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NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME

FULL PUBLIC REPORT

Reactive Orange DER 8089

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Director
Chemicals Notification and Assessment

FULL PUBLIC REPORT

Reactive Orange DER 8089

1. APPLICANT

Ciba Specialty Chemicals of 235 Settlement Rd., THOMASTOWN, VIC 3074 has submitted a standard notification statement in support of their application for an assessment certificate for Reactive Orange DER 8089.

2. IDENTITY OF THE CHEMICAL

The chemical name, CAS number, molecular and structural formulae, molecular weight, spectral data and details of impurities present, along with the details of the Predicted Environmental Concentration (PEC) calculations have been exempted from publication in the Full Public Report and the Summary Report.

Marketing Name:	Reactive Orange DER 8089 Lanosol Black CE Cibacron Black LS-N
Other Names:	FAT 45' 176/A FAT 40' 560/A Lanosol Orange 8089
Method of Detection and Determination:	UV/visible spectrophotometry Infrared spectrometry ^{1}H nmr spectrometry reference spectra have been provided by the notifier

3. PHYSICAL AND CHEMICAL PROPERTIES

The physical and chemical properties given below are for the product Orange DER 8089, containing > 50 % notified chemical, rather than for the notified chemical. The notified chemical is in a mixture of at least 29 reaction products (identified by HPLC) which are not individually isolated.

Appearance at 20°C and 101.3 kPa:	orange brown powder
Melting Point:	> 400°C (see comments below)

Specific Gravity: 1.7 at 20°C

Vapour Pressure: 1×10^{-9} kPa at 20°C (calculated)

Water Solubility: 339 g/L at 20°C

Particle Size: Median mass distribution 47 μm
9.9 % < 10 μm (respirable)

Size Range (μm)	Mass %
< 0.36	0.13
0.36 – 0.75	0.43
0.75 – 1.55	0.64
1.55 – 3.09	1.30
3.09 – 6.18	3.64
6.08 – 12.01	8.39
12.01 – 24.63	14.99
24.63 – 63	29.61
63 – 100	26.45
100 – 200	13.87
> 200	0.55

Surface Tension: 70 mN/m at 20°C for a 1 g/L solution

Partition Co-efficient (n-octanol/water): $\log P_{ow} < -5.4$

Hydrolysis as a Function of pH:

$T_{1/2}$ at pH 4.0	12234 hours at 25°C
	120.6 hours at 70°C
	> 40 hours at 80°C
$T_{1/2}$ at pH 7.0	84.8 hours at 25°C
$T_{1/2}$ at pH 9.0	< 24 hours at 25°C

Adsorption/Desorption:

Soil Type	K_{oc} (mL/g)
Loamy sand	1105
Sandy loam	1156
Silt loam	1249

Dissociation Constant:	group	pK_a	method
	sulphuric acid 1	~ -5	general estimation
	sulphuric acid 2	~ -5	general estimation
	azobenzene 1	< 0	general estimation
	aniline 1	< 0.8	Hammett
	aniline 2	< 3.25	Hammett
	sulphonic acid	-6.3	Hammett

Flash Point: none observed

Flammability Limits: not highly flammable

Autoignition Temperature: 289°C

Explosive Properties: not explosive

Reactivity/Stability: not reactive

Comments on Physico-Chemical Properties

No melting point was observed below 400°C; decomposition was observed at approximately 270°C. An extrapolated boiling point of 692°C was derived from the vapour pressure calculations.

The maximum water solubility of Reactive Orange DER 8089 was determined to be 330 g/L at 20°C using the flask method (OECD TG 105). This solubility resulted for test solutions of a concentration of 2.0 g test substance in 5.0 mL water. The notifier indicated that test solutions with 1.0 and 0.5 g test substance in 5.0 mL water, corresponding to concentrations of 200 and 100 g/L, respectively, show a sediment after centrifugation. The pH of the solutions above was approximately 5.0.

The notified chemical was determined by the notifier according to OECD TG 111 to be hydrolytically stable at 20°C and pH 4.0 but unstable at pH 9.0.

The partition coefficient Log P_{ow} of Reactive Orange DER 8089 between n-octanol and water was not determined. A pre-test indicated that the notified chemical had a very good solubility in water and a very poor solubility in n-octanol, indicating a partition coefficient below -2. Therefore, neither the HPLC method according to OECD TG 117 nor the flask shaking method according to OECD TG 107 were applicable for the determination. The Log P_{ow} of Reactive Orange DER 8089 was estimated to be <-5.4 from its solubility in n-octanol and water.

