000 mg/kg/day group with the controls.

In the stomach, an increase in incidence and severity of hyaline droplets in the glandular mucosa was observed for all treated groups; vacuolation and inflammatory cells in the submucosa of the glandular stomach were observed at 200 and 1000 mg/kg/day, with an increase in incidence and severity with increased dose. An increase in severity of hyaline droplets in the tubular cells of the kidney was observed for the 1000 mg/kg/day group; this was accompanied by multifocal tubular degeneration. Brownish exogenous pigment was also observed in the kidneys of the 1000 mg/kg/day females. Exogenous pigment was also observed in intestinal segments and several lymph nodes and in the alveolar macrophages of the lung in one animal, primarily at 1000 mg/kg/day.

Following the recovery period, the 1000 mg/kg/day animals showed hyaline droplets in the glandular mucosa of the stomach, and similar kidney findings to those following the treatment period were made. The degree of multifocal tubular degeneration was increased relative to that observed at the end of the treatment period. Also the incidence and severity of exogenous pigment were increased compared with the end of the main test; pigment was recorded in the alveolar macrophages of the lung in one animal. One female also showed a basophilic focus in the liver, and multifocal hepatocellular vacuolisation was present at increased incidence and severity relative to controls.

Comment:

The increase in hyaline droplets in the glandular mucosa was observed at all treatment levels, and a No Observed Effect Level (NOEL) could not be established. This observation may be considered an adaptive change for dealing with exogenous material. Other changes observed at 200 mg/kg/day (vacuolation and inflammatory cells in the stomach) were of minor degree. The changes observed in the kidneys at 1000 mg/kg/day were not reversible after the recovery period and corresponded to the observation of blood in the urine.

Result:
A No Observed Adverse Effect Level (NOAEL) of 200 mg/kg/day was established in the study.

9.3 Genotoxicity

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

Strains:	*Salmonella typhimurium*: TA98, TA100, TA1535, TA1537 *Escherichia coli*: WP2 *uvrA*
Concentration range:	33.3, 100, 333.3, 1000, 2500 and 5000 μg/plate
Metabolic Activation System:	rat liver S9 fraction from animals pretreated with phenobarbital and β-napthoflavone; 15 % in standard cofactors
Test method:	OECD TG 471 and TG 472
Comment:	two independent tests were performed, using both the plate incorporation and pre-incubation methods no toxic effects, either in the presence or absence of metabolic activation, occurred at the dose levels used no substantial increase in the number of revertants was seen at any of the doses used, either in the absence or presence of metabolic activation appropriate positive controls were used and produced clear positive results, indicating that the test system responded appropriately
Result:	the notified chemical was not mutagenic in the bacterial strains tested in the absence or presence of metabolic activation provided by rat liver S9 fraction

9.3.2 Chromosomal Aberration Assay in Chinese Hamster V79 Cells (CCR, 1997)

Cells:	Chinese Hamster V79

Metabolic Activation System: rat liver S9 fraction from animals pretreated with phenobarbital and β-napthoflavone

Test method: OECD TG 473

Dosing schedule:

Metabolic Activation	*Experiment Number*	*Test concentration (μg/mL)*	*Controls*
-S9	1	treatment time = 18 hours 0-500 μg/mL; 50, 300 and 500 μg/mL evaluated treatment time = 28 hours 0-500 μg/mL; 500 μg/mL evaluated	Positive: EMS 600 μg/mL Negative: water
	2	treatment time = 18 hours 30-750 μg/mL; 50, 300 and 500 μg/mL evaluated treatment time = 28 hours 30-750 μg/mL; 500 μg/mL evaluated	
+S9	1	treatment time = 4 hours; total 18 hours 5-300 μg/mL; 10, 30 and 100 μg/mL evaluated treatment time = 4 hours; total 28 hours 5-300 μg/mL; 100 μg/mL evaluated	Positive: CP 0.71 μg/mL Negative: water
	2	treatment time = 4 hours; total 18 hours 2-200 μg/mL; 50, 100 and 200 μg/mL evaluated treatment time = 4 hours; total 28 hours 2-200 μg/mL; 100 and 200 μg/mL evaluated	

