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NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME

FULL PUBLIC REPORT

Notified Chemical in Yellow TZ 4210

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Director
Chemicals Notification and Assessment

FULL PUBLIC REPORT

Notified Chemical in Yellow TZ 4210

2. APPLICANT

Ciba Specialty Chemicals Pty Ltd 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 Yellow TZ 4210".

2. IDENTITY OF THE CHEMICAL

Claims were made and accepted for the identity of the notified chemical to be exempt from public publication in the Full Public Report. The data items were:

chemical name;
CAS number;
molecular and structural formulae;
molecular weight;
spectral data;
purity and impurities; and
import volume.

Trade Name:	Yellow TZ 4210, Cibacron Yellow W-R 200% (contains Yellow TZ 4210)
Other Names:	FAT 40549/A
Molecular Weight	>1 000 g/mol
Method of Detection and Determination:	appropriate methods include: physical testing; infrared (IR) spectroscopy; ultraviolet/visible (UV/Vis) spectroscopy; nuclear magnetic resonance (NMR) spectroscopy

3. PHYSICAL AND CHEMICAL PROPERTIES

Information for the physical and chemical properties was derived for the notified chemical, Yellow TZ 4210, which contains the notified chemical.

Appearance at 20°C and 101.3 kPa: red/brown odourless powder

Melting Point: > 400°C (capillary method)

Density: 1.76 x 10^3 kg/m^3 at 19.5°C (pycnometer)

Vapour Pressure: 2.2 x 10^{-30} kPa at 25°C (calculated)

Water Solubility: > 385 g/L at 21°C

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

Hydrolysis as a Function of pH:
$T_{1/2}$ = 312 days at 25°C, pH 4.0
$T_{1/2}$ > 1 year at 25°C, pH 7.0
$T_{1/2}$ = 81 days at 25°C, pH 9.0

Adsorption/Desorption: not provided (see comments below)

Dissociation Constant:

Reaction Centre	*Nature of the Reaction Centre*	*Acid Dissociation Constant*	
1	AR-SO_3H	pK_a (1)	-6.0
2	AR-SO_3H	pK_a (2)	-5.8
3	AR-SO_3H	pK_a (3)	-5.1
4	AR-SO_3H	pK_a (4)	-3.3
5	R-O-SO_3H	pK_a (5)	-0.5
6	R-$CONH_2$	pK_a (6)	6.9
7	AR-NCHO-R	pK_a (7)	16.8

Surface Tension: 51.9 mN/m at 1 g/L, 20°C (ring tensiometer)

Flash Point: not flammable

Flammability Limits: not flammable

Autoignition Temperature: not auto-flammable

Explosive Properties: not explosive

Reactivity/Stability: notified chemical is considered stable under conditions of intended use

Particle size distribution:

Range (µm)	Mass (%)
<0.36*	0.03
0.36 - 0.75*	0.12
0.75 - 1.54*	0.27
1.54 - 3.07*	0.52
3.07 - 6.14*	1.20
6.14 - 11.93*	3.79
11.93 - 24.46	8.92
24.46 - 63	30.22
63 - 100	35.46
100 - 200	18.96
>200	0.51

* respirable fraction (< 10 µm): 6%

Comments on Physico-Chemical Properties

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

The vapour pressure was determined from the calculated boiling point using the Modified Watson Correlation. The notified chemical is not volatile.

The water solubility was determined to be at least 385 g/L. Further tests could not be carried out because the highly viscous solution could not be exactly measured.

Adsorption/desorption data were not provided. High water solubility and a low partition coefficient would normally indicate low affinity for soil or sediment. The notifier has indicated the notified chemical is unlikely to bind/adsorb strongly to soil and to move with the ground water. However, it is expected that the chemical will bind to positively charged substances such as clay particles (Weber, 1991).

The notified chemical contains four sulfonic acid and one sulfate functionality that will be expected to completely dissociate under environmental conditions. The important pKs are the strongly acidic sulfite groups that will render the molecule fourfold negatively charged over the whole environmentally relevant pH region. The possible protonation of the weakly basic amino group will have negligible effect in the lowest pH region (below pH≈2).

The notified chemical is surface active at a concentration of 1 g/L. By definition, a chemical has surface activity when the surface tension is less than 60 mN/m (EEC, 1992).

4. PURITY OF THE CHEMICAL

Degree of Purity: exempt information

5. USE, VOLUME AND FORMULATION

Use
The notified chemical will not be manufactured in Australia but will be imported as a component of the end-use dye Cibacron Yellow H-W 200%. The dyestuff is a reactive dye used for the purpose of colouring cellulose textiles by the exhaust dyeing method. Cibacron Yellow W-R 200% is estimated by the notifier to comprise 10% of total dyestuff used at the dyehouse. The dye has a fixation performance of 75%.