The adsorption/desorption of Reactive Orange DER 8089 were determined in a screening test OECD TG 106 by the batch equilibrium method using three soils.

Soil	*Clay %*	*Silt %*	*Sand %*	*Organic C g/100 g soil*	*pH*	*Cation exchange capacity meq/100 g soil*	*K_{oc} mL/g*
Speyer-loamy sand	5.1	5.6	89.3	2.29	6.0	9.7	1105
Les Barges-silt loam	19.4	60.1	20.5	3.80	6.9	25.4	1156
Sisseln-sandy loam	15.9	16.1	68.0	1.57	7.1	13.8	1249

The soils chosen represented a range of organic carbon content, pH, cation exchange capacity and clay content. Under the conditions of the test the amount of Reactive Orange DER 8089 adsorbed to soil was 82.05 %, 89.89 % and 77.30 % for Speyer, Les Barges and Sisseln, respectively. Desorption of adsorbed substance was 16.74 %, 24.03 % and 23.83 % from soils Speyer, Les Barges and Sisseln, respectively. The calculated adsorption/desorption coefficients K_{oc} for the three soils Speyer, Les Barges and Sisseln were 1105 mL/g, 1156 mL/g and 1249 mL/g, respectively. The notified chemical can be regarded as having low mobility in soils and can be expected to be absorbed to the non-organic carbon content of the soils.

The notifier has indicated that the behaviour of the notified substance in aqueous solutions is dominated by the strongly acidic $ArSO^{3-}$ and RSO^{4-} groups with pKa of ~ -5 and -6.3, respectively. The molecule is 3-fold negatively charged and is present in anionic form over the environmental pH range. It would be difficult to estimate an overall dissociation constant, K, for the notified chemical, as it contains the strong acidic groups $ArSO^{3-}$ and RSO^{4-} as well as two basic $ArNH_2$ groups with pKa estimated by the notifier to be < 0.8 and < 3.25, respectively.

The surface tension of Reactive Orange DER 8089 was determined in a screening test OECD TG 115 to be 70 mN/m at a 0.1 % concentration at 20ºC. Based on the criteria outlined in the OECD TG 115 Reactive Orange DER 8089 should not be regarded as a surface-active substance.

Flammability testing was carried out according to method A10 in EEC Directive 92/69.

4. PURITY OF THE CHEMICAL

Degree of Purity: 97.7 %

The notified chemical is in a mixture of at least 29 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, Orange DER 8089.

Composition

Chemical Name	***Weight %***
main product (notified chemical)	> 50 %

organic byproducts	< 20 %
inorganic co-products	< 20 %

Hazardous Impurities: none detected

Non-hazardous Impurities (> 1% by weight): none

Additives/Adjuvants: The notified chemical will be imported as a component of the commercial black dyes Lanosol Black CE (for dyeing wool) and Cibacron Black LS-N (for dyeing cotton), which consist of a mixture of azo dyes (> 70 %) with dispersing agents, buffers, antidusting additive and moisture.

One azo dye component in Lanosol Black CE and Cibacron Black LS-N, 2,7-naphthalenedisulphonic acid, 4-amino-5-hydroxy-3,6-bis[[4-[[2-(sulphooxy)ethyl]-sulphonyl]phenyl]azo]-, tetrasodium salt, is a skin sensitiser and possible respiratory sensitiser.

5. USE, VOLUME AND FORMULATION

The notified chemical will not be manufactured in Australia. It will be imported as a component at less than 10 % of the formulated reactive dyestuffs Lanosol Black CE and Cibacron Black LS-N. These are reactive black dyes for application to wool and cotton by the exhaust dyeing method. 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 1 to 10 tonnes per year.

6. OCCUPATIONAL EXPOSURE

Routes of Exposure
The notified chemical will be imported as a powdered solid which will be dissolved in 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 the preparation will not contain inhalable particles in relevant amounts. Dust production from the dyes containing the notified chemical has been measured by a light scattering technique to company quality control standards. 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. The notifier indicated that 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.

End Use
The following procedures are carried out at a small number of customer facilities. The notifier estimates that less than 100 workers throughout Australia will be exposed to the notified chemical during these activities.

Weighing and mixing
At the customer facilities, the powdered dye will be weighed out from the 30 kg containers in 1 kg lots (less than 100 g notified chemical), and 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 would be carried out under local exhaust ventilation. The process will involve one operator per site, on a daily basis. The exposure to the notified chemical will be to the powdered solid, as well as to a < 0.02 % (w/v) aqueous solution.

