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NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME

FULL PUBLIC REPORT

C.I. REACTIVE GREEN 29

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

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, Worksafe Australia, 92-94 Parramatta Road, Camperdown NSW 2050, between the hours of 10.00 a.m. and 12.00 noon and 2.00 p.m. and 4.00 p.m. each week day except on public holidays.

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Director
Chemicals Notification and Assessment

FULL PUBLIC REPORT

C.I. REACTIVE GREEN 29

1. APPLICANT

Sandoz Australia Pty Ltd, 675-685 Warrigal Rd, Chadstone, Victoria, 3148

2. IDENTITY OF THE CHEMICAL

C.I. Reactive Green 29 has been classified as hazardous by Worksafe Australia due to its skin sensitisation and eye irritation properties. However, for commercial reasons, the chemical identity, methods of detection and determination, and spectral data have been granted exemption from publication in the Full Public Report and Summary Report. The conditions of this being permitted are:

- A descriptive generic name be used to identify the substance in public reports and the MSDS,
- The relevant employee unions shall be informed of the conditions of use of C.I. Reactive Green 29,
- The full chemical name shall be provided to any health professionals in the case of a legitimate need where exposure to the chemical may involve a health risk,
- The full chemical name shall be provided to those on site who are using the chemical and to those who are involved in planning for safe use, etc. in the case of a legitimate need,
- The Director of NICNAS will release the full chemical name etc in the case of a request from a medical practitioner,
- Confidentiality will expire after a 3 year period,
- The chemical be identified as a sensitiser in the Health Effects Section of the MSDS, and that reference to its assessment by NICNAS be made on the MSDS,
- These conditions shall be published in the Chemical Gazette.

Trade name:	Drimarene Brilliant Green R-4G CDG
Other name:	C.I. Reactive Green 29
Molecular weight:	1530
Methods of detection and determination:	UV/Vis, IR and NMR spectra (qualitative); spectrophotometry (quantitative)

3. PHYSICAL AND CHEMICAL PROPERTIES

Appearance at 20°C and 101.3 kPa:	Dark green granules
Melting Point:	> 250°C
Specific Gravity/Density:	1670 kg/m^3 at 20°C
Water Solubility:	29 - 60 g/L at 19.7 - 20.7°C
Partition Co-efficient (n-octanol/water) log P_{ow}:	≤ -4.3 at 20°C
Fat solubility:	< 3 X 10^{-4} g/L at 37°C
Surface Tension:	66.2 mN/m (1 g/L, 20°C). The notified chemical is not a surface active agent.
Soil adsorption/ desorption	Test not performed. The dye is expected to have a low affinity to soil.
Flammability Limits:	Not flammable
Autoignition Temperature:	> 400°C
Explosive Properties:	Not explosive
Particle size distribution:	range: 4.6 - 118.4 µm mean: 21.6 µm

Comments on physico-chemical properties

Data for some items were not provided for acceptable reasons. These were:

Vapour Pressure:	Expected to be negligible based on the high melting point and high molecular weight.
Hydrolysis as a function of pH:	The notified chemical is recommended for application processes which have to be carried out at pH 6.0 and 10.5 and at temperatures up to 80°C. In view of the chemical stability of the notified substance a measurable hydrolysis is not expected.
Dissociation Constant:	This test was not performed. It is argued that the dye is a tetra sulphonic acid salt and the dissociation constant should be typical of these functionalities.
Reactivity:	Based on the structural formula exothermic reaction is not expected with flammable material (no reactive groups would support oxidation). This is supported by the chemical's lack of flammability and explosive properties together with the very high autoignition temperature.

4. PURITY OF THE CHEMICAL

Degree of purity : 73% (Limits: 68 - 78%)

Toxic or hazardous impurities: None known

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

Chemical Name	CAS No.	Weight Percentage
Sodium Chloride	7647-14-5	10 - 20%
Water	7732-18-5	6 - 16%
Ammonium Sulphate	7783-20-2	0.5 - 1.5%

Additives/ Adjuvants: None

5. INDUSTRIAL USE

The notified chemical is to be used to dye cotton textiles.