The notified chemical is intended for use as a colourant in textile dyes for the exhaust dyeing method at a concentration of < 1%. 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.

Volume
Exact import volumes are exempt information. The notified chemical will be imported in 30 kg antistatic lined sealed cardboard containers. The notifier anticipates that the dyestuff will be sold directly to approximately fifteen dyehouses, in city and country locations. In addition, a small amount of Cibacron Yellow W-R 200% will be repacked into 5 and 10 kg containers for the purpose of supplying samples or material for mill trials. Repacking will be carried out by Ciba Specialty Chemicals at one warehouse.

Formulation
No formulation of the notified chemical is to take place in Australia.

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 non-dusting additive was not provided. Workers who will handle the notified chemical include transport 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 the imported containers will need to be repacked into smaller containers. If packs need to be broken, then repacking will occur in a 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 mechanical ventilation booth, and workers will wear safety glasses, protective long impervious neoprene or rubber gloves, overalls and industrial footwear. 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 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. This manual process was not described. Once mixed, the notified chemical is at less than 1%. During handling of the mixed dye solutions, the operators wear overalls, gloves and safety glasses.

An occupational exposure survey in US indicated that workers involved in weighing activities were exposed to a mean concentration of airborne commercial dye dust of approximately 0.18 mg/m^3 in 24 hours. The notified chemical comprises a maximum of 10% of the various dyes used at the dyehouses. It has a respirable fraction of 6% for the dust. Based on an inhalation rate of 10 m^3 per person per shift, a typical body weight of 70 kg, the maximum exposure of the notified chemical is estimated to be 0.15 µg/kg/day.

Dyeing
Dyeing and fixation are carried out in a closed system so there is little opportunity for occupational exposure during this process. 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 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

Cibacron Yellow H-W 200% will not be sold to the public. Public exposure to the notified chemical during storage, repacking, distribution and dyeing processes is expected to be negligible. In the case of accidents, spills will be collected and disposed of by secure landfill or incineration as indicated in the Material Safety Data Sheet (MSDS).

At a fixation rate of 75%, about 25% would be discharged in the dyehouse effluent. Waste water from dyehouses is expected to be treated in waste treatment plants before being released to receiving waters. Traces of the notified chemical remaining in empty packaging (estimated to be 5 g/container) will be disposed of to approved landfill. Public exposure from disposal should be minimal.

There will be extensive dermal contact with the dyed fabrics by the general public. However, the dye will be strongly bound to the fibre (unfixed dye is removed from the textile by a hot and soap solution before drying), and the dye is unlikely to be dermally absorbed. Public exposure is expected to be low.

8. ENVIRONMENTAL EXPOSURE

Release

The bulk of the dye will become chemically fixed to the cellulose textiles, and in this state is not expected to impact on the environment. The result of fastness performance tests shows that a high order of fastness rating is achieved in all cases. After application to fabrics, the

dye undergoes a chemical change involving chemical bonding with hydroxy groups on the cellulose fibres.

The major environmental exposure to dye will come from effluent discharge from dyehouses and waste water treatment systems. Other releases will be limited to traces remaining from repacking operations and clean-up of any spills, and from trace residues in empty packaging (estimated to be a maximum of 0.1%, based on previous similar notifications by the notifier).

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

Fate

The dye normally released in water as effluent from the dyehouse is expected to be the major environmental exposure. The dye may either partition to sediment or stay in the aqueous compartment. Hobbs (1988) reports that reactive dyes have been found not to 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 and a small amount of hydrogen chloride. Disposal by landfill will be at a secured site, so the risk of leaching to the water table is significantly reduced.

The biochemical oxygen demand (BOD) of the dye was tested and the five day study showed the BOD_5 was 2 mg O_2/g. The chemical oxygen demand (COD) was determined to be 759 mg O_2/g. The dye was found to be not readily biodegradable in the OECD 301F Test (Manometric Respirometry Test) for ready biodegradability. When measured as dissolved organic carbon (DOC) and expressed as percentage elimination, biodegradation amounted to 2% at the end of the 28-day exposure to micro-organisms from a domestic sewage treatment plant, no inhibition on the activity of the bacteria was observed in this test. The dye's inherent biodegradability was -3% after 28 days according to the test procedure that followed OECD 302B guidelines (Zahn-Wellens/EMPA Test).

Although the dye is not readily biodegradable, the potential for bioaccumulation is low due to the low partition coefficient (log P_{OW} < -2.0) and very high water solubility of the substance. Hydrophilic dyes with log P_{OW} < 3 have been shown not to bioaccumulate (Yen et al, 1991). Also, biological membranes are not permeable to chemicals of very large molecular size and therefore bioaccumulation of the notified polymer is not expected (Gobas et al, 1986; Anliker et al, 1988).