The notifier states that the workers involved in the weighing and mixing procedures will wear protective gloves and glasses.

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 two workers per site per shift (three shifts per day) will be exposed during these activities. The dyeing cycle may be carried out up to 14 times per shift.

The notifier states that operators of the dyeing machines wear personal protective equipment including gloves and safety glasses when potentially in contact with the solution, including during transfer of the cloth to the dryer.

Laboratory
The notifier estimates that two laboratory technicians in each customer facility will be involved in quality control checks on the dye solution, and sometimes on the wet or dry dyed

cloth. Laboratory technicians will only be exposed to the dye in solution form or fixed to cloth during these activities. Gloves will be used while handling the solution or cloth.

7. PUBLIC EXPOSURE

The dyes containing the notified chemical will not be available to the public. It is expected that during transport, repackaging and storage, exposure of the general public to the notified chemical will be minimal, except in the event of an accidental spill. Releases should damped down to prevent dust and spills should be scooped into chemical waste containers for disposal. Entry into surface waters should be prevented.

Public exposure to the notified substance via the dermal route is expected to be widespread as dyed wool and cotton fabrics will made into clothing to be sold to the public.

8. ENVIRONMENTAL EXPOSURE

Release

The dye will be used at dyehouses at both city and country locations. The bulk of the dye will become chemically fixed to the fibres of wool and cotton, and in this state is not expected to impact on the environment. The notifier has submitted evidence to show that the dye has a fixation performance of 98 % to wool. The fixation to cotton was assumed by the notifier to be lower at 76 - 77 %. No evidence was provided to support this claim.

The major environmental exposure to dye will come from effluent discharge from mills and their wastewater treatment systems. Other releases will be limited to traces from repacking operations and clean up of any spills, and from trace residues in empty packaging.

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

Fate

The bulk of the dye will become chemically fixed to the fibres of wool and cotton, while the remainder would be rinsed into wastewater. The fate of the majority of the notified substance is linked with the fate of the textile fibre and in this state is not expected to impact on the environment. Eventually the textile will enter the waste disposal stream for recycling, or ultimately for disposal as waste in landfill. Once in the landfill sites movement of the chemical by leaching is not expected because of the expected high binding affinity to the non-organic component of soil.

The dye released in mill effluent water is expected to be the major source of environmental exposure. The dye may either partition to the non-organic component of sediment, or stay in the aqueous compartment. Any dye that binds to the sludge during the waste treatment process would be disposed of through incineration or landfill. Incineration of the dye will produce oxides of carbon, nitrogen and sulfur. Disposal by landfill will be at a secured site.

The dye is not readily biodegradable. When measured as Biochemical Oxygen Demand (BOD) in a Manometric Respirometry Test (Dietschy, 1997) according to OECD TG 301F and expressed as percentage elimination, biodegradation was 0 % over the 28-day exposure to micro-organisms from a domestic sewage treatment plant. The dye's inherent biodegradability was measured according to OECD TG 302B (Zahn-Wellens/EMPA Test). (Grutzner, 1997b) When measured as Dissolved Organic Carbon (DOC) and expressed as percentage elimination, biodegradation was 18 % over the 28-day exposure to micro-organisms from a domestic sewage treatment plant.

Although the dye is only slightly biodegradable, the potential for bio-accumulation is low due to the low partition coefficient (Log P_{ow} < -5.4) of the substance and its high adsorption to sludge and other surfaces. Also, hydrophilic dyes with log P_{ow} < 3 have been shown not to bioaccumulate (Yen et al., 1991).

Residues that persist after sewage treatment will enter marine and freshwater environments in solution from city and country wastewater treatment systems. The concentrations are expected to be low because of the very high fixation rate for wool and moderate fixation rate for cotton in the initial process, the expected movement to sediment/sludge and the high dilution rates in the release processes.

Residues from empty containers are expected by the notifier to be small because of the universal practice of thoroughly emptying out all drums to remove as much of the high cost dyestuff as possible.