6. OCCUPATIONAL EXPOSURE

The notified chemical is to be imported in plastic bags (25 kg nett weight) sealed inside steel drums at a rate of 250 kg for the first year increasing to 380 kg in the following four years.

Normally customers will be expected to order 25 kg drums of the dye. However, if a customer orders a quantity smaller than 25 kg, 1 storeman at the notifier' s site will weigh the required amount into a rigid plastic container. This is done under local exhaust ventilation.

It is estimated that 5 dyehouses will use the notified chemical. At each dyehouse, 1 storeman and 1 operator will be exposed to the dye. Exposure is expected to be limited to 1 hour per day. The dye is weighed out, dissolved in water and added to the dyebath.

The dye is manufactured in a non-dusting form, that is, fine granules. According to the notifier, the dye ' kitchens' in the dyehouses will, most likely, not be equipped with local exhaust ventilation.

According to the manufacurer' s information sheet, the dye is instantaneously soluble in cold water which reduces the time for preparation of padliquors and consequently, the potential for exposure. Following dissolution in a premix tank, the dye solution is added to the dyebath by pump or gravity feed. The dyebath is usually, but not always, enclosed.

7. PUBLIC EXPOSURE

The notified chemical is a reactive dye, and in its final form is covalently bound to the textile substrate. Thus, although the public contact with fabrics/textiles which have been coloured with the dye is expected to be high, exposure to the notified chemical is expected to be negligible.

In the case of accidental spillage during transport, the public may be exposed to the notified chemical. This is minimised by the recommended practices for storage and transportation.

8. ENVIRONMENTAL EXPOSURE

. Release

The notifier has indicated that the dye has a 35% level of fixation on the fibres. This level of fixation is significantly lower than normal for reactive dyes. The remainder will be discharged into the sewer.

. Fate

The dye that is chemically bound to fibres is not expected to adversely impact on the environment.

The unfixed residues from dyeing operations will enter the aquatic environment after discharge from the textile mills and subsequent treatment at sewage treatment plants. As a result of the dye's low K_{ow} and hydrolytic stability, it is likely that significant quantities will remain in the aquatic phase. Furthermore, reactive dyes have been found not to strongly adsorb to sludge (1) in model systems.

The dye was tested for both its ready and inherent biodegradability using standard test methods. The ready biodegradability test (EEC test method C.6, closed bottle test) gave a negative result (-61% and -19% for 1.0 mg/L and 3.0 mg/L respectively), while the inherent biodegradability test (OECD Test Guideline 303B) showed 15% degradation within 28 days. As the results of the OECD test 209 below showed no toxicity to waste water bacteria (see environment effects), the negative result for the ready biodegradation is unlikely to be due to toxicity of the dye. The dye is not ready biodegradable but is slightly inherently degradable, thus significant degradation is unlikely in sewage treatment plants. However, the inherent biodegradation test showed that the dye should slowly degrade in the environment.

After treatment in the sewage plant, the dye will enter either freshwater or marine environments in solution. The dye is reasonably stable to aerobic conditions but azo dyes are susceptible to reductive degradation under anaerobic conditions, characteristic of sediments (2). The half life of this degradation was found to be between 2 and 16 days for several sulphonic azo dyes (3), thus no significant increase in concentration overtime is expected. One possible route for the dye to enter the sediments is by precipitation of its calcium salts, as several calcium salts of sulphonic dyes are known to be insoluble (3) at modest concentrations. However, apart from precipitation as the calcium salt, the hydrophilic nature of C.I. Reactive Green 29 should limit the affinity for soil and sediment and thus the dye should remain mainly in the aquatic compartment.

The bioaccumulation potential of the dye was not investigated due to its very low partition coefficient (log Pow < -4.3), as allowed by the Act. This, together with the high water solubility and low fat solubility, indicate that bioaccumulation should not occur.