Residues that persist after sewage treatment will enter marine or freshwater environments in solution (from city and country waste water treatment systems, respectively). A possible route of entry of the dye to the sediment is by the precipitation of its calcium salts, as several calcium salts of sulphonic dyes are known to be insoluble at modest concentrations (Weber, 1991). Degradation of such dyes in sediment water systems proceeded with a half-life of 2-16 days. Accordingly, no significant increase in dissolved concentrations over time is

predicted, while residues bound to sediment are expected to undergo reductive degradation.

9. EVALUATION OF TOXICOLOGICAL DATA

9.1 Acute Toxicity

Summary of the acute toxicity of Yellow TZ 4210

Test	*Species*	*Outcome*	*Reference*
Acute oral toxicity	rat	LD_{50} > 2 000 mg/kg	(Arcelin, 1995)
Acute dermal toxicity	rat	LD_{50} > 2 000 mg/kg	(Arcelin, 1996a)
Skin irritation	rabbit	non-irritant	(Braun, 1996a)
Eye irritation	rabbit	eye irritant with persistent efects	(Braun, 1996b)
Skin sensitisation	guinea pig	non-sensitiser	(Arcelin, 1996b)

9.1.1 **Oral Toxicity** (Arcelin, 1995)

Species/strain:	Rat/HanIbm: WIST (SPF)
Number/sex of animals:	5/males, 5/females
Observation period:	15 days
Method of administration:	Oral gavage; test substance was dissolved in bi-distilled water.
Clinical observations:	None
Mortality:	None
Morphological findings:	None
Test method:	limit test, OECD TG 401 (Organisation for Economic Co-operation and Development, 1995-1996)
LD_{50}:	> 2 000 mg/kg
Result:	Yellow TZ 4210 was of very low acute oral toxicity in a limit test in rats.

9.1.2 Dermal Toxicity (Arcelin, 1996a)

Species/strain:	Rat/HanIbm: WIST (SPF)
Number/sex of animals:	5/males, 5/females
Observation period:	15 days
Method of administration:	Single dose (in water) applied to a clipped area of skin on back of animal and covered with a semi-occlusive dressing. Dressing removed and skin washed with lukewarm water 24 hours after application.
Clinical observations:	Orange discolouration of the skin persisted in all animals throughout the study. Scales were noted in 1 male and 1 female animal between test day 4 and 10. The slight weight loss in one female animal was considered to be due to the semi-occlusive dressing.
Mortality:	None
Morphological findings:	None
Test method:	limit test, OECD TG 402 (Organisation for Economic Co-operation and Development, 1995-1996)
LD_{50}:	> 2 000 mg/kg
Result:	Yellow TZ 4210 was of low acute dermal toxicity in a limit test in rats.

9.1.3 Inhalation Toxicity

An acute inhalation study was not performed as less than 6% 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/CRL:KBL(NZW)BR
Number/sex of animals:	1 male, 2 females
Observation period:	72 hours
Method of administration:	Gauze patches bearing 0.5 g of the test article (in water) were applied to a shaved area of dorsal skin. The semi-occlusive dressing was removed after 4 hours and the skin washed with lukewarm water.
Clinical observations:	Orange discolouration of the skin persisted throughout the study. There were no Draize scores (Draize, 1959) greater than 0.
Test method:	OECD TG 404 (Organisation for Economic Co-operation and Development, 1995-1996)
Result:	Yellow TZ 4210 was non-irritating to rabbit skin

9.1.5 Eye Irritation (Braun, 1996b)

Species/strain:	Rabbit/CRL:KBL(NZW)BR
Number/sex of animals:	1 male, 2 females
Observation period:	21 days
Method of administration:	0.1 g of the notified chemical was placed in the conjunctival sac of the left eye of each animal. The right eye served as the control.

	Time after instillation													
Animal	***1 hr***		***24 hrs***		***48 hrs***		***72 hrs***		***7 days***		***14 days***		***21 days***	
Cornea	***o***		***o***		***o***		***o***		***o***		***o***		***o***	
1	0		0		0		0		0		0		0	
2	0		0		0		0		0		0		0	
3	0		0		0		0		0		0		0	
Iris														
1	0		0		0		0		0		0		0	
2	0		0		0		0		0		0		0	
3	0		0		0		0		0		0		0	
Conjunctiva	***r***	***c***	***r***	***c***	***r***	***c***	***r***	***c***	***r***	***c***	***r***	***c***	***r***	***c***
1	2	2	2	0	1	0	1	0	0	0	0	0	0	0
2	2	2	1	0	1	0	0	0	0	0	0	0	0	0
3	2	2	1	0	1	0	0	0	0	0	0	0	0	0

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

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

Result: The primary irritation score was determined to be 0.78 for conjunctival effects. Persistent colouration was observed in all animals to the end of the observation period. This included light orange to orange-brown staining of the conjunctiva to day 14, followed yellow staining from then on. Staining of the sclera of all animals was present on day 21 after application. Yellow TZ 4210 is an eye irritant with persistent effects.

