File No: NA/855

January 2001

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME

FULL PUBLIC REPORT

1-Naphthalenesulfonic acid, 4-amino-3-[[4-[[2-(sulfooxy) ethyl] sulfonyl] phenyl] azo]-disodium salt

This Assessment has been compiled in accordance with the provisions of the *Industrial Chemicals (Notification and Assessment) Act* 1989 (the Act) and Regulations. This legislation is an Act of the Commonwealth of Australia. The National Industrial Chemicals Notification and Assessment Scheme (NICNAS) is administered by the National Occupational Health and Safety Commission which also conducts the occupational health & safety assessment. The assessment of environmental hazard is conducted by the Department of the Environment and the assessment of public health is conducted by the Department of Health and Aged Care.

For the purposes of subsection 78(1) of the Act, copies of this full public report may be inspected by the public at the Library, National Occupational Health and Safety Commission, 92-94 Parramatta Road, Camperdown NSW 2050, between the following hours:

Monday - Wednesday	8.30 am - 5.00 pm
Thursday	8.30 am - 8.00 pm
Friday	8.30 am - 5.00 pm

Copies of this full public report may also be requested, free of charge, by contacting the Administration Coordinator on the fax number below.

For enquiries please contact the Administration Coordinator at:

Street Address: 92 -94 Parramatta Rd CAMPERDOWN NSW 2050, AUSTRALIA
Postal Address: GPO Box 58, SYDNEY NSW 2001, AUSTRALIA
Telephone: (61) (02) 9577 9514 FAX (61) (02) 9577 9465

Director
Chemicals Notification and Assessment

TABLE OF CONTENTS

FULL PUBLIC REPORT

1-Naphthalenesulfonic acid, 4-amino-3-[[4-[[2-(sulfooxy) ethyl] sulfonyl] phenyl] azo]-disodium salt

1. APPLICANT

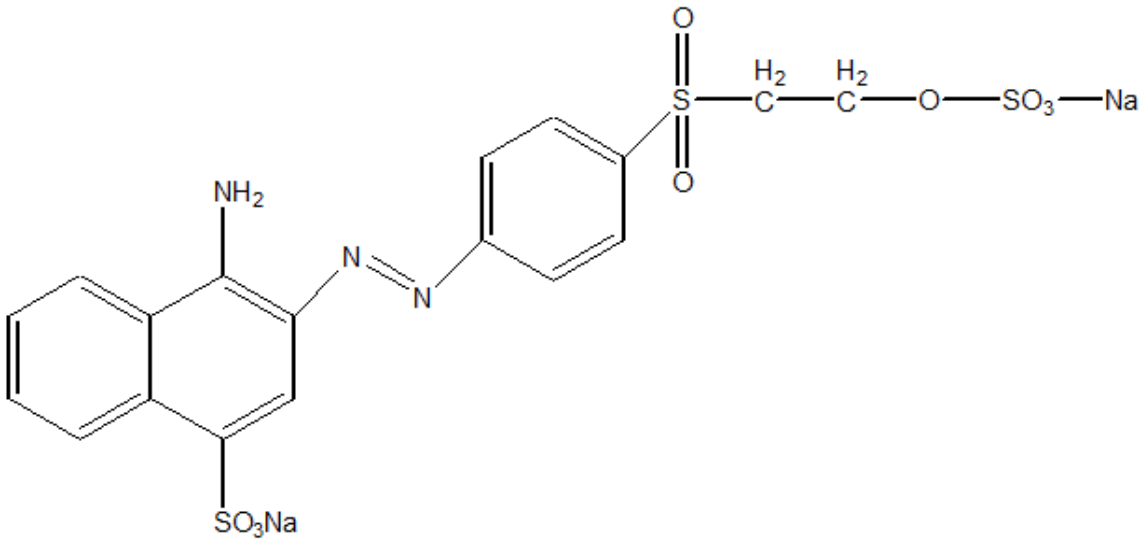
Bayer Australia Limited of 633-47 Springvale Road MULGRAVE VIC 3170 (ACN 000 138 714) has submitted a standard notification statement in support of their application for an assessment certificate for 1-Naphthalenesulfonic acid, 4-amino-3-[[4-[[2-(sulfooxy) ethyl] sulfonyl] phenyl] azo]-disodium salt.

2. IDENTITY OF THE CHEMICAL

The notifier has not applied for any information to be been exempted from publication in the Full Public Report and the Summary Report.

Chemical Name:	1-Naphthalenesulfonic acid, 4-amino-3-[[4-[[2-(sulfooxy) ethyl] sulfonyl] phenyl] azo]-disodium salt.
Chemical Abstracts Service (CAS) Registry No.:	250688-43-8
Other Names:	4-Amino-3-[4-(2-sulfooxy-ethanesulphonyl)-phenylazo]-naphthaline-1-sulfonic acid, sodium salt.
Marketing Name:	Reactive Orange DYPR 1410; Remazol Black NF liquid (containing 9.55% Reactive Orange DYPR 1410)
Molecular Weight:	559.5
Method of Detection and Determination:	IR, UV-Vis and NMR.
Spectral Data:	IR, UV-Vis and NMR spectra were provided.
Molecular Formula:	$C_{18}H_{15}N_3O_9S_3Na_2$

Structural Formula:



3. PHYSICAL AND CHEMICAL PROPERTIES

Most data on physical and chemical properties were determined from the notified chemical.