EMS - ethyl methanesulphonate
CP - cyclophosphamide

Observations: precipitation was observed at 200 μg/mL in the presence of S9; cytotoxic effects were observed in a pretest after treatment with 1000 μg/mL and above (in the absence of S9) and 300 μg/mL and above (in the presence of S9)

no substantial reductions in mitotic index were observed in experiment 1 except in the presence of S9 at 28 hours after treatment with 100 μg/mL (mitotic index 63 % of control); at 300 μg/mL the mitotic index was strongly reduced; in experiment 2 reduction in mitotic index was observed at the highest evaluated concentration in all cases; cytotoxicity as indicated by a reduction in cell numbers occurred at the highest evaluated concentration except in Experiment 1 in

	the presence of S9 at 28 hours after treatment with 100 μg/mL no reproducible increases in the frequency of structural chromosome aberrations or polyploid metaphases were observed in the presence or absence of metabolic activation; in experiment 1 in the 18 hour harvest in the absence of S9, statistically significant increases in the frequency of structural chromosome aberrations for 300 μg/mL (3 % aberrant cells) were attributed to the low solvent control values (0.0 % aberrant cells), as the values are within the historical control range of 0 – 4 %; accordingly these observations were not considered biologically significant a statistically significant increase in aberration frequency (19.5 % exclusive gaps) and cells carrying exchanges (8.5 %) was seen in experiment 2 at 28 hours in the presence of metabolic activation statistically significant increases in cells showing structural chromosome aberrations occurred for the positive control substances, indicating that the test system responded appropriately
Comment:	precipitation and high cytotoxicity (cell number 18.2 % of solvent control) were seen under the conditions where an increase in aberration frequency was observed; no similar results were obtained for 18 hr exposure at 200 μg/mL or 28 hour exposure at 100 μg/mL
Results:	the notified chemical gave equivocal evidence for induction of structural chromosome aberrations in the presence of metabolic activation

9.4 Overall Assessment of Toxicological Data

The notified chemical was of very low acute oral toxicity (LD_{50} > 2000 mg/kg) and low dermal toxicity (LD_{50} > 2000 mg/kg) in rats. It was non-irritating to rabbit skin, and a slight irritant to rabbit eyes. The notified chemical also produced persistent staining of skin and the conjunctiva, while staining of eyelid hairs and the sclera resolved between 7 and 14 days. It was not sensitising to the skin of guinea pigs.

In a 28 day repeat dose oral study, organ discolouration and stomach effects were seen at all treatment doses with increasing incidence and severity. Kidney effects were seen at the highest dose of 1000 mg/kg/day. These were not fully reversible after a 14 day recovery period. In addition, a number of changes in haematological and clinical biochemistry parameters were seen at this dose, along with increased blood urine. As the changes in the stomach were of minor degree and may be considered an adaptive change, a NOAEL of 200

mg/kg/day was established in the study. The results of the study also showed that the notified chemical may be absorbed through the gastrointestinal tract, as discolouration and exogenous pigment were observed in other internal organs.

In a bacterial point mutation study, no evidence of mutagenicity was observed. In an in vitro chromosome aberration study, some evidence of clastogenicity was found in the presence of metabolic activation, although the result was not fully reproducible and was found at a dose where very high cytotoxicity along with precipitation was evident.

Based on the above results, the notified chemical is not classified as a hazardous substance in accordance with the NOHSC *Approved Criteria for Classifying Hazardous Substances* (Approved Criteria) (NOHSC, 1999a). While irreversible staining (persisting beyond 21 days) was observed in the eye irritation test, and a classification of R41: 'Risk of serious damage to eyes' may be consistent with the Approved Criteria, the irreversible staining was confined to the conjunctiva and therefore would not be considered to comprise serious damage.

10. ASSESSMENT OF ENVIRONMENTAL EFFECTS

Test	*Species*	*Results*
Acute Toxicity	Zebra fish *Brachydanio rerio*	LC_{50} (96 h) >100 mg/L NOEC (96 h) = 100 mg/L
Acute Toxicity	*Daphnia magna*	EC_{50} (48 h) >100 mg/L
Growth Inhibition	Algae *Scenedesmus subspicatus*	E_bC_{50} >100 mg/L E_rC_{50} >100 mg/L NOEC = 100 mg/L
Inhibition	Aerobic Waste Water Bacteria	EC_{50} (30 min) >100 mg/L

* NOEC - no observable effect concentration

Fish

Fish toxicity testing was carried out according to OECD TG 203 (Ciba, 1996a). The study was conducted at 0 and 100 mg/L nominal concentration. Every 24 hours the temperature, oxygen and pH in the test tanks were measured. The small variations observed in these parameters were acceptable. No mortalities were observed during the 96 hour experiment and the fish continued to swim normally throughout the study. Since a LC_{50} end point was not observed in the study, the nominal LC_{50} is greater than 100 mg/L, while the NOEC was estimated to be 100 mg/L, the mean nominal concentration. The measured concentrations of the three replicate test solutions were 97.2, 100.5 and 104.2 mg/L.