9. EVALUATION OF TOXICOLOGICAL DATA

Under the *Industrial Chemicals (Notification and Assessment) Act, 1989,* toxicity data are not required for new chemicals intended to manufactured or imported at a rate of less than 1 tonne per year. However, the studies evaluated below were available and were submitted as part of the notification statement.

9.1 Acute Toxicity

Table 1 Summary of the acute toxicity of C.I. Reactive Green 29

Test	Species	Outcome	Reference
Acute oral toxicity	Rat	LD_{50} > 5000 mg/kg	(4)
Acute dermal toxicity	Rat	LD_{50} > 2000 mg/kg	(5)
Skin Irritation	Rabbit	Not irritant	(6)
Eye irritation	Rabbit	Moderate to Severe Irritant	(7)
Skin sensitisation	Guinea pig	Potent sensitiser	(8)

9.1.1 Oral Toxicity (4)

This study was conducted in accordance with OECD guideline No. 401 (9). Wistar rats (5/sex) received a single dose of 5000 mg/kg in water.

No signs of ill health or behavior changes and no deaths were noted over 14 days. At necropsy dark colouration of both kidneys and greenish discolouration of mesenteric lymph nodes were noted in females only.

It can be concluded that the acute oral LD_{50} for the notified chemical in rats is > 5000 mg/kg.

9.1.2 Dermal Toxicity (5)

This study was conducted in accordance with OECD guideline No. 402 (10). Wistar rats (5/sex) received a single dose of 2000 mg/kg applied under a gauze patch.

No clinical signs of toxicity or behavioural changes were noted and no deaths occurred during the study. However, erythema was observed on the treated skin area of 2/5 males and 4/5 females.

It can be concluded that the acute dermal LD_{50} for the notified chemical in rats is > 2000 mg/kg.

9.1.3 Skin Irritation (6)

This study was conducted in accordance with OECD guideline No. 404 (11). Three female New Zealand White rabbits received a dose of 0.5 g of the notified chemical moistened with water.

No erythema or oedema was observed in any animal up to 72 hours post-treatment.

In can be concluded that the notified chemical is not a skin irritant in rabbits.

9.1.4 Eye Irritation (7)

This study was conducted in accordance with OECD guideline No. 405 (12). Three female New Zealand White rabbits received a dose of 84 mg of the notified chemical. Observations were made at 1, 24, 48 and 72 hours, 7, 14 and 21 days post-treatment.

Slight corneal opacity was observed in all rabbits at 48 and 72 hours, in 2/3 rabbits at 7 days and in one rabbit at 14 days.

Moderate effects on the iris (injection) were observed in all animals at 24 hours, in 2/3 rabbits at 48 hours and in 1/3 rabbits at 72 hours.

Conjunctival redness could not be scored at 1 hour due to green discolouration of the ocular tissues. The conjunctivae of all animals were a diffuse beefy red in 2/3 rabbits at 24 hours and in all animals at 48 and 72 hours. Conjunctival redness was classified as a diffuse crimson colour in 1/3 rabbits at 24 hours and in all animals at 7 days. By 14 days conjunctival redness was slight.

Swelling with lids about half closed was observed in all animals at 24 hours. Swelling was not as severe (obvious with partial eversion of the lids) at 1, 48 and 72 hours, was slight in 2/3 rabbits at 7 days and was not observed in any animal at 14 days.

It can be concluded that the notified chemical is a moderate to severe eye irritant in rabbits.

9.1.5 Skin Sensitisation (8)

This study was conducted in accordance with OECD guideline No. 406 (13) following the adjuvant method of Magnusson and Kligman (14) using female himalayan guinea pigs (10 controls, 20 experimental).

Induction was achieved with injection of a 5% solution and topical exposure to a 50% solution of the notified chemical. For challenge, concentrations of the notified chemical used were 10, 25 and 50%.