9.1.6 Skin Sensitisation (Arcelin, 1996b)

Species/strain: Guinea pigs/Himalayan spotted

Number of animals: 30 females (10 control, 20 test)

Induction procedure:

Day 1: 3 pairs of intradermal injections:

- 0.1 mL of 1:1 mixture of Freund's Complete Adjuvant (FCA) and saline
- 0.1 mL of 5% concentration of test substance in bi-distilled water
- 0.1 mL of 5% concentration of test substance in 1:1 mixture of FCA and saline

Day 8: Occluded application of 50% concentration of test substance in bi-distilled water for 48 hours.

Challenge procedure:

Day 22: Occluded application of 25% concentration of test substance in bi-distilled water for 48 hours.

Challenge outcome:

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

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

Test method: OECD TG 406 (Organisation for Economic Co-operation and Development, 1995-1996)

Comments: as the test substance stained the skin dark red, it was not possible to determine whether erythema was present; however, no oedema was observed

Result: Yellow TZ 4210 is not a skin sensitiser in guinea pigs.

9.2 Repeated Dose Toxicity (Schmid et al, 1996)

Species/strain:	Rat/HanIbm: WIST (SPF)
Number/sex of animals:	30 male, 30 female (5/sex/group)
Method of administration:	Oral (gavage)
Dose/Study duration::	The notified chemical was administered daily for a total of 28 days: control: 0 mg/kg/day low dose: 50 mg/kg/day mid dose: 200 mg/kg/day high dose: 1000 mg/kg/day All animals were sacrificed at the end of the treatment period, with the exception of 5/sex from control and high dose groups, which were maintained for an additional 2 week recovery period before sacrifice.
Clinical observations:	No deaths occurred during the test period. There were no signs of clinical toxicity. There were no effects on body weight or food consumption.
Clinical chemistry/Haematology	A number of minor changes were noted in both male and female high dose animals: • a slight increase in the total bilirubin concentration; • a more intense yellow colour of the plasma; and, • a yellow to light orange discolouration of the urine. These findings were reversed at the end of the recovery period. At the end of the recovery period, a slightly higher score for blood in urine of high dose males was recorded.
Histopathology:	Treatment related findings were confined to the kidneys and consisted of an increase in the incidence and severity of lipofuscin pigment in the proximal renal tubular epithelium in the high dose group. Lipofuscin pigment was present at the end of the recovery period. It was also present to a minor degree in some rats in other treatment groups and controls.

Test method:	OECD TG 407 (Organisation for Economic Co-operation and Development, 1995-1996)
Result:	The increase in the incidence and severity of lipofuscin pigment in the proximal tubular renal epithelium in all high dose animals is derived from oxidation of tissue lipids and probably represents an increased metabolic turnover in the affected cells. The NOEL was established to be 200 mg/kg based on this renal effect. Although the study authors considered these effects to be related to the notified chemical, the NOAEL was determined to be 1 000 mg/kg/day because of the absence of related morphological changes.

9.3 Genotoxicity

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

Strains:	*Salmonella typhimurium* TA 1535, TA 1537, TA 98, TA 100, *Escherichia coli* WP2, WP2 uvrA
Concentration range:	33.3, 100, 333.3, 1 000, 2 500, 5 000 µg/plate with or without metabolic activation
Test method:	OECD TG 471 (Organisation for Economic Co-operation and Development, 1995-1996)
Result:	The notified chemical was not mutagenic in the bacterial strains tested in the presence or absence of metabolic activation provided by rat liver S9 fraction.