Appearance at 20°C & 101.3 kPa:	Orange powder (the notified chemical), A black odourless liquid (product containing the notified chemical).
Melting Point:	Decomposed when >170°C
Specific Gravity:	1 490 kg/m^3
Vapour Pressure:	< 10^{-4} kPa at 25°C
Water Solubility:	255.6 g/L at 20°C (readily soluble).
Partition Co-efficient (n-octanol/water):	log P_{ow}=-5.0 at 20°C

Hydrolysis as a Function of pH:

pH 4	pH 7	pH 9
50°C, $t_{½}$=155.26 h	35°C, $t_{½}$=17.72 h	50°C, $t_{½}$=2.4 h
60°C, $t_{½}$=60.82 h	50°C, $t_{½}$=2.99 h	
70°C, $t_{½}$=30.45 h		
25°C, $t_{½}$=1572 h*	25°C, $t_{½}$=64.0 h*	

* extrapolated data

Adsorption/Desorption:	The binding to organic matter in soil is expected to be low.

Dissociation Constant:	pK_a=1.0 to 5.0 (arylamine group); pK_a=-1.0 to 1.0 (sulfonate group); pK_a=-4.0 to -2.0 (sulfate group).
Particle size:	20-200 μm.
Flash Point:	Not determined.
Flammability Limits:	Not flammable.
Autoignition Temperature:	>404°C
Explosive Properties:	Not explosive.
Reactivity/Stability:	Stable under the conditions of intended use.
Surface Tension:	70.0 mN/m at 20°C.

3.1 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 water solubility was determined using the flask method guideline 92/69/EEC A.6 (Santos and Muhlberger, 1999a). At 255.6 g/L the chemical would be considered readily soluble.

Hydrolysis as a function of pH was determined using guideline 92/69/EEC C.7 (Muhlberger and Henkelmann, 1999). At pH 4 and 7 the half-lives were determined via extrapolation of the test results. At pH 9 after 2.4 hours the test material had decomposed by more than 50%. The results indicate that the rate of hydrolysis at pH 7–9 range is rapid, while at pH 4 it is slow (ie approximately 225 days).

Partition Coefficient was determined using guideline 92/69/EEC A.8 (Santos and Muhlberger, 1999b). Test solutions were prepared by dissolving dye in n-octanol. Aliquots (2 mL) of the n-octanol phase were extracted with water. The water phase was run though a HPLC. The solubility of the active ingredient in n-octanol was 2.336 mg/L. This value was used to calculate the log P_{ow} of -5.0.

Adsorption/Desorption was not performed. High water solubility and a low partition coefficient normally indicates low affinity for soil or sediment.

The dissociation constant was not determined. However the notifier provided the dissociation constants for functional groups in the chemical.

The surface tension was determined using guideline 92/69/EEC (1992) Annex (Hoffmann, 1999). The surface tension at 1.0 g/L of the notified chemical was measured as 70 mN/m, indicating that the material is not surface active.

4. PURITY OF THE CHEMICAL

Degree of Purity: 73±2%

Hazardous Impurities: None.

Non-hazardous Impurities (> 1% by weight):

Chemical name: Water
Weight percentage: 4.4
CAS No.: 7732-18-5

Chemical name: Sodium chloride
Weight percentage: 9.2
CAS No.: 7647-14-5

Chemical name: Sodium sulfate
Weight percentage: 2.4
CAS No.: 7757-82-6

Additives/Adjuvants: None

Comments

Based on the HPLC results, the notified chemical also contains 10.8% unidentified organics. The chemical identifications, physical and chemical properties, and hazardous nature of these organics are unknown.

5. USE, VOLUME AND FORMULATION

The notified chemical, Reactive Orange DYPR 1410, is to be used for the colouration of cellulose textile materials.

The notifier estimates that the notified chemical will be imported at 3 tonnes per year over the first 5 years.

Reactive Orange DYPR 1410 will be imported as a component (9.55%) of the product Remazol Black NF. Remazol Black NF is formulated in a liquid form and packaged in 1 000 L Schutz containers. The dye product will be used for the colouration of cellulose textiles by the exhaust dye method only.

6. OCCUPATIONAL EXPOSURE

The notifier provided number and category of workers who could be exposed to the notified chemical.

Category	*Number*	*Duration*		*Concentration of the notified chemical*
		Hours/day	*Days/year*	
Transport & storage	4-6	2	10	9.55%
Weighing & mixing	60	0.5	200	9.55%
Laboratory technician	5	0.5	100	9.55% & <1%
Dyeing	160	1.0	200	<1%
Curing/rinsing/drying	100	0.5	200	<1%

Transport and Storage
Remazol Black NF liquid will be imported in 1 000 L Schutz containers. Transport and storage workers will unload and transfer the containers to the notifier's warehouse by road. The containers will be stored under cover in a bunded area. These workers could only be exposed to the notified chemical in the case of an accident where the packaging was breached.

Weighing and Mixing
There will be 2 workers per shift (2 shifts a day) at each dyehouse involving weighing and mixing. Approximately 2 kg notified chemical per batch will be measured (using metered dosing equipment) and pumped from the Schutz container into an enclosed dilution tank to prepare the dye solution. This is expected to be done about ten times per shift. The diluted dye solution containing less than 1% notified chemical will be pumped into the dyeing machine.

Occupational exposure to the notified chemical will be to the dye product containing 9.55% notified chemical, as well as to <1% in the dye solution, mainly by dermal and ocular routes. The notifier states that the workers involved in the weighing and mixing procedures will follow existing procedures in the dyehouses that require workers to wear overalls, protective gloves and glasses, and use respiratory protection. Mechanical ventilation of the weighing area will remove any build up of dye mist.