The induction phase resulted in slight to well-defined skin irritation. After taking account of control responses, all animals displayed a positive reaction following challenge with the 10% and 25% concentrations and 18/20 animals were positive following challenge with the 50% concentration.

It can be concluded that the notified chemical is a potent skin sensitiser in guinea pigs.

9.2 28-Day Repeated Dose Oral Toxicity (15)

This study was conducted in accordance with OECD guideline No. 407 (16). Wistar rats (5/sex/dose with an extra 5/sex given a 14 day recovery period at doses of 0 and 1000 mg/kg/day) recceived doses of 0, 50, 200 or 1000 mg/kg/day by gavage.

No mortality ocurred during the study and no effects were observed on body weight, body weight gain and food consumption.

No treatment-related clinical signs of toxicity or behavioural changes were observed.

Minor statistically significant differences in haemotological parameters were either not dose-related or were due to control values outside the historical range.

No toxicologically significant changes in clinical biochemistry parameters were observed.

Macroscopic examination revealed green staining of various organs attributed to the green colour of the notified chemical. No other treatment-related effects were noted. Microscopic examination also did not reveal any treatment related effects.

It can be concluded that there no target organ toxicity occurs in rats treated with repeated doses up to 1000 mg/kg/day of the notified chemical for 28 days.

9.3 Genotoxicity

9.3.1 Salmonella typhimurium Reverse Mutation Assay (17)

This study was conducted in accordance with OECD guideline No. 471 (18). *Salmonella typhimurium* strains TA 1535, TA 1537, TA 98 and TA 100 were treated with doses up to 5000 µg/plate in the presence or absence of metabolic activation provided by rat liver S9.

No treatment-related increases in the number of prototrophic back mutants were observed in any strain. Responses to the postive control mutagens 4-nitro-o-phenylene diamine, sodium azide and 2-aminoanthracene were as expected.

It can be concluded that the notified chemical is unlikely to be mutagenic in *Salmonella typhimurium.*

9.3.2 Micronucleus Assay in the Bone Marrow Cells of the Mouse (19)

This study was conducted in accordance with OECD guideline No. 474 (20) using Charles River NMRI mice (10 animals/test group, 1000 PCE scored per animal).

No treatment-related increase in the frequency of micronuclei was observed at a dose of 5000 mg/kg with sampling times of 24, 48 and 72 hours post-treatment. The positive control substance, cyclophosphamide induced micronuclei as expected.

It can be concluded that the notified chemical is unlikely to be clastogenic in mice.

9.4 Overall Assessment of Toxicological Data

The notified chemical exhibited low oral and dermal toxicity in rats, did not exhibit toxic effects when administered to rats for 28 days, was not a skin irritant in rabbits and was not mutagenic in bacteria or clastogenic in mice. However, the notified chemical was a moderate to severe eye irritant in rabbits and was a potent skin sensitiser in guinea pigs.

10. ASSESSMENT OF ENVIRONMENTAL EFFECTS

While the notification is for < 1 tonne and the results of ecotoxicity tests are not a schedule requirement, the notifier has provided the following test results.

Ecotoxicity tests were performed using technical grade (73%) C.I. Reactive Green 29 dye and the results (table 2) were provided by the notifier. No precipitates or other irregularities were noted in these tests and the concentrations were measured at the conclusion of tests and the actual concentration were within 5% of the nominal concentrations. These tests were performed in accordance with standard EEC test methods or OECD test guidelines, and at facilities complying with OECD principles of GLP.

Table 2

Species	Test	Result
Zebra fish, *Brachydanio reno*	96 hour acute EEC TG C1	NOEC < 10 mg/L LC_{50} = 16.7 mg/L
Daphnia, *Daphnia magna*	48 hour immobilisation EEC TG C2	NOEC = 32 mg/L EC_{50} = 46.2 mg/L
Bacteria, from aerobic waste water	Inhibition of Microbial Activity, OECD TG 209	NOEC = 50 mg/L EC_{50} > 100 mg/L

The above results show that C.I. Reactive Green 29 is slightly toxic to fish and daphnia. The dye does not affect aerobic waster water bacterial respiration at 50 mg/L, indicating that it is unlikely to effect bacteria in the sewage system. Based on these results, chronic effects would not be expected at the estimated environmental concentrations.