Daphnia

The acute toxicity study of daphnia with the notified chemical was based on OECD TG 202 (Ciba, 1996b). The concentrations used were 0 and 100 mg/L with the temperature, oxygen

and pH in the test tanks being measured at the start and the end of the test. These parameters were in the range required by the guidelines for the validity of the test. The mobility of the daphnia was not observed to be affected during the study. The notifier has estimated the nominal EC_{50} to be greater than 100 mg/L, while the NOEC was determined to be 100 mg/L, the mean nominal concentration. The measured concentrations of the two replicate test solutions were 94.6 and 95.9 mg/L.

Algae

The fresh water algal (*Scenedesmus subspicatus*) growth inhibition test with the notified chemical was based on OECD TG 201 (RCC Umweltchemie, 1997d). The concentrations tested were 0, 1.0, 3.2, 10, 32 and 100 with an initial cell density of 10000 cells/mL. HPLC was used to determine the actual measured concentrations of the chemical in the test solutions. The mean measured concentrations were found to be 91 to 96 % of the nominal concentrations and there was no inhibition of growth. The EC_{50} for the reference compound, 3,5-dichlorophenol, was 10.4 mg/L.

Bacteria

The influence of the notified chemical on the respiration rate of activated sludge was investigated according to OECD TG 209 (RCC Umweltchemie, 1997e). The concentrations studied were 3.2, 10, 32, 50 and 100 mg/L with an incubation period of 30 minutes. A concentration of 200 mg/L activated sludge was used. The temperature, pH and oxygen concentration were measured and a reference substance (3,5-dichlorophenol) was used. At the highest concentration of the test material the inhibition observed was 1.7 %, and the EC_{50} is estimated to be greater than 100 mg/L.

Conclusion

The ecotoxicity data indicate that based on the conditions of individual tests, the notified chemical is practically non-toxic to fish, daphnia, algae and waste water bacteria.

11. ASSESSMENT OF ENVIRONMENTAL HAZARD

The majority of the notified chemical will ultimately be released to landfill or incinerated once the textile to which the dyestuff has been applied is discarded. While the notified chemical is water soluble, it is bound to the textile so little release is expected to occur. Except in the case of accidental release during transport, the primary source of release of the notified chemical will be via the waste water discharged from dyehouses.

Up to 1300 kg of the notified chemical is expected to enter the aquatic compartment per year as a result of the use of the dye. Ecotoxicity data indicate that the notified chemical is practically non-toxic to fish, daphnia, algae and waste water bacteria.

The notifier has indicated that approximately 2 ML of washwater is generated daily, therefore an estimated predicted environmental concentration would be:

Estimated amount of notified chemical used per day	5 kg

Fixation Rate	87.4%
Amount of unfixed notified chemical	0.63 kg
Volume of wash water per day	1 ML
Concentration of notified chemical in wash water	0.63 mg/L
Dilution factor in STP	1:50
Concentration of notified chemical in STP	12.6 µg/L
Dilution factor in receiving water	1:3
Concentration of notified chemical in receiving water	4.2 µg/L

This PEC calculation assumes that none of the notified chemical is removed during treatment of the different waste effluents and represent the worst case scenario for a country dyehouse where the receiving waters from the STP may be limited at times.

The calculation shows that the exposure to fish, daphnia, algae and waste water treatment bacteria is at levels unlikely to cause any significant effect. The safety factor of aquatic organisms for exposure to the notified chemical is approximately 23000. 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 slow biotic and abiotic degradation and 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.

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

When used as indicated in the notification, the notified chemical is unlikely to present a hazard to the environment.

12. ASSESSMENT OF PUBLIC AND OCCUPATIONAL HEALTH AND SAFETY EFFECTS

Hazard Assessment
The acute toxicity of FAT 40557/A is low, and it is not an irritant to the skin of rabbits. It is a slight irritant to the eyes of rabbits. The notified chemical does cause persistent staining of eye tissues, although the staining is not of sufficient severity to warrant health effects classification. The safety phrases S25: 'Avoid contact with eyes', S26: 'In case of contact with eyes, rinse immediately with plenty of water and contact a doctor or Poisons Information Centre' and S39: 'Wear eye/face protection' should be applied to the notified chemical. The notified chemical was negative in an adjuvant type skin sensitisation study in guinea pigs, however, as a general rule, worker exposure to reactive dyes of this type should be strictly controlled because of their potential skin and respiratory sensitisation effects.

For longer-term systemic effects, the NOAEL is 200 mg/kg/day, based on kidney effects and haematological, clinical biochemistry and urine changes observed at 1000 mg/kg/day in a 28 day oral rat study. As discolouration of organs and adaptive stomach changes were observed at all doses no NOEL can be established.