9.3.2 Chromosomal Aberration Assay in Chinese Hamster V79 Cells (Czich, 1996)

Cell culture:	Chinese hamster V79 cells
Dosing Schedule:	Without S9 mix: 10 - 300 µg/mL, treatment time 18 hours and 28 hours With S9 mix: 10 - 100 µg/mL, treatment time 4 hours
	The concentration range in the experiments was

	limited by a precipitation of the test substance in the test medium which started at concentrations of 300 µg/mL without S9 mix and 100 µg/mL with S9 mix. For all treatment groups, cells were prepared 18 hours and 28 hours after the start of treatment and scored for structural chromosomal aberrations.
Test method:	OECD TG 473 (Organisation for Economic Co-operation and Development, 1995-1996)
Comments:	In the presence of S9 mix and at the 28 hour interval , there were dose dependent increases in cells carrying structural chromosome aberrations after treatment with the test article (5.5% at 50 µg/mL and 14.5% at 100 µg/mL in experiments 1, and 2.0% at 50 µg/mL and 2.5% at 100 µg/mL in experiment II). Although these increases were within the historical control data range, they were regarded as being biologically relevant, as the quality of the aberrations found (multiple aberrant cells, exchanges) indicated a strong substance-induced effect on the DNA. In the absence of S9 mix, the mitotic index was reduced after treatment in both experiments with the highest evaluated concentration at each fixation interval. However no induction of chromosomal aberrations was observed.
Result:	the notified chemical induced structural chromosomal aberrations in the Chinese hamster V79 cells in the presence of S9 metabolic activation.

9.3.3 Micronucleus Assay in the Bone Marrow Cells of the Mouse (Volkner, 1996)

Species/strain:	mouse/NMRI
Number and sex of animals:	6/sex/group
Doses:	24 h preparation interval: 200, 670 and 2 000 mg/kg; 48 h preparation interval: 2 000 mg/kg; vehicle: deionised water

Method of administration:	oral administration
Test method:	OECD TG 474 (Organisation for Economic Co-operation and Development, 1995-1996)
Comment:	Comparing with vehicle controls, there was no significant enhancement in the frequency of the detected micronuclei at any preparation interval after administration of the test substance and with any dose level used. The positive control, cyclophosphamide, caused a distinct increase of induced micronucleus frequency.
Result:	Yellow TZ 4210 was non-mutagenic in this *in vivo* micronucleus assay.

9.4 Overall Assessment of Toxicological Data

Yellow TZ 4210 exhibited very low acute oral toxicity and low acute dermal toxicity in rats (LD_{50} > 2 000 mg/kg in both tests). Inhalation toxicity tests were not carried out by the notifier as less than 6% of the dye powder is in the respirable range. In addition, the product contains an antidusting agent which reduces the potential for dust formation. Yellow TZ 4210 was non-irritating to rabbit skin, but was an eye irritant with persistent effects in rabbits. It was not a skin sensitiser in guinea pigs.

Repeated oral administration of doses ranging from 50 to 1 000 mg/kg/day of Yellow TZ 4210 to rats resulted in an increase in the incidence and severity of lipofuscin pigment in the proximal renal tubular epithelium at the high dose. The effect is derived from the oxidation of tissue lipids and probably represents an increased metabolic turnover in the affected cells. The NOEL was 200 mg/kg/day.

No mutagenicity was observed in a reverse mutation assay in bacteria. However, a weakly positive result was obtained for Yellow TZ 4210 in an *in vitro* chromosomal aberration assay. An *in vivo* study, a micronucleus assay in mouse bone marrow cells, was provided by the notifier. Yellow TZ 4210 did not significantly enhance the frequency of detected micronuclei at either the 24 or 48 hours preparation interval up to 2 000 mg/kg. Therefore, Yellow TZ 4210 is unlikely to be mutagenic.

Based on the results of the animal studies summarised above, Yellow TZ 4210 would be classified as a hazardous substance on the basis of persistent eye effects according to the NOHSC *Approved Criteria for Classifying Hazardous Substances* (National Occupational Health and Safety Commission, 1994a).

10. ASSESSMENT OF ENVIRONMENTAL EFFECTS

The following ecotoxicity studies have been supplied by the notifier. The tests were performed in compliance with OECD/EEC Test Methods and according to OECD Principles of Good Laboratory Practices.

Ecotoxicity Test Results

Test	*Species*	*Results (Nominal)*
Acute Toxicity (semi static) (OECD TG 203)	Rainbow trout *Oncorhynchus mykiss*	96 h LC_{50} > 100 mg/L 96 h LC_0 > 100 mg/L
Acute Toxicity -Immobilisation Test (Static Test) (OECD TG 202)	Water Flea (*Daphnia magna*)	48 h EC_{50} > 100 mg/L 48 h NOEC > 100 mg/L
Growth Inhibition - Growth (μ) & Biomass (b) (Static Test) (OECD TG 201)†	Green Algae (*Scenedesmus subspicatus*)	<u>Experiment A</u> $E\mu C_{50}$ = 89.9 mg/L (21.9-n.d. mg/L)# E_bC_{50} = 10.9 mg/L (1.0-299 mg/L) LOEC = 3.2 mg/L <u>Experiment B</u> $E\mu C_{50}$ = 377 mg/L (164-1699 mg/L) E_bC_{50} = 21.9 mg/L (11.5-55.8 mg/L)
Respiration Inhibition (OECD TG 209)	Activated Sludge - Aerobic Waste Water Bacteria	3 h IC_{50} > 100 mg/L

\# 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 substance had no toxic effects on the test fish up to concentration of nominal 100 mg/L. As such, the only concentration tested in the definitive study was 100 mg/L.