11. ASSESSMENT OF ENVIRONMENTAL HAZARD

As indicated above, 35% of the dye is fixed in the exhaust dyeing process, thus 65% of the dye used could be discharged into effluents of the dyehouses where it is used. The notifier has calculated the concentration of discharge for a typical dyehouse. The calculations presented by the company are based on a batch size of 100 kg of fabric and are as follows:

Use of C.I. Reactive Green 29 per batch = 2 kg

Amount of dye used per batch (63% pure) = 1.3 kg

Fixation rate of 35%, quantity passing to effluent = 0.845 kg

Total volume of wash waters =10,500 L

Effluent concentration from dye bath =80 ppm

Dilution in other waste waters of the dyehouse @ 10:1 =8 ppm

Dilution in receiving waters @ 10:1 =0.8 ppm

The assumptions made are very conservative, for example dilution in the dyehouse effluent is normally be considered at least ten times greater. The EPA has extended this calculation from the dyehouse to include dilution in the sewage and the receiving waters for both a coastal city based and an inland rural based dyehouse.

Dilution in sewage treatment plants for:

Rural treatment plant 5 ML per day = 260 ppb

City based 250 ML per day = 5.2 ppb

In final receiving waters:

Inland waterway (3:1 dilution) = 90 ppb

Ocean discharge from city (10:1 dilution) = 0.5 ppb

These calculations are based on no removal of C.I. Reactive Green 29 through adsorption to sludge in the sewage treatment plant due to its high water solubility (29-60 g/L) and low partition coefficient (log Pow =<-4.3). The calculations give expected environmental concentrations significantly below the LC_{50} for fish (16.7 mg/L) and the EC_{50} for daphnia (46.2 mg/L) and are unlikely to significantly affect algae.

The dye is not expected to accumulate in the sediment nor bioaccumulate.

Spills of the dye should not present an environmental hazard when cleaned up according to the MSDS sheets.

12. ASSESSMENT OF PUBLIC AND OCCUPATIONAL HEALTH AND SAFETY EFFECTS

In common with a number of other reactive dyes, the notified chemical is likely to be a skin sensitiser in humans and should be considered a potential respiratory sensitiser in addition to being a moderate to severe eye irritant. A discussion of the health effects of reactive dyes is provided in Information Notice No. 3 of the Ecological and Toxicological Association of the Dyestuffs Manufacturing Industry supplied by the notifier.

The notified chemical is imported as fine granules which are stated by the manufacturer to be non-dusting. This suggests that inhalational exposure is unlikely to occur.

When the dye is in aqueous solution, skin contact is possible. Transfer from the premix tank where the dye is dissolved to the dyebath is by pump or gravity feed. Thus the potential for spillage or splashing appears to be controlled. Dissolution of the dye in cold water is said to be instantaneous and mists are not formed during mixing.

Most dyebaths using the notified chemical are closed systems although there are open dyebaths in some dyehouses.

Because the amount of the notified chemical to be imported is a maximum of 380 kg per year, a high level of exposure of workers is not expected.

Although the notified chemical should be regarded as a potential respiratory sensitiser, the risk of respiratory sensitisation would appear to be low given that the dye is in a non-dusting form and is used in relatively small amounts. There is clearly a risk of skin sensitisation and moderate to severe eye irritation from contact with the dye in solution and personal protective equipment as outlined below should be used.

Based on the provided information and the intended usage, the notified chemical does not appear to represent a significant hazard to public health.