Dyeing
The dye solution (<1% notified chemical) will be manually transferred to a feed tank then automatically sprayed onto cloth on a continuous roller in an enclosed dyeing machine. Skin contamination may occur during handling of the dye solution. There is further possibility of worker exposure if the cloth becomes tangled and the machine has to be opened to realign it on the rollers. The machine is also opened on a regular basis to clean loose fibres out of the filter with a hose. It is estimated that about 8 workers per shift (two shifts per day) will be exposed during these activities. Exposure episodes would be frequent, given the frequency of use of the dye. Individual exposure times may be no more than several minutes per day as the dye application occurs for 20 minutes of the 3 hour dyeing cycle.

The used dye solution will then go into the waste stream. The dyed cloth is fixed at low pH, then washed in warm soapy water to remove any free dye at the end of the dyeing cycle. Dyeing machines are regularly washed by hosing. The fixation level is given as >80% (data not provided).

Operators of the dyeing machines wear gloves and glasses when handling dye solutions and for threading the cloth. During cleaning up and maintenance in dyeing plants, personnel will be wearing overalls, protective gloves and glasses and respiratory protection.

Drying
In each dyehouse, 4 operators per shift (two shifts a day) are normally involved in drying treated cloth. This involves manually loading the wet dyed cloth into dryers. The concentrations of free dye at this time are expected to be low as the dye is fixed to the cloth and the excess has been washed out during the dyeing process. The exposure for each worker to this particular dye is expected to be 45 minutes per shift, mainly by dermal route.

Drying will be performed by the same personnel in the dyeing, cleaning up and maintenance processes. The workers involved in drying will wear the same PPE as dyeing machine operators.

Laboratory
The notifier estimates that 2 laboratory technicians in each customer facility (dyehouse) and also 5 technicians at the importer facility will be involved in weighing and mixing small samples of the dye for colour matching. The exposure time is estimated to be several minutes per day. Laboratory technicians may be exposed to the notified chemical in either dye product or dye solution by dermal and/or ocular routes during these activities.

Laboratory technicians are expected to wear lab coats, gloves and safety glasses as standard protective measures.

7. PUBLIC EXPOSURE

The notified chemical, as a component (9.55%) of Remazol Black NF, will be imported into Australia in 1 000 L Schutz containers and stored at the notifier's warehouse. To this point, the public could only be exposed to the notified chemical in the event of an accidental spill. In the event of a spill, the notified chemical should be contained with absorbent material and prevented from entering drains. Disposal should occur according to local laws and regulations. The product will be sold to dyehouses, where dyeing solutions containing less than 1% notified chemical will be used for dyeing textile fabrics. The public will come in contact with fabrics that have been dyed. However, since the dye will be covalently bound to fabrics, the risk to the general public is considered to be low.

8. ENVIRONMENTAL EXPOSURE

8.1 Release

Most dye will become chemically fixed to the cellulosic textiles and is not expected to impact on the environment. After application to fabrics, the dye forms chemical bonds with hydroxy groups on the cellulose fibres.

The rinsate generated from fabric rinsing should only contain approximately 20% of the dye. This will be combined with other waste water (including equipment cleaning and spill clean-up waste) from the dyehouse and will be disposed of to the sewer as industrial washwater. This will represent the major route of environmental exposure.

Any solid waste generated at the dyehouse will go to a secure landfill.

It has been estimated that 0.5% of container contents will remain as residue. This equates to 15 kg of waste notified chemical annually. This residue will be washed out and the containers recycled. The resultant washwater will be disposed of by a licensed waste disposal contractor to a secure landfill.

8.2 Fate

Approximately 20%, 600 kg annually, of the imported notified chemical will end up in dyehouse waste water. This equates to an annual PEC of approximately 1 µg/L in receiving waters to the sewer and is expected to be the major environmental exposure.

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

Notified chemical in dye residue released into landfill would be expected to be mobile and degrade very slowly via biotic and abiotic processes. However, with disposal to secure landfill, the risk of leaching to the water table will be significantly reduced.

Once the dye has fixed to the cloth it will be stable. Therefore the dye and cloth will share the same fate. The dye would remain inert on cloth disposed of to landfill.

Ready Biodegradability of the notified chemical was tested by the modified closed bottle method under guideline 92/69/EEC C.4-E (Caspers and Muller, 1999). Aniline (99.5% pure) was used as the reference substance at 2.0 mg/L, and had a degradation of 85% within 14 days. Secondary effluent from a domestic sewage treatment plant was used as the inoculum, at 5 mL/L. After 28 days only 1% of the theoretical oxygen consumption occurred, therefore the notified chemical cannot be considered as readily biodegradable.

The log P_{OW} of –5.0 suggests that the notified chemical is hydrophilic and will be mobile in soil and sediments. Although the notified chemcial is not readily biodegradable, the potential for bioaccumulation is low due to the low partition coefficient and ready water solubility. Hydrophilic dyes with log $P_{OW} < 3$ have been shown not to bioaccumulate (Yen, 1991).

9. EVALUATION OF TOXICOLOGICAL DATA

All the toxicological studies on the notified chemical were conducted under the guidelines of OECD/EEC/USEPA with GLP compliance by Hoechst Marion Roussel Deutschiand GmbH in Germany.