The results are all related to nominal concentrations of the notified substance. The analytically determined test substance concentrations in the test media varied in the range of 89% to 94% of the nominal value at the start of the test period. During the 96 hours of the test the concentration of the test substance dropped to 59% of the nominal value. Assuming

the reaction product may also have a toxic effect the sum of the reaction product and the test substance was between 89-94% and as such, all biological results are related to nominal concentrations.

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. The results are all related to nominal concentrations of the notified substance. The analytically determined test substance concentrations in the test media varied in the range of 92% to 96% of the nominal value at the start of the test period. During the 48 hours of the test the concentration of the test substance dropped to between 55 and 62% of the nominal value. Assuming the reaction product may also have a toxic effect the sum of the reaction product and the test substance was in all treatments above 90%.

The 24 h and 48 h LC_{50} and NOEC were determined to be > 100 mg/L as no daphnids were noted to be immobilised at the highest concentration tested. One daphnid in each of the control and 46 mg/L treatment was observed to be immobile at the 24 h and 48 h observation. This appears to have been an occurrence unrelated to the chemical concentration and has no bearing on the final results and as such all biological results are related to nominal concentrations.

A *Daphnia sp.* reproduction test was not supplied. However, based on the low acute toxicity to both fish and daphnids, it is not expected to produce reproduction effects on daphnids.

Algae

Nominal concentrations of 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 in the range from 99% to 103% on the nominal values. During the 72 hours of the test the concentration of the test substance dropped to between 55 and 62% of the nominal value. Assuming the reaction product may also have a toxic effect the sum of the reaction product and the test substance was above 90% and as such, all biological results are related to nominal concentrations.

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 3.2 mg/L. As such, the 72 h NOEC was determined to be 0.9 mg/L. The EC-values (indicated in the above table) were calculated for the algal biomass (b) and the growth rate (μ) after 72 hours. There was no observed difference in the shape of algal cells when compared to those growing in the control.

In experiment part B, the algae grew in test water without the test substance. 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 1.0 mg/L. The EC_{50} values and the percentage inhibition of the algal growth rate (μ) after 72 hours of exposure in this experiment were of a slightly lesser magnitude than in experiment part A.

The modified growth inhibition test showed that there were differences between the growth of *Scenedesmus subspicatus* under the two different test regimes. Growth inhibition 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 directly in the test media with the dissolved test substance were greater at some concentrations than the 10 % allowed under the test protocol. Therefore, the notifier claims that the real toxic effect of the notified chemical cannot be excluded at the test concentrations of 1.0, 10 and 32 mg/L.

In conditions where release to the environment occurs, algistatic effects from reduced light incidence as well as the algitoxic effects of the chemical may still lead to an undesirable environmental impact if exposure is continuous. Therefore, it is necessary to take into account the combined effects on the test algae. Thus, the notified chemical can be considered as slightly toxic to algae.

Microorganisms

The inhibitory effect of the notified chemical on aerobic waste water bacteria (activated sludge from a domestic waste water treatment plant) was investigated in a respiration test. The notified chemical 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, with a final 3 hour IC_{50} greater than 100 mg/L.

Conclusion

The ecotoxicity data for the notified substance indicate that it is practically non-toxic to fish, aquatic invertebrates and microorganisms, and slightly toxic to algae (with effects on biomass and growth rate). Reproductive effects on aquatic invertebrates are not expected.

11. ASSESSMENT OF ENVIRONMENTAL HAZARD

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

The notifier has specified that a limited number of dyehouses (approx. 15) in city and country areas will be using the notified dye. The environmental hazard has been determined for two dyehouses located in two general locations, one metropolitan based dyehouse and the other country based. The Predicted Environmental Concentration (PEC) is estimated below.