9. EVALUATION OF TOXICOLOGICAL DATA

9.1 Acute Toxicity

Summary of the acute toxicity of Reactive Orange DER 8089

Test	*Species*	*Outcome*	*Reference*
acute oral toxicity	rat	LD_{50} > 2000 mg/kg	(Allard, 1996)
acute dermal toxicity	rat	LD_{50} > 2000 mg/kg	(Arcelin, 1997a)
skin irritation	rabbit	non-irritant	(Braun, 1997b)
eye irritation	rabbit	non-irritant	(Braun, 1997a)
skin sensitisation	guinea pig	not sensitising	(Arcelin, 1997b)

9.1.1 Oral Toxicity (Allard, 1996)

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

Number/sex of animals: 5/sex

Observation period: 15 days

Method of administration: gavage, 20 % (w/v) aqueous solution

Test method:	limit test, OECD TG 401
Mortality:	no deaths occurred during the study
Clinical observations:	no clinical signs of toxicity were observed during the study
Morphological findings:	no gross abnormalities were observed at necropsy
Comment:	one female showed a slight loss of body weight between days 8 and 15
LD_{50}:	> 2000 mg/kg
Result:	the notified chemical was of very low acute oral toxicity in rats

9.1.2 Dermal Toxicity (Arcelin, 1997a)

Species/strain:	rat/HanIbm: WIST (SPF)
Number/sex of animals:	5/sex
Observation period:	15 days
Method of administration/dose:	semi-occluded patch; 24 hour exposure dose 2000 mg/kg; test material applied as a 50 % (w/v) aqueous suspension
Mortality:	no deaths occurred during the study
Clinical observations:	no clinical signs of toxicity were observed during the study; orange discolouration and scales were observed on all animals as local effects of the test substance
Morphological findings:	no gross abnormalities were observed at necropsy
Test method:	limit test, OECD TG 402
LD_{50}:	greater than 2000 mg/kg
Result:	the notified chemical was of low dermal toxicity in rats

9.1.3 Inhalation Toxicity

The notifier has concluded that, because the commercial product is treated with an antidusting additive, inhalation would not be a major exposure route. No inhalation toxicity test reports were provided.

9.1.4 Skin Irritation (Braun, 1997b)

Species/strain:	rabbit/New Zealand White
Number/sex of animals:	1 male, 2 female
Observation period:	3 days
Method of administration:	0.5 g of test material, moistened with bi-distilled water, was applied to a clipped intact region of the dorsal skin and secured under a gauze patch with a semi-occlusive dressing for 4 hours; at the end of this time residual material was removed with lukewarm water; animals were examined for skin reaction 1, 24, 48 and 72 hours following application of the test substance
Test method:	OECD TG 404
Observations:	Draize scores were all zero at all time intervals; light orange staining of the treated skin persisted throughout the study
Result:	the notified chemical was not irritating to the skin of rabbits

9.1.5 Eye Irritation (Braun, 1997a)

Species/strain:	rabbit/New Zealand White
Number/sex of animals:	1 male, 2 female
Observation period:	14 days
Method of administration:	0.1 g of test material applied as supplied into conjunctival sac of the left eye of each animal; the contralateral eye served as the control; animals were examined for eye lesions 1, 24, 48 and 72 hours after test substance application; further observations were made after 7 and 14 days
Test method:	OECD TG 405
Observations	chemosis was observed in one animal and discharge was observed in all of the animals 1 hour after application of the test material; all Draize scores were zero for the remainder of the study; light orange staining of the conjunctivae and sclera of the treated eyes was observed in all animals at 24 and 48 hours; staining was restricted to the nictating membrane and lid hairs after this time; staining of the nictating membrane had cleared by day 14

Result: the notified chemical was not irritating to the eyes of rabbits

9.1.6 Skin Sensitisation (Maximisation Test) (Arcelin, 1997b)

Species/strain: guinea pig/Ibm: GOHI SPF

Number/sex of animals: 10 males test group, 5 males 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 bi-distilled water
- the test material diluted to 5 % by emulsion with 1:1 (v/v) mixture of Freund's Complete Adjuvant and physiological saline

day 8 a filter paper patch with 0.3 g of test material (50 % (w/v) in bi-distilled water) 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 water only was used in place of the aqueous solution of test material in both induction phases

Challenge procedure:

day 22 a 24 hour occluded application of 0.2 mL of a 25 % solution of the test material was applied to the shaved flank of both test and control animals

Test method: OECD TG 406

Comment: no skin response to the challenge application of test material was observed in either the test or control animals

Result: the notified chemical was not sensitising to the skin of guinea pigs

9.2 Repeated Dose Toxicity (Allard et al., 1997)

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

Number/sex of animals: group 1: 10/sex

group 2: 5/sex
group 3: 5/sex
group 4: 10/sex

Method of administration: gavage, notified chemical dissolved in bi-distilled water to give a dose level of 10 mL/kg/day

Dose/Study duration:
group 1: 0 mg/kg/day
group 2: 50 mg/kg/day
group 3: 200 mg/kg/day
group 4: 1000 mg/kg/day

the study duration was 28 days; 5 animals per sex in groups 1 and 4 were then allowed to recover for 14 treatment free days

Test method: OECD TG 407

Clinical observations:

One female in the main 1000 mg/kg/day group died during blood sampling on the day of scheduled necropsy. No other deaths occurred during the study.