9.1 Acute Toxicity

Summary of the acute toxicity of Reactive Orange DYPR 1410

Test	*Species*	*Outcome*	*Reference*
acute oral toxicity	rat	LD_{50} > 2 000 mg/kg	(Jensch, 1999a)
acute dermal toxicity	rat	LD_{50} > 2 000 mg/kg	(Jensch, 1999b)
skin irritation	rabbit	Slightly irritating	(Kreiling, 1999a)
eye irritation	rabbit	Slightly irritating	(Kreiling, 1999b)
skin sensitisation	guinea pig	Non sensitising	(Jensch, 1999c)

9.1.1 Oral Toxicity (Jensch, 1999a)

Species/strain:	Rat/Sprague Dawley.
Number/sex of animals:	5/sex.
Observation period:	14 days.
Method of administration:	The notified chemical (20% in water) was given by gavage at 2 000 mg/kg.
Test method:	OECD TG 401.
Mortality:	None.
Clinical observations:	One female had weight loss between Day 8 and 15. Reddish discoloured faeces were observed in some male and female rats after dosing. All clinical signs were reversed after Day 2.
Morphological findings:	No visible changes in macroscopic examination.
Comment:	None.
LD_{50}:	> 2 000 mg/kg.
Result:	The notified chemical was of very low acute oral toxicity in rats.

9.1.2 Dermal Toxicity (Jensch, 1999b)

Species/strain:	Rat/Sprague Dawley.
Number/sex of animals:	5/sex.
Observation period:	14 days.
Method of administration:	The notified chemical in water (5:3, w/v) was applied under an occlusive dressing at 2 000 mg/kg for 24 hours.
Test method:	OECD TG 402
Mortality:	None.
Clinical observations:	One female had weight loss between Day 1 and 8. Orange discoloured skin was observed in animals frm Day 2 till end of the study.
Morphological findings:	No visible changes in macroscopic examination.
Comment:	None.
LD_{50}:	> 2 000 mg/kg.
Result:	The notified chemical was of low dermal toxicity in rats.

9.1.3 Inhalation Toxicity

No inhalation toxicity study was provided.

9.1.4 Skin Irritation (Kreiling, 1999a)

Species/strain:	Rabbit/New Zealand White.
Number/sex of animals:	3 females.
Observation period:	72 hours.
Method of administration:	The notified chemical (0.5 g) in water (0.3 mL) was applied under a semi-occlusive dressing for 4 hours.
Test method:	OECD TG 404.

Draize scores:

	Time after treatment (hours)			
	0.5-1	***24***	***48***	***72***
Erythema				
1	[a]1	1	0	0
2	1	2	1	0
3	1	1	0	0
Oedema				
1	0	0	0	0
2	0	1	0	0
3	0	0	0	0

[a] see Attachment 1 for Draize scales

Individual mean scores were 0.3:1:0.3 and 0:0.3:0 for erythema and oedema, respectively.

Comment: The skin of the animals were discoloured orange during the whole observation period.

Result: The notified chemical was slightly irritating to the skin of rabbits.

9.1.5 Eye Irritation (Kreiling, 1999b)

Species/strain: Rabbit/New Zealand White.

Number/sex of animals: 3 females.

Observation period: 72 hours.

Method of administration: The notified chemical (100 mg) was administered to the conjunctival sac of the left eye. The untreated eye served as a control.

Test method: OECD TG 405.

Draize scores:

	Time after instillation											
Animal	***1 hour***			***1 day***			***2 days***			***3 days***		
Cornea	***o***		***a***	***o***		***a***	***o***		***a***	***o***		***a***
1	[1]0		0	0		0	0		0	0		0
2	0		0	0		0	0		0	0		0
3	0		0	0		0	0		0	0		0
Iris												
1		0			0			0			0	
2		0			0			0			0	
3		0			0			0			0	
Conjunctiva	***r***	***c***	***d***	***r***	***c***	***d***	***r***	***c***	***d***	***r***	***c***	***d***
1	1	1	0	2	1	0	1	0	0	0	0	0
2	1	1	1	1	1	0	0	0	0	0	0	0
3	1	1	1	0	0	0	0	0	0	0	0	0

[1] see Attachment 1 for Draize scales
o = opacity a = area r = redness c = chemosis d = discharge

Individual mean scores were 0:0:0, 0:0:0, 0:0:0, 1:0.3:0, 0.3:0.3:0 and 0:0:0 for cornea opacity, cornea area, iris, redness, chemosis and discharge, respectively.

Comment: Up to 48 hours post-dosing, conjunctivae of the rabbits showed definitely injected blood vessels up to a diffuse crimson red colour and very slight swelling. Two rabbits had reddish or substance coloured discharge 1 hour after dosing. All signs of irritation were reversed at 72 hours.

Result: The notified chemical was slightly irritating to the eyes of rabbits.

9.1.6 Skin Sensitisation (Jensch, 1999c)

Species/strain/sex: Female guinea pig/Pirbright-White.

Number of animals: Test group: 10;
Control group: 5.

Induction procedure:

test group: Intradermal Induction:
day 1 Three pairs of intradermal injections (0.1 mL) into the dorsal area in the vicinity of the shoulders:
- Freund's complete adjuvant (FCA) 1:1 in deionized

water;
- 5% the notified chemical in deionized water;
- 5% the notified chemical in a 50:50 mixture of FCA and deionized water.

day 8 — Topical Induction:
A 48-hour occluded application of 0.5 mL of the notified chemical (25% in water) to the treated area.

control group: — Treated similarly to the test animals using deionized water instead of the notified chemical in the intradermal injections and topical application.