Predicted Environmental Concentration (PEC)

Calculation Factor	*Country Dyehouse (high use)*	*City Dyehouse*
typical use of product expected per day (700 kg cloth)	60 kg	30 kg
amount of notified chemical (at 73%)	43.8 kg	21.9 kg
concentration in wastewater (fixation rate 75%)	11 kg	5.5 kg
quantity of water used incl. wash-off water	60 000 L/day	35 000 L/day
effluent concentration in product-specific wash-water	183 mg/L	157 mg/L
dilution factor in dyehouse by other wash-waters	1:70	1:100
influent concentration	2.6 mg/L	1.57 mg/L
dilution factor in sewage treatment plant[1]	1:10	1:100
concentration balance in effluent from sewage treatment plant	0.26 mg/L	0.016 mg/L
dilution factor in receiving waters	1:2 (river)	1:10 (ocean)
PEC in receiving waters	0.13 mg/L (0.13 ppm)	1.6 µg/L (1.6 ppb)
safety factor (EC_{50}/PEC) for exposure to most sensitive aquatic organism, algae[2] (72 h E_bC_{50} ≈ 10.9 mg/L)	83.4	6,800

[1] The dilution at a rural town could reasonably be expected to be about 5-6 ML/day, while for a major city, say Sydney, it would be between 150-500 ML/day.
[2] The growth of Green algae was inhibited by 50% after 72 hr at a test concentration of 10.9 mg/L.

These calculations assume that no dye is removed in treatment of the different waste effluents and represent the worst case scenario for dyehouses. The "typical use of dye expected per day" amount was supplied by the notifier, and is expected to be a representation of maximum use.

The calculations show that the exposure to fish, daphnia, algae and waste water treatment bacteria is at levels unlikely to cause any significant effect. At higher release rates, there is still unlikely to be any significant effect on these species. Once in the aquatic environment, the chemical is expected to swiftly dilute to undetectable concentrations, and undergo biotic and abiotic degradation and adequate safety factor exists for use in country locations.

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

12. ASSESSMENT OF PUBLIC AND OCCUPATIONAL HEALTH AND SAFETY EFFECTS

Yellow TZ 4210 was of very low acute oral toxicity and low dermal toxicity in rats. It was an eye irritant with persistent effects in rabbits, but not a skin irritant in rabbits or a skin sensitiser in guinea pigs. In a 28 day oral repeat dose study in rats, NOEL was determined to be 200 mg/kg/day based on increased incidence and severity of pigment deposits in the kidneys at the next highest dose. Yellow TZ 4210 did not induce mutations in bacteria *in vitro* or in mouse bone marrow cells *in vivo*, however it induced chromosome aberrations in Chinese hamster V79 cells in the presence of S9 *in vitro*. Yellow TZ 4210 is classified as a hazardous substance based on the persistent eye 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. The chemical has very low vapour pressure and the formulation presented has a very high percentage (>94%) of particles beyond 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. The notifier indicated that cases of skin and respiratory sensitisation have been observed with some 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

Aware that the chemical can cause persistent effects in eyes, may be mutagenic and may be implicated in sensitising effects, workers repacking from 30 kg to 5 and 10 kg containers need to be adequately protected against topical and systemic exposure. Repacking workers are estimated to work infrequently on this task. The exposure controls identified, namely mechanical ventilation, protective gloves, safety glasses and overalls, should offer adequate protection during this task.

End use

The weighing operators have potentially the highest exposure to the notified chemical. Give the health effects outlined above, it is essential that effective control measures operate in the weighing and dye mixing and using areas. Local exhaust ventilation is provided at the weighing and mixing areas. As inhalation toxicity data are not available for the notified chemical, and there is some potential for inhalational exposure to the notified chemical during the weighing operation, the level of dust in the workplace should be maintained at as low a concentration as possible and personal protective equipment should be worn where necessary to minimise exposure. As the weighing and mixing workers wear a respirator, overalls and gloves to minimise exposure, the risk of adverse health effects during this operation is expected to be low.

The occupational health risk for wash-off and drier operators is low once the notified chemical is dissolved into water, as the dyeing processes are largely automated and the maximum concentration of the notified chemical during the dyeing process is less than 1%. In addition, exposure times for operators of the dyeing machines and driers are expected to be short (several minutes per hour). The dye is shortly fixed and becomes irreversibly bound to the fabric. The main route of exposure at this stage will still be dermal and the workers will wear gloves.

Based on data reported to be from a US air monitoring study, the notifier estimated that the average daily inhalation exposure of a worker during weighing would be 0.00015 mg/kg/day. Based on a NOEL of 200 mg/kg/day for kidney effects, a margin of exposure (MOE) for this estimate is 1.3×10^6. If an additional safety factor of 10 is applied to take into account a longer weighing operation and/or a greater amount of dye in the Australian work situation, then an MOE of 1.3×10^5 would still indicate that the risk of adverse health effects resulting from inhalational exposure during weighing is of low concern.

The Material Safety Data Sheet (MSDS) for Cibacron Yellow W-R 200% alerts workers to the fact that they may experience skin and respiratory sentitisation when handling reactive dyes such as those. The MSDS further recommends that such sensitised workers should cease working with the dyes.