No clinical signs were observed in the 50 mg/kg/day and 200 mg/kg/day groups. Dark faeces were observed for the 1000 mg/kg/day animals from day 13 of treatment to day 1 of the recovery period.

No treatment related effects on food consumption or body weights were observed.

Clinical chemistry/Haematology

No treatment related changes in haematology parameters were observed.

No treatment related changes in clinical biochemistry parameters were seen in the 50 mg/kg/day and 200 mg/kg/day groups. For the 1000 mg/kg/day animals, compared with the controls, there were a number of small statistically significant changes. Total bilirubin was moderately increased in both males and females. Uric acid and triglycerides were slightly increased in the females, and total cholesterol, phospholipids, total protein and globulin were slightly increased in the males. Slightly lower potassium was also observed in the males. Orange discolouration of plasma was also observed. Following the recovery period all parameters except the potassium level in males were similar to the controls, and plasma discolouration was no longer observed.

No statistically significant differences in urine chemistry were observed. Urine was discoloured, ranging from deep yellow to deep orange, for 3 out of 5 males and 1 out of five females of the 50 mg/kg/day group, 4 out of 5 males and 2 out of five females of the 200 mg/kg/day group, and all animals of the 1000 mg/kg/day group. Urine discolouration was found to be reversible after the recovery period.

Gross pathology:

No major differences in absolute or relative organ weights compared with the controls were found in any group. No treatment related abnormalities were observed.

Histopathology:

The incidence and severity of microscopic findings was similar for treated animals and controls and was within the historical range for this age and strain of rats.

Result:

A No Observed Adverse Effect Level (NOAEL) was established at 200 mg/kg/day, based on the treatment-related changes in clinical signs and serum biochemistry at 1000 mg/kg/day. A No Observed Effect Level (NOEL) was not established due to the presence of treatment-related urine discolouration at the lowest dose tested i.e. 50 mg/kg/day.

9.3 Genotoxicity

9.3.1 *Salmonella typhimurium* and *Escherichia coli* Reverse Mutation Assay (Wollny, 1997)

Strains:	*Salmonella typhimurium*: TA98, TA100, TA1535, TA1537 *Escherichia coli*: WP2, WP2 *uvrA*
Concentration range:	33, 100, 333, 1000, 2500 and 5000 μg/plate
Metabolic Activation System:	rat liver S9 fraction from animals pretreated with phenobarbital and β-napthoflavone
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 a small increase in the number of revertants (up to 1.8 times solvent control) was seen in several of the tests at the highest doses (2500 and 5000 μg/plate); the increases were not observed for both experiments except in the case of the *E. coli* strains in the presence of S9; the observed increases were not statistically significant 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 Aberrations in Chinese Hamster V79 Cells *In Vitro* (Czich, 1997)

Cells:	Chinese Hamster V79
Doses:	test material 3-500 μg/mL, 3-300 μg/mL (without metabolic activation) 3-500 μg/mL (with metabolic activation) positive controls ethylmethane sulphonate (EMS) 600 μg/mL (without metabolic activation) cyclophosphamide (CPA) 0.71 μg/mL (with metabolic activation)
Metabolic Activation System:	rat liver S9 fraction from animals pretreated with phenobarbital and β-napthoflavone
Test method:	OECD TG 473
Treatment Regime:	with metabolic activation: test material or positive control added to cell cultures in serum free medium, with 50 μL/mL S9 mix, for 4 hours; the cells were then washed and cultured in fresh complete medium to a total time of 18 or 28 hours without metabolic activation: test material or positive control added to cell cultures in complete medium for a total time of 18 or 28 hours without a change of medium colcemid was added to all cultures 2.5 hours before harvest to arrest cells in metaphase
Observations:	precipitation was observed 4 hours after the start of treatment for concentrations of 30 μg/mL and above; cytotoxic effects were observed in a pretest after treatment with 300 μg/mL and above (in the absence of S9) and 100 μg/mL and above (in the presence of S9) no substantial reductions in mitotic index were observed in any of the evaluated cultures, except for experiment 1 in the presence of S9 at 18 hours after treatment with 500 μg/mL (mitotic index 55.6 % of control); at the next highest dose, the mitotic index was reduced to < 7 % of controls