13. RECOMMENDATIONS

To minimise occupational exposure to C.I. Reactive Green 29 the following guidelines and precautions should be observed:

. good general and local exhaust ventilation should be provided in weighing areas;

. particular care should be taken to avoid spillage or splashing of the dye solution;

. production of mists in the workplace during mixing operations should be avoided;

. good personal hygeine should be practiced to minimise the potential for ingestion;

. when handling the dye personal protective equipment which conforms to and is used in accordance with Australian Standards (AS) for eye protection (AS 1336, AS 1337) (21,22), impermeable gloves (AS 2161) (23) protective clothing (AS 3765.1, 3765.2) (24,25) and, if there is any possibility of dust generation, respiratory protection (AS 1715) (26), should be worn;

. a copy of the Material Safety Data Sheet should be easily accessible to employees.

14. MATERIAL SAFETY DATA SHEET

The attached Material Safety Data Sheet (MSDS) for C.I. Reactive Green 29 was provided in an acceptable format (EC format).

This MSDS was provided by Sandoz Australia Pty Ltd as part of their notification statement. The accuracy of this information remains the responsibility of Sandoz Australia Pty Ltd.

15. REQUIREMENTS FOR SECONDARY NOTIFICATION

Under the *Industrial Chemicals (Notification and Assessment) Act 1989*, secondary notification of C.I. Reactive Green 29 shall be required if any of the circumstances stipulated under subsection 64(2) of the Act arise. No other specific conditions are prescribed.

16. REFERENCES

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3. Weber, E. J., Environmental Toxicology and Chemistry, 10, 609-618, 1991.

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5. *Assessment of Acute Dermal Toxicity with Brilliant Green K-RWa 6083 in the Rat*, RCC Notox Project No. 064597, data on file, Sandoz Chemikalien AG, Basel, Switzerland, 1991.

6. *Primary Skin Irritation/Corrosion Study with Brilliant Green K-RWa 6083 in the Rabbit*, RCC Notox Project No. 064608, data on file, Sandoz Chemikalien AG, Basel, Switzerland, 1992.

7. *Acute Eye Irritation/Corrosion Study with Brilliant Green K-RWa 6083 in the Rabbit*, RCC Notox Project No. 064619, data on file, Sandoz Chemikalien AG, Basel, Switzerland, 1992.

8. *Assessment of Contact Hypersensitivity to Brilliant Green K-RWa 6083 in the Albino Guinea (Maximisation Test)*, RCC Notox Project No. 051997, data on file, Sandoz Chemikalien AG, Basel, Switzerland, 1991.

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12. OECD Guidelines for Testing of chemicals - *Acute Eye Irritation/Corrosion No: 405*, 1987.

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17. *Salmonella typhimurium Reverse Mutation Assay with Brilliant Green K-RWa 6083,* CCR Project 219600, data on file, Sandoz Chemikalien AG, Basel, Switzerland, 1992.

18. OECD Guidelines for Testing of chemicals - *Salmonella typhimurium, Reverse Mutation Assay No: 471*, 1983.

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20. OECD Guidelines for Testing of chemicals - *Micronucleus Test No: 474*, 1983.

21. Australian Standard 1336-1982, *Recommended Practices for Eye Protection in the Industrial Environment*, Standards Association of Australia Publ., Sydney, 1982.

22. Australian Standard 1337-1984, *Eye Protectors for Industrial Applications*, Standards Association of Australia Publ., Sydney, 1984.

23. Australian Standard 2161-1978, *Industrial Safety Gloves and Mittens (excluding Electrical and Medical Gloves)*, Standards Association of Australia Publ., Sydney, 1978.

24. Australian Standard 3765.1-1990, *Clothing for Protection Against Hazardous Chemicals, Part 1: Protection Against General or Specific Chemicals*, Standards Association of Australia Publ., Sydney, 1990.

25. Australian Standard 3765.2-1990, *Clothing for Protection Against Hazardous Chemicals, Part 2: Limited Protection Against Specific Chemicals*, Standards Association of Australia Publ., Sydney, 1990.

26. Australian Standard 1715-1991, *Selection, use and maintenance of Respiratory Protective Devices*, Standards Association of Australia Publ, Sydney 1991.