Challenge procedure:

day 22 — Test and Control animals:
A 24 hour occluded application of 25% of the notified chemical in water (0.5 mL) to the left flank of each animal.

day 29 — Repeated dermal challenge treatment as Day 22.

Test method: OECD TG 406.

Challenge outcome:

	Test animals		***Control animals***	
Challenge concentration	***24 hours****	***48 hours****	***24 hours***	***48 hours***
25% (first challenge)	**0/9	0/9	0/5	0/5
25% (second challenge)	0/9	0/9	0/5	0/5

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

Comment: One animal of the test group died on Day 10. No treatment related toxic effects could be found.

Result: The notified chemical was not sensitising to the skin of guinea pigs at a challenge concentration of 25%.

9.2 Repeated Dose Toxicity (Hofmann, 1999)

Species/strain: Rat/Sprague Dawley

Number/sex of animals: 5 sex/group

Method of administration: Oral (gavage) administration daily for 28 days.

Dose/Study duration: Group 1: 0 mg/kg/day;

Group 2: 62.5 mg/kg/day;
Group 3: 250 mg/kg/day; and
Group 4: 1 000 mg/kg/day
(vehicle: water).

Groups 1 and 4 had additional 5/sex as recovery groups which were terminated after a recovery period of 14 days.

Test method: OECD TG 407.

Clinical observations:
No death occurred throughout the study. Bodyweights, and food and water consumption remained unaffected by the treatment. No treatment related toxic effects in behaviour, neurotoxicological examinations, or state of health were observed in both treatment groups and the recovery groups.

Clinical chemistry/Haematology
There was an increase in total bilirubin level in Group 4 males. Increases in protein and albumin values were also found in Group 3 (without dose-dependance) and 4 males. The recovery Group 4 had increases in cholesterol levels in males and in direct bilirubin levels in females.

A slight increase in leukocyte counts was found in Group 4 females, and a decrease in red blood cell and reticulocyte counts were observed in Group 4 males. No statistically significant changes were found in the recovery groups.

Discoloured urine was seen in Group 4 males. The sediments were inconspicuous.

Histopathology:
No treatment related changes were observed in histopathological examinations.

Comment:
A decrease in relative brain weight was seen in Group 4 females probably due to the higher bodyweight in this group. An increase in relative testes weight was found in the recovery Group 4 males probably due to the lower bodyweight of this group.

Result:
The No Observed Effect Level (NOEL) is established to be 250 mg/kg/day based on the clinical chemistry and haematology changes at the next highest dose.

9.3 Genotoxicity

9.3.1 *Salmonella typhimurium* and *Escherichia coli* Reverse Mutation Assay (Stammberger, 1999a)

Strains: *S. typhimurium* TA100, TA1535, TA1537 and TA98;
E. coli WP2uvrA.

Metabolic activation: Liver fraction (S9 mix) from rats pretreated with Aroclor

1254

Concentration range: 0, 50, 160, 500, 1 600 and 5 000 μg/plate (one preincubation test also included 3 000 μg/plate for TA100).

One plate incorporation test and 1 preincubation test were conducted, each concentration was tested in triplicate, either with or without metabolic activation.

Positive controls (without metabolic activation):
Sodium azide for TA100 and TA1535;
9-aminoacridine for TA1537;
2-nitrofluorene for TA98; and
1-methyl-3-nitro-1-nitrosoguanidine (MNNG) for WP2uvrA.

Positive control (with metabolic activation):
2-aminoanthracene for all strains.

Test method: OECD TG 471

Comment: Three preincubation tests were conducted with TA100. In the first preincubation test, the positive control was non-mutagenic with S9-mix. In the second preincubation test, an increase of revertant count was seen at the highest concentration with S9-mix. In the third preincubation test, a concentration of 3 000 μg/plate was included, and the results confirmed that the notified chemical was not mutagenic in TA100 in the presence of S9-mix.

There were no significant increases in revertant colony numbers at any concentration, in the presence or absence of metabolic activation.

Concurrent positive controls used in the tests induced marked increases in the frequency of revertant colonies and the activity of the S9 fraction was found to be satisfactory. Historic data were included to support the findings.

Result: The notified chemical was non mutagenic under the conditions of the test.

9.3.2 Chromosomal Aberration Assay in Chinese Hamster Cells (Stammberger, 1999b)

Cells: V79 Chinese hamster lung fibroblasts

Metabolic activation system: Liver fraction (S9 mix) from rats pretreated with Aroclor 1254

Dosing schedule:

Metabolic Activation	Experiment/ Study Number	Test concentration (μg/mL)	Controls
-S9	1	treatment time = 3 hours 0, 250, 500, 1 000, 2 500 and 5 000 μg/mL.	Positive: EMS Negative: solvent
	2	treatment time = 20 hours 0, 25, 50, 100, 250 and 375* μg/mL.	
+S9	1	treatment time = 3 hours 0, 250, 500, 1 000, 2 500 and 5 000 μg/mL.	Positive: CP Negative: solvent

* - not evaluated due to high toxicity
EMS - ethyl methanesulphonate
CP - cyclophosphamide
Cell culture medium was used as the solvent.

Test method: OECD TG 473

Comment: In the preliminary test, precipitation occurred at 2 000 and 1 000 μg/mL in 3 and 20 hour experiments, respectively. Dose related cytotoxicity was observed at ≥2 000 μg/mL following 3 hour treatment, and at ≥500 μg/mL following 20 hour treatment without S9-mix. In the presence of S9-mix, mild toxicity was observed at ≥2 000 μg/mL.