	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 2 in the 28 hour harvest in the absence of S9, statistically significant increases in the frequency of structural chromosome aberrations for 10 and 100 μg/mL (3 % aberrant cells) were attributed to the low solvent control values (0.5 % aberrant cells), as the values are within the historical control range of 0 – 4 %; accordingly these observations were not considered biologically significant
	statistically significant increases in cells showing structural chromosome aberrations occurred for the positive control substances, indicating that the test system responded appropriately
Comment:	due to the high cytotoxicity observed at the doses higher than those evaluated, it was not possible to evaluate doses where there was a significant reduction in the mitotic index
Results:	the notified substance did not induce structural chromosome aberrations in the presence or absence of metabolic activation

9.4 Overall Assessment of Toxicological Data

The acute oral toxicity of the notified chemical in rats is very low (LD_{50} > 2000 mg/kg) and the acute dermal toxicity in rats is low (LD_{50} > 2000 mg/kg). It was found to be non-irritating to rabbit skin. It was not a skin sensitiser in guinea pigs.

The notified chemical is not irritating to rabbit eyes, although some staining was observed. The staining of the sclera and of the conjunctiva with the exception of the nictating membrane was resolved by 72 hours; the staining of the nictating membrane was resolved by 14 days after application. The eye staining is therefore persistent, but is not of sufficient severity to warrant classification as producing a risk of serious eye damage.

No acute inhalation study on the notified chemical was provided by the notifier.

In a 28 day oral repeat dose study in rats, a NOAEL of 200 mg/kg/day was established, based on changes in clinical biochemistry seen in the 1000 mg/kg/day animals. The changes in clinical biochemistry, with the exception of lower potassium levels in males, were reversible after 14 days recovery. A NOEL was not established due to the presence of treatment-related urine discolouration at the lowest dose tested i.e. 50 mg/kg/day.

The notified chemical was not mutagenic in bacterial test systems, and it did not induce chromosomal aberrations in Chinese hamster V79 cells *in vitro*.

10. ASSESSMENT OF ENVIRONMENTAL EFFECTS

Test	*Species*	*Nominal Test Concentrations*	*Results*
acute toxicity (static test) (OECD TG 203)	zebra fish *(Brachydanio rerio)*	45, 100 mg/L	96 h LC_{50} > 33 mg/L
acute toxicity – immobilisation (semi-static test) (OECD TG 202)	water flea *Daphnia magna*	300 mg/L	48 h EC_{50} > 259 mg/L
growth inhibition - growth (μ) biomass (b) (static test) (OECD TG 201)	green algae *(Scenedesmus subspicatus)*	1.0, 3.2, 10, 32, 100 mg/L	Experiment A $E\mu C_{50}$ > 100 mg/L E_bC_{50} = 40.9 mg/L Experiment B $E\mu C_{50}$ > 100 mg/L E_bC_{50} > 100 mg/L LOEC = 3.2 mg/L
respiration inhibition (OECD TG 209)	activated sludge-aerobic waste water bacteria	1.0, 3.2, 10, 32, 50, 100 mg/L	3 h IC_{50} > 100 mg/L

Fish
The acute toxicity of the notified dye to Zebra fish was determined in a 96 hour static test (Bottcher, 1997). No intoxication symptoms were observed and no fish died during the 96 hour test period. The 96 hour LC_{50} of the notified dye was determined to be > 33 mg/L, the average measured concentration during the test period.

The test concentration was taken from a stock solution made up by dissolving 0.5 g of test substance in 200 mL of water. This was homogenised for 5 minutes by ultrasonication then made up to 500 mL. The notifier did not indicate whether there was undissolved sediment in the test media, though undissolved material, assumed to be impurities, was encountered in solubility tests (see Section 3 above). The analytically determined test substance concentrations in the test media samples varied from 10 % to 70 % of the nominal values. These values were determined by spectrophotometric measurements.

Aquatic Invertebrates
The acute toxicity of the notified dye to *Daphnia magna* was determined in a 48 hour semi-static test (Hertl, 1997a). No immobilisation was observed and no daphnia died during the 48 hour test period. The 48 hour LC_{50} of the notified dye was determined to be > 259 mg/L, the average measured concentration during the test period. During the renewal period of 24 hours the test substance concentration decreased to 72 % of nominal. Again the notifier gave no indication whether there was undissolved sediment in the test media.