Public health

There will be extensive dermal contact with dyed fabrics by the general public. However, since the notified chemical has a low partition coefficient, a relatively high molecular weight, and is strongly bound to the fibres, the notified chemical is unlikely to be dermally absorbed. The notified chemical is not a skin irritant or sensitiser. The proposed use of the notified chemical is not expected to pose a significant hazard to public health.

13. MATERIAL SAFETY DATA SHEET

The MSDS for the notified chemical was provided in accordance with the *National Code of Practice for the Preparation of Material Safety Data Sheets* (National Occupational Health and Safety Commission, 1994c).

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 Yellow TZ 4210, the following guidelines and precautions should be observed:

- Safety goggles should be selected and fitted in accordance with Australian Standard (AS) 1336 (Standards Australia, 1994) to comply with Australian/New Zealand Standard (AS/NZS) 1337 (Standards Australia/Standards New Zealand, 1992);
- Inhalational exposure to potentially harmful dusts should be kept to a minimum and respiratory protection (selected and fitted) according to AS/NZS 1715 (Standards Australia/Standards New Zealand, 1994a) meeting the requirements of AS/NZS 1716 (Standards Australia/Standards New Zealand, 1994b);
- Industrial clothing should conform to the specifications detailed in AS 2919 (Standards Australia, 1987) and AS 3765.1 (Standards Australia, 1990);
- Impermeable gloves or mittens should conform to AS 2161 (Standards Australia/Standards New Zealand, 1998);
- All occupational footwear should conform to AS/NZS 2210 (Standards Australia/Standards New Zealand, 1994c);
- Spillage of the notified chemical should be avoided. Spillages should be cleaned up promptly and 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 became sensitised to reactive dyes such as Cibacron W-R 200% should not continue to handle such dyes 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.

16. REFERENCES

Anliker, R, Moser, P & Poppinger, D (1988) "Bioaccumulation of dyestuffs and organic pigments in fish. Relationships to hydrophobicity and steric factors". *Chemosphere* 17(8): 1631-1644.

Arcelin, G (1995) "Acute Oral Toxicity Study with FAT 40'544/A in Rats". RCC Project 609682.

Arcelin, G (1996a) "Acute Dermal Toxicity Study with FAT 40'549/A in Rats". RCC Project 617534.

Arcelin, G (1996b) "Contact Hypersensitivity to FAT 40'549/A in Albino Guinea Pigs". RCC Project 617545.

Braun, WH (1996a) "Primary Skin Irritation Study with FAT 40'544/A in Rabbits". RCC Project 609693.

Braun, WH (1996b) "Primary Eye Irritation Study with FAT 40'544/A in Rabbits". RCC Project 609704.

Czich, A (1996) "Chromosome Aberration Assay in Chinese Hamster V79 Cells *in vitro* with FAT 40'549/A". CCR Project 550210.

Draize JH (1959) Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics. Association of Food and Drug Officials of the US, 49: 2-56.

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

Gobas FAPC, Opperhuizen A & Hutzinger O (1986). "Bioconcentration of hydrophobic chemicals in fish: relationship with membrane permeation". *Environmental Toxicology and Chemistry* 5: 637-646.

Hobbs, SJ (1988). "Industry Category Document: UK dye production and use in the textile industry". Department of the Environment (UK).

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

National Occupational Health and Safety Commission (1994b) List of Designated Hazardous Substances [NOHSC:10005(1994)]. Canberra, Australian Government Publishing Service.

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

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

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

Standards Australia (1987) Australian Standard 2919-1987, Industrial Clothing. Sydney, Standards Association of Australia.

Standards Australia (1990) Australian Standard 3765.1-1990, Clothing for Protection against Hazardous Chemicals Part 1 Protection against General or Specific Chemicals. Sydney, Standards Association of Australia.

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

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

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

Standards Australia/Standards New Zealand (1994b) Australian/New Zealand Standard 1716-1994, Respiratory Protective Devices. Sydney/Wellington, Standards Association of Australia/Standards Association of New Zealand.

Standards Australia/Standards New Zealand (1994c) Australian/New Zealand Standard 2210-1994, Occupational Protective Footwear. Sydney/Wellington, Standards Association of Australia/Standards Association of New Zealand.

Standards Australia/Standards New Zealand (1998) AS/NZS 2161.2:1998 Occupational protective gloves, Part 2: General requirements, Standards Australia/Standards New Zealand.

Volkner, W (1996) Micronucleus assay on bone marrow cells of the mouse. CCR Project 630415.

Wollny, H (1996) *'Salmonella typhimurium* and *Escherichia coli* Reverse Mutation Assay with FAT 40549/A". CCR Project 550209.

Yen, CP, Perenich, TA, & Baughman, GL (1991). "Fate of Commercial Disperse Dyes in Sediments". *Environmental Toxicology and Chemistry,* 10: 1009-1017.

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