In the main test, the mitotic index was reduced at the highest doses in both experiments with and without S9-mix. Treatment did not induce relevant increase in the number of polyploid cells. Significant increases (7-10%) in aberration rate were observed at 2 500 and 5 000 μg/mL (cytotoxic doses) following 3 hour treatment with and without S9-mix. No significant aberration rate increase was found following treatment at 20 hours without S9-mix.

Positive controls used in the test caused marked increases in the incidence of aberrant cells and the activity of the S9

	fraction was found to be satisfactory. Historic data were provided with the report.
Result:	The notified chemical was clastogenic under the conditions of the test.

9.3.3 Micronucleus Assay in the Bone Marrow Cells of the Mouse (Stammberger, 1999c)

Species/strain:	Mouse/NMRI.
Number and sex of animals:	5/sex/group.
Doses:	0 and 2 000 mg/kg (vehicle: water).
Method of administration:	The notified chemical was administered twice at an interval of 24 hours orally (gavage). Positive control: CP was give once orally (by gavage) at 50 mg/kg. All animals were sacrificed 24 hours after dosing.
Test method:	OECD TG 474.
Comment:	All animals survived after treatment with no clinical signs of toxicity except discoloured faeces and urine seen after second dosing. No significant increase in micronucleated polychromatic erythrocytes (PCEs) due to the treatment with the notified chemical. The ratio of PCEs to total erythrocytes remained unaffected. The positive control caused a significant increase in micronucleated PCEs.
Result:	The notified chemical was non clastogenic under the conditions of the test.

9.4 Overall Assessment of Toxicological Data

The notified chemical was of very low acute oral toxicity (LD_{50}>2 000 mg/kg) and low acute dermal toxicity (LD_{50}>2 000 mg/kg) in rats. The notified chemical was a slight skin and eye irritant in rabbits. In guineapigs, there was no evidence of dermal sensitisation in an adjuvant type test at a challenge concentration of 25%.

Oral administration of the notified chemical to rats at dose levels of 0, 62.5, 250 and 1 000 mg/kg/day for 28 consecutive days revealed no toxicologically significant findings in clinical signs, neurobehavioural effects or mortality. Clinical chemistry test found increases in total bilirubin and protein in males of the high dose group. Discoloured urine was also seen in the high dose males. All changes were reversed during the recovery period. No treatment related pathological or histopathological changes were found. The No Observed Effect Level (NOEL) determined for this 28 day oral study was 250 mg/kg/day.

There were three genotoxicity studies provided for the notified chemical. The notified chemical was not considered mutagenic in the bacterial reverse mutation test. In the *in vitro* chromosomal aberration test an increase in aberration rate of 7-10% was detected at cytotoxic doses of 2 500 and 5 000 µg/mL following a 3 hour exposure. Given the fact that the chemical was only clastogenic *in vitro* at concentrations that were cytotoxic and resulted in precipitation, the notified chemical is not considered to be a genotoxic hazard. This is further supported by the absence of an increase in chromosomal aberration rate or cytotoxicty at 2 500 and 5 000 µg/mL in the 20 hour *in vitro* test and a negative result in the *in vivo* micronucleus study.

Several azo dyes are human and experimental carcinogens. However, based on available data, the notified chemical is not classified as a hazardous substance according to the NOHSC *Approved Criteria for Classifying Hazardous Substances Classifying Hazardous Substances* (NOHSC, 1999).

10. ASSESSMENT OF ENVIRONMENTAL EFFECTS

It should be noted that the following ecotoxicity data are for the parent chemical. The tests were carried out according to OECD Test Methods by Caspers and Muller (1999).

Species	*Test*	*Concentrations[a] (mg/L)*	*Result (mg/L)*
Zebra fish (*Brachydanio rerio*)	96 h static acute	0 and 100	NOEC ≥ 79.6 LC_{50} > 100
Water Flea (*Daphnia magna*)	48 h acute	0 and 100	NOEC ≥ 86 EC_{50} ≥ 118
Algae (*Scenedesmus suspicatus*)	72 h growth	0, 1.9, 4.3, 9.4,20.7, 45.5 and 100	E_RC_{50} > 110.4 E_BC_{50} > 110.4 NOEC = 51.6 LOEC = 110.4
Activated Sludge	3 hr	0, 100, 1000 and 10 000	EC_{50} > 10 000

[a]test concentrations are nominal only.

Fish toxicity testing was carried out according to Council Directive 92/69/EEC C.1 (1992). The study was conducted at 0 and 100 mg/L nominal concentration. Every 24 hours the temperature, oxygen and pH in the test tanks were measured. The small variations observed in these parameters were acceptable. Only one fish mortality was observed during the 96

hour experiment at the 48 hour observation. The remaining fish continued to swim normally throughout the study. Since a LC_{50} end point was not observed in the study, the nominal LC_{50} is greater than 100 mg/L, while the NOEC was estimated to be greater than 79.6 mg/L, the mean measured concentration.

The daphnia acute toxicity study was based on Council Directive 92/69/EEC C.2 (1992). The concentrations used were 0 and 100 mg/L, and temperature, oxygen and pH in the test tanks were measured every 24 hours. These parameters did not vary over the 48 hours of the study. The mobility of the daphnia was not observed to be affected during the study. The notifier has estimated the nominal EC_{50} to be greater than 118 mg/L, while the NOEC was determined to be greater than 86 mg/L, the mean measured concentration.