A reproduction test was not supplied. However, based on the low acute toxicity to both fish and daphnia, no reproduction effects on daphnia at likely environmental levels are expected.

Algae

The influence of Reactive Orange DER 8089 on the growth of the green algae *Scenedesmus subspicatus* was investigated in a 72 hour static test (Hertl, 1997b).

The test included two parts:

Part A: the algae grew in test media with suspended dyestuff in Erlenmeyer flasks, each placed in a black cylinder. The cylinders were covered with glass dishes, containing untreated test water

Part B: the glass dishes above the cylinders contained the coloured test media suspensions without algae. In the Erlenmeyer flasks below, the algae grew in test water without dyestuff, however, under changed light conditions due to the filter effect of the coloured test media in the glass dishes above.

The nominal test concentrations were 1.0, 3.2, 10, 32 and 100 mg/L. All test media were slightly to strongly coloured by the test substance.

During the 72 hour test period a decrease of test substance in the test media to 42 – 94 % of the nominal values.

The E_bC_{50} for parts A and B were determined to be 40.9 and > 100 mg/L, respectively. These results demonstrate that the observed biomass inhibition effect of Reactive Orange DER 8089 on *Scenedesmus subspicatus* was due to a direct toxic effect and not due to an indirect effect of light absorption in the test media. The lowest observed effect concentration was 3.2 mg/L.

It should be noted that for environmental purposes, growth inhibition whether due to either chemical or physical factors, is still of relevance. Algistatic effects may still lead to an undesirable environmental impact if exposure is continuous.

Microorganisms
The inhibitory effect of the notified substance on aerobic wastewater bacteria, activated sludge from a domestic wastewater treatment plant, was investigated in a respiration test (Grutzner, 1997a). The notified substance showed very little inhibition (-3.8 % to 4.9 %) on the respiration rate at concentrations ranging from 3.2 mg/L to 100 mg/L. The final 3 hour IC_{50} was determined to be > 100 mg/L. Measured concentrations did not appear in the test report so the IC_{50} may be below 100 mg/L due to some dissipation.

Conclusion
The ecotoxicity data for the notified substance indicates that it is slightly toxic to fish and algae and practically non-toxic to aquatic invertebrates and microorganisms.

11. ASSESSMENT OF ENVIRONMENTAL HAZARD

The environmental hazard from the dye, when fixed to textile fibres, is rated as low.

Estimations of the Predicted Environmental Concentrations (PEC) for use in wool and cotton textile dyeing modeled on three sites, each using 5000 kg (for wool) or 8333 kg (for cotton) per year of the commercial form of the notified chemical have been made using data provided by the notifier. The assumed fixation rates were 98 % for wool and 76 – 77 % for cotton.

Wool Textiles

Calculation Factor	*City dyehouse*	*Country dyehouse*
Commercial dyestuff consumed per day	1.55 kg	1.55 kg
Fixation rate	98 %	98 %
Substance not fixed to fibres	0.031 kg	0.031 kg
Mill effluent per day	1000000 L	1000000 L
Dye concentration in effluent	0.031 mg/L	0.031 mg/L
Dilution in STP	250 fold	50 fold
Concentration after dilution in STP	0.00012 mg/L	0.00062 mg/L
Dilution factor in receiving waters	1:10	1:3
Predicted Environmental Concentration	1.2×10^{-5} mg/L	2.1×10^{-4} mg/L
Safety factor for exposure of most sensitive aquatic organism *Algae* (E_bC_{50} = 40.9 mg/L)	> 1000000	> 190000

Cotton Textiles

Calculation Factor	*City dyehouse*	*Country dyehouse*
Commercial dyestuff consumed per day	2.58 kg	2.58 kg
Fixation rate	Assume 76-77 %	Assume 76-77 %
Substance not fixed to fibres	0.59 kg	0.59 kg
Mill effluent per day	1000000 L	260000 L
Dye concentration in effluent	0.59 mg/L	2.28 mg/L
Dilution in STP	250 fold	37 fold
Concentration after dilution in STP	0.0024 mg/L	0.061 mg/L
Dilution factor in receiving waters	1:10	1:3
Predicted Environmental Concentration	2.4×10^{-4} mg/L	0.02 mg/L
Safety factor for exposure of most sensitive aquatic organism *Algae* (E_bC_{50} = 40.9 mg/L)	> 170000	> 2000

The calculations show that the exposure to fish, daphnia, algae and wastewater treatment bacteria is at levels unlikely to cause any significant effect. Once in the aquatic environment, the chemical is also expected to swiftly dilute to undetectable concentrations, dissipate to sediment and undergo slow biotic and abiotic degradation.