The powdered solid includes approximately 2 % of powder within the respirable range and the majority of the remainder is within the inspirable range and may be deposited in the respiratory tract. The notifier indicates that the imported product contains an anti-dusting additive but no indication of the efficacy of the additive has been provided. The low acute dermal toxicity and high molecular weight of the main component of FAT 40557/A would suggest that significant absorption via the skin is unlikely.

Occupational Health and Safety
Occupational exposure to the notified chemical can be divided into exposure to the powdered solid, the dye solution, and to the dyed cloth. The amount of free dye in the washed, dyed cloth is expected to be small. The dust will be a potential hazard by inhalation and by dermal and ocular exposure. Contact with the solution will be more easily avoided, but dermal and ocular exposure to drips and splashes will be possible. After fixation to the textile, the potential hazard should be negligible. In all cases, contact of solid or dissolved dye with the eyes should be avoided.

The notifier provided an estimate of exposure of workers to notified chemical dust during weighing under worst case conditions and in the absence of exhaust ventilation and respiratory protection. The calculation indicated a lifetime inhalation exposure of 0.00064 mg/kgbw/day. No inhalation toxicity studies on the notified chemical have been submitted by the notifier and comparison to the NOAEL (200 mg/kgbw/day) derived from oral exposure in rats may be misleading.

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

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

End Use
The workers involved in weighing and mixing the dye will be exposed to the powdered solid, and also to the dye solution. The notifier indicates that existing dyehouse procedures require the wearing of overalls, protective gloves, glasses and respiratory protection while weighing and mixing the dye. Mechanical ventilation of the weighing area is also provided. Also, an antidusting additive is one component of the commercial dye formulations. The personal protective equipment is required for protection against a variety of dyes, and should provide dermal, ocular and respiratory protection. The MSDS indicates that a dust mask should be used in conjunction with local exhaust ventilation, and a half face mask in the absence of local exhaust ventilation.

The dyeing machine operators will be not exposed to the dye solution under normal circumstances, as the dye solution will be transferred within an enclosed system. There is a possibility of dermal or ocular exposure if the dyeing system has to be opened in the case of a malfunction. The exposure time for the operators is expected to be short. Gloves, overalls and

safety glasses will be worn by these workers while handling the dye. Therefore the exposure and subsequent health risk for these workers will be low.

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

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

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

Public Health
The notified chemical is a component in a dye product used only by industrial users. Public exposure is therefore limited to dermal contact with dyed material. In such material the dye is fixed to the cloth and is generally not biologically available. The notified chemical has a low acute dermal toxicity and is not an irritant nor a skin sensitiser. The notified chemical gave equivocal evidence of clastogenicity, however, this was at high concentrations in which the public is unlikely to encounter. Therefore the notified chemical is not likely to pose a threat to public health.

13. RECOMMENDATIONS

Regulatory controls

- The NOHSC Chemicals Standards Sub-committee should consider the following health hazard classification for the notified chemical and the commercial dye containing the notified chemical:
  - S25: Avoid contact with eyes
  - S26: In case of contact with eyes, rinse immediately with plenty of water and contact a doctor or Poisons Information Centre
  - S39: Wear eye/face protection

Control Measures

Occupational Health and Safety

- Employers should implement the following engineering controls to minimise occupational exposure to the notified chemical in the commercial reactive dyestuff:
  - a downdraft weighing booth or efficient local exhaust ventilation should be used during operations involving handling the powdered dyestuff

- Employers should ensure that the following personal protective equipment is used by workers to minimise occupational exposure to the notified chemical in the commercial reactive dyestuff:
  - face shield or safety goggles
  - respiratory protection while handling the notified chemical in powder form

– protective gloves
– industrial clothing

Guidance in selection of personal protective equipment can be obtained from Australian, Australian/New Zealand or other approved standards.

- A copy of the MSDS should be easily accessible to employees.

- If products and mixtures containing the notified chemical are classified as hazardous to health in accordance with the NOHSC *Approved Criteria for Classifying Hazardous Substances*, workplace practices and control procedures consistent with provisions of State and Territory hazardous substances legislation must be in operation.

13.1 Secondary notification

The Director of Chemicals Notification and Assessment must be notified in writing within 28 days by the notifier, other importer or manufacturer:

(1) Under Section 64(2) of the Act:
- if any of the circumstances listed in the subsection arise.

The Director will then decide whether secondary notification is required.

No additional secondary notification conditions are stipulated.

14. MATERIAL SAFETY DATA SHEET

The MSDS for the formulated reactive dyestuff containing the notified chemical (Cibacron Orange CR-N) was provided in a format consistent with the *National Code of Practice for the Preparation of Material Safety Data Sheets* (NOHSC, 1994).

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.

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Attachment 1

The Draize Scale (Draize, 1959) 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 (Draize *et al*., 1944) 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

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