The fresh water algal (*Scenedesmus subspicatus*) growth inhibition test was based on Council Directive 92/69/EEC (1992). The concentrations tested were 0, 1.9, 4.3, 9.4, 20.7, 45.5 and 100 with an initial cell density of 10 000 cells/mL. The results of the study were calculated from the analytically determined TOC values. The EC_{50} was less than 110.4 mg/L and the NOEC of the notified chemical 51.6 mg/L by the Dunnett-test.

The influence of the notified chemical on the respiration rate of activated sludge was investigated according to Council Directive 88/302/EEC or OECD 209 test method. The concentrations studied were 100, 1 000 and 10 000 mg/L with an incubation period of 3 hours. A concentration of 320 mg/L activated sludge was used. The temperature, pH and oxygen concentration were measured and a reference substance (3,5-dichlorophenol) was used. At the highest concentration of the test material, the inhibition observed was 1.2%, so the EC_{50} is estimated to be greater than 10 000 mg/L.

Based on the ecotoxicity data provided, the notified chemical is practically non-toxic to fish, daphnia, algae and waste water bacteria.

11. ASSESSMENT OF ENVIRONMENTAL HAZARD

The majority of the notified chemical will ultimately be released to landfill or incinerated once the dyed fabric is discarded. While the notified chemical is water soluble, when bound to the fabric, little release is expected. Except in the case of accidental release during transport, the primary source of release of the notified chemical will be via the waste water discharged from dyehouses. This release will be widespread and diffuse, and is unlikely to lead to toxic concentrations of the chemical.

Approximately 600 kg of the notified chemical is expected to enter the aquatic compartment per year as a result of the use of the dye. Ecotoxicity data indicate that the notified chemical is practically non-toxic to fish, daphnia, algae and waste water bacteria.

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

Volume of notified chemical (NC) imported	3 000 kg/year
Number of days NC used	230/year
Estimated amount of NC used per day	3 000/230 = 13.04 kg
Fixation Rate	80%

Amount of unfixed notified chemical	2.61 kg
Volume of wash water per day	2 ML
Concentration of notified chemical in wash water	1.305 mg/L
Dilution factor in STP	1:125 ML
Concentration of notified chemical in STP	10.4 µg/L
Dilution factor in receiving water	1:10
Concentration of NC in receiving water	1.04 µg/L

Taking into account the purity (73%) of the notified chemical the concentration of the active ingredient in the receiving water would be approximately 0.75 µg/L. These calculations assume that none of the notified chemical is removed during treatment of the different waste effluents and represent the worst case scenario for dyehouses.

The calculation shows 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 slow biotic and abiotic degradation, suggesting an adequate safety factor for use in country locations.

The only other source of environmental contamination is from accidental spills and disposal of packaging. The Material Safety Data Sheet (MSDS) contains adequate information to enable clean-up personnel to limit the environmental exposure and therefore limit the environmental effects.

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

When used as indicated, the new chemical is unlikely to present a hazard to the environment.

12. ASSESSMENT OF PUBLIC AND OCCUPATIONAL HEALTH AND SAFETY EFFECTS

The notified chemical was of very low acute oral toxicity and low acute dermal toxicity in rats, and was a slight irritant to the skin and eyes of rabbits. The high molecular weight of the notified chemical suggests that absorption via the skin is unlikely. The notified chemical was negative in a Magnusson and Kligman maximisation skin sensitisation study in guinea pigs.

In a 28 day repeat dose study in rats, changes in clinical chemistry and haematology parameters occurred at 1000 mg/kg/day. The NOEL was 250 mg/kg/day. The notified chemical was not mutagenic in an Ames test performed in *S. typhimurium* and *E. coli* strains. Following repeat testing in the *in vitro* chromosome aberration test, from the *in vivo* mouse micronucleus assay, it is concluded that the notified chemical is not clastogenic.

The notified chemical is not classified as a hazardous substance according to the NOHSC *Approved Criteria for Classifying HazardousSubstances*(NOHSC, 1999) based on the provided data. 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.

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

Occupational exposure to the notified chemical can be divided into exposure to the dye product containing 9.55% notified chemical, the <1% dye solution, and to the dyed cloth. The product containing the notified chemical is a liquid. Dermal and ocular exposure to drips and splashes is possible. In all cases, contact of dissolved dye with the skin and eyes should be avoided.

The workers involved in weighing and mixing the dye will be exposed to the 9.55% and <1% 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 should be provided. The combination of personal protective equipment and ventilation is expected to provide adequate protection from the notified chemical.

The dyeing machine operators will be exposed to the dye solution containing <1% notified chemical while manually filling the feed tank, and loading and realigning the fabric. 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.

Once the dyed cloth is fixed and washed, the amount of free dye is expected to be very low. The workers involved in drying the dyed and washed cloth will have little exposure as the excess dye will be removed from the cloth prior to this stage. After fixation to the textile, the potential hazard should be negligible.

Measures should also be implemented in the disposal of the notified chemical to ensure that exposure is avoided.

Laboratory workers will be exposed to small quantities of the notified chemical for short periods. The exposure could be in a variety of ways. Standard laboratory practices and personal protective equipment should be adequate to minimise health risk to those workers.

Public health
The notified chemical is imported at 9.55% in a dye product, sold to dyehouses and used to colour cellulose textiles. Except in the event of an accidental spillage during transportation, the public will only come in contact with the notified chemical once it has been covalently bound to fabric. Therefore, the risk of the notified chemical to the general public is considered to be low.