The only other source of environmental contamination is from accidental spills and disposal of packaging. The information provided in the MSDS is adequate to enable clean-up operators to limit the environmental exposure and environmental effects.

In the event of accidental spillage of the dyestuff into waterways, the chemical is expected to disperse into the water due to its high water solubility but also settle out onto sediments. If

the dyestuff is spilt on land, either during usage or transport, it is expected that the chemical would become immobilised in the soil layer. Contaminated soil can then be collected and disposed of to landfill.

Solid waste consigned to landfill, either from spillages or from residues in packaging, would be expected to be retained at the landfill sites and not be mobile. Movement of the chemical by leaching from landfill sites is not expected because of its lack of mobility due to its high binding affinity to soil.

Given the above, environmental exposure and the overall environmental hazard is expected to be low.

12. ASSESSMENT OF PUBLIC AND OCCUPATIONAL HEALTH AND SAFETY EFFECTS

The acute toxicity of Reactive Orange DER 8089 is low, and it is not an irritant to the skin or 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 notified chemical was negative in a Buehler 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. The imported products containing the notified chemical also contain a dye which is a known sensitiser.

For longer-term systemic effects, the NOAEL is 200 mg/kg/day, based on clinical biochemistry changes observed at 1000 mg/kg/day in a 28 day oral rat study. As discolouration of urine was observed at all doses no NOEL can be established. No long term toxicological studies such as a worker health study were provided.

The powdered solid as produced includes 9.9 % 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 and that the efficacy of the additive is tested during quality control procedures. The low acute dermal toxicity and high molecular weight of the main component of Reactive Orange DER 8089 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 1 % 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.

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 downdraught weighing booth, which would be expected to substantially reduce dust exposure.

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 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.

The notifier recommends an exposure limit of 1 mg/m^3. This is based on a UK chemical industry limit for reactive dyes which are not respiratory sensitisers.

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
Public contact will occur from touching dyed fabric in clothes. Greater than 98 % of the dye is fixed to wool and 76 – 77 % to cotton fabrics by covalent bonding. Tests including alkali and hot (70°C) water washes, peroxide, chlorine and sea water treatments have shown high levels of colour fastness. Consequently, the dye fixed to fabrics will be biologically unavailable and the potential for public exposure to the notified chemical throughout all phases of its life cycle is considered to be low.

13. RECOMMENDATIONS

To minimise occupational exposure to Reactive Orange DER 8089 the following guidelines and precautions should be observed:

- Respiratory protection should be used while handling the powdered dyestuffs; a particulate filter should be used if local exhaust ventilation is present, otherwise a half face mask according to Australian Standard (AS) 1716 (Standards Australia/Standards New Zealand, 1994a) should be used;

- 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);

- Industrial clothing should conform to the specifications detailed in AS 2919 (Standards Australia, 1987) and AS 3765.2 (Standards Australia, 1990); impermeable gloves should conform to AS/NZS 2161.2 (Standards Australia/Standards New Zealand, 1998); all occupational footwear should conform to AS/NZS 2210 (Standards Australia/Standards New Zealand, 1994b);

- An exposure limit of 1 mg/m^3 is specified by the notifier, based on a UK chemical industry limit; nevertheless, given the potential for skin and respiratory sensitisation, exposure to the notified chemical in the workplace should be controlled to be as low as is reasonably achievable;

- Individuals who become sensitised should not continue to handle the notified chemical;

- Spillage of the notified chemical should be avoided. Spillages should be cleaned up promptly with absorbents which should be put into containers for disposal;

- Good personal hygiene should be practised to minimise the potential for ingestion;

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

If the conditions of use are varied, then greater exposure of the public may occur. In such circumstances, secondary notification may be required to assess the hazards to public health.

14. MATERIAL SAFETY DATA SHEET

The MSDS for the notified chemical was provided in a format consistent with the *National Code of Practice for the Preparation of Material Safety Data Sheets* (National Occupational Health and Safety Commission, 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.

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.

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Attachment 1

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

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

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

CORNEA

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

CONJUNCTIVAE

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