13. RECOMMENDATIONS

To minimise occupational exposure to 1-naphthalenesulfonic acid, 4-amino-3-[[4-[[2-(sulfooxy) ethyl] sulfonyl] phenyl] azo]-disodium salt the following guidelines and precautions should be observed:

- Respiratory protection should be selected and fitted according to Australian/New Zealand Standard (AS/NZS) 1715 (Standards Australia/Standards New Zealand, 1994b); safety goggles should be selected and fitted in accordance with AS 1336 (Standards Australia, 1994) to comply with 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 3765.1 (Standards Australia, 1990); impermeable gloves should conform to AS/NZS 2161.2 (Standards Australia/Standards New Zealand, 1998); all occupational footwear should conform to AS/NZS 2210 (Standards Australia/Standards New Zealand, 1994a);

- 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;

- 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.

If products containing the notified chemical are hazardous to health in accordance with the NOHSC *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 1999), workplace practices and control procedures consistent with State and Territory hazardous substances regulations must be in operation.

14. MATERIAL SAFETY DATA SHEET

The MSDS for the notified chemical and the product containing the notified chemical were provided in a format consistent with the *National Code of Practice for the Preparation of Material Safety Data Sheets* (NOHSC, 1994).

These MSDS were provided by the applicant as part of the notification statement. The MSDS for the product containing the notified chemical 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, the director must be informed if any of the circumstances stipulated under subsection 64(2) of the Act arise, and secondary notification of the notified chemical may be required. No other specific conditions are prescribed.

16. REFERENCES

Caspers and Muller (1999): Investigation of the Ecological Properties of Reactiv-orange DYPR 1410. Bayer AG.

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

Hoffmann, Dr H (1999): Surface Tension: Aventis Research & Technologies GmbH & Co KG, Analytical Technologies, Frankfurt am Main.

Hofmann, Th (1999) Reactiv-Orang DYPR 1410, Testing for subacute oral toxicity in the male and female rat, Report number 98.0172, Hoechst Marion Roussel Deutschland GmbH, Germany.

Jensch, U (1999a) Reactiv-Orang DYPR 1410, Testing for acute oral toxicity in the male and female Sprague Dawley rat, Report number 98.0979, Hoechst Marion Roussel Deutschland GmbH, Germany.

Jensch, U (1999b) Reactiv-Orang DYPR 1410, Testing for acute dermal toxicity in the male and female Sprague Dawley rat, Report number 98.1004, Hoechst Marion Roussel Deutschland GmbH, Germany.

Jensch, U (1999c) Reactiv-Orang DYPR 1410, Testing for sensitising properties in the Pirbright-White guinea pig in the maximization test, Report number 98.0490, Hoechst Marion Roussel Deutschland GmbH, Germany.

Kreiling, R (1999a) Reactiv-Orang DYPR 1410, Testing for primary dermal irritation in the rabbit, Report number 98.1001, Hoechst Marion Roussel Deutschland GmbH, Germany.

Kreiling, R (1999b) Reactiv-Orang DYPR 1410, Testing for primary eye irritation in the rabbit, Report number 98.1025, Hoechst Marion Roussel Deutschland GmbH, Germany.

Muhlberger, B and Henkelmann, B (1999): Abiotic Degradation – Hydrolysis as a Function of pH: Aventis Research & Technologies GmbH & Co KG, Analytical Technologies, Frankfurt am Main.

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

National Occupational Health and Safety Commission (1999) Approved Criteria for Classifying Hazardous Substances [NOHSC:1008(1999)]. Australian Government Publishing Service, Canberra.

Santos, A and Muhlberger, B (1999a): Water Solubility: Aventis Research & Technologies GmbH & Co KG, Analytical Technologies, Frankfurt am Main.

Santos, A and Muhlberger, B (1999b): Partition Coefficient – N-octanol/Water: Aventis Research & Technologies GmbH & Co KG, Analytical Technologies, Frankfurt am Main.

Stammberger, I (1999a) Reactiv-Orang DYPR 1410, Bacterial reverse mutation test, Report number 98.0008, Hoechst Marion Roussel Deutschland GmbH, Germany.

Stammberger, I (1999b) Reactiv-Orang DYPR 1410, In vitro mammalian chromosome aberration test in V79 Chinese hamster cells, Report number 98.0044, Hoechst Marion Roussel Deutschland GmbH, Germany.

Stammberger, I (1999c) Reactiv-Orang DYPR 1410, Mammalian erythrocyte test in male and female NMRI mice, Report number 98.0240, Hoechst Marion Roussel Deutschland GmbH, Germany.

Standards Australia (1990) Australian Standard 3765.1-1990, Clothing for Protection against Hazardous Chemicals Part 1 Protection against general or specific chemicals. Standards Association of Australia.

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

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

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

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

Standards Australia/Standards New Zealand (1998) Australian/New Zealand Standard 2161.2-1998, Occupational protective gloves, Part 2: General requirements. Standards Association of Australia/Standards Association of New Zealand.

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 (Draize, 1959) for evaluation of skin reactions is as follows:

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

The Draize scale (Draize *et al*., 1944) for evaluation of eye reactions is as follows:

CORNEA

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

CONJUNCTIVAE

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

Draize, J. H., Woodward, G., Calvery, H. O. (1944) Methods for the Study of Irritation and Toxicity of Substances Applied Topically to the Skin and Mucous Membranes, J. Pharmacol. Exp. Ther. 82 : 377-390

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

MSDS