File No.: NA/225

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME

FULL PUBLIC REPORT

FAT 45'164/A

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.

For Enquiries please contact the Administration Coordinator at:

Street Address: 92 Parramatta Rd Camperdown, NSW 2050, AUSTRALIA
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Telephone: (61) (02) 565-9466 **FAX (61) (02) 565-9465**

Director
Chemicals Notification and Assessment

FULL PUBLIC REPORT

FAT 45'164/A

1. APPLICANT

Ciba-Geigy Australia Ltd of 235 Settlement Road, Thomastown VIC 3074 have submitted a Standard Notification for the assessment of FAT 45'164/A.

2. IDENTITY OF THE CHEMICAL

Other names:	Reactive Red RUE 55 FAT 45'164/A
Trade names:	Commercial form Cibacron Red LS-6G

Method of detection and determination:

FAT 45'164/A may be detected by UV/VIS, IR and NMR spectroscopy and HPLC. Determination of the impurities may be performed by atomic absorption spectroscopy, elemental analysis, X-Ray fluorescence, flame ionisation analysis, ion chromatography, and thin layer chromatography.

3. PHYSICAL AND CHEMICAL PROPERTIES

Appearance at 20°C and 101.3 kPa:	Green black powder
Odour:	None
Melting Point :	> 300°C
Specific Gravity:	0.842 at D_4^{21}
Vapour Pressure:	< 4.6×10^{-4} Pa at 25°C
Water Solubility:	> 233 g/L at 20°C, pH = 6.9 (the substance forms a paste at 400 g/L).
Fat Solubility:	< 0.1 mg/100g at 37°C
Partition Co-efficient (n-octanol/water) log P_{ow}:	Could not be determined because the substance was not found in the octanol phase. Calculated as = -14.3

Hydrolysis as a function of pH:	pH 7, 50°C - hydrolysis < 10% after 5 days 25°C - half-life > 1 year (est.) pH 4, 25°C - half-life 77 hours (3.2 days) (est.) pH 9, 25°C - half-life 1228 hrs (51.2 days) (est.)
Adsorption/Desorption:	Not provided
Dissociation Constant:	Not provided
Flash Point:	Not provided
Flammability Limits:	Not provided
Combustion Products:	Oxides of carbon, sulphur, and possibly fluorinated compounds.
Pyrolysis Products:	Not provided
Decomposition Temperature:	Not provided
Decomposition Products:	Not provided
Autoignition Temperature:	Not provided
Explosive Properties:	Not provided
Reactivity/Stability:	Not provided
Particle size distribution:	> 500 µm 28.5% 250 - 500 µm 29.2% 106 - 250 µm 30.43% < 106 µm 11.90%
Surface Tension:	72.8 mN/m at 1.012 g/L (not a surfactant).

Comments on physico-chemical properties:

Adsorption/Desorption : It is claimed that the dye would exhibit "very strong adsorption on strongly silty sand and weak sandy loam". This is possible since a study of highly sulphonated *bis*(azo) dyes has shown that these chemicals sorb to sediment (1). However, the degree to which they will absorb to soils in the Australian environment is unknown. The high solubility (> 233 g/L), very low partition coefficient (log P_{ow} < -10), low fat solubility and lack of surface activity of the notified chemical would tend to indicate low absorption.

Hydrolysis and dissociation : The compound's hydrolytic stability indicated that it would be stable only at a neutral pH to slightly alkaline pH. The half-life of 3.2 d at 25°C and a pH of 4 is considered fast, while the half-life of 51.2 d at 25°C and a pH of 9 is considered moderate. The structure of the notified chemical suggests that it is likely to dissociate under environmental conditions.

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Dissociation Constant:	Not provided
Flash Point:	Not provided
Flammability Limits:	Not provided
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Pyrolysis Products:	Not provided
Decomposition Temperature:	Not provided
Decomposition Products:	Not provided
Autoignition Temperature:	Not provided
Explosive Properties:	Not provided
Reactivity/Stability:	Not provided
Particle size distribution:	> 500 µm 28.5% 250 - 500 µm 29.2% 106 - 250 µm 30.43% < 106 µm 11.90%
Surface Tension:	72.8 mN/m at 1.012 g/L (not a surfactant).

Comments on physico-chemical properties:

Adsorption/Desorption : It is claimed that the dye would exhibit "very strong adsorption on strongly silty sand and weak sandy loam". This is possible since a study of highly sulphonated *bis*(azo) dyes has shown that these chemicals sorb to sediment (1). However, the degree to which they will absorb to soils in the Australian environment is unknown. The high solubility (> 233 g/L), very low partition coefficient (log P_{ow} < -10), low fat solubility and lack of surface activity of the notified chemical would tend to indicate low absorption.

Hydrolysis and dissociation : The compound's hydrolytic stability indicated that it would be stable only at a neutral pH to slightly alkaline pH. The half-life of 3.2 d at 25°C and a pH of 4 is considered fast, while the half-life of 51.2 d at 25°C and a pH of 9 is considered moderate. The structure of the notified chemical suggests that it is likely to dissociate under environmental conditions.

4. PURITY OF THE CHEMICAL

Degree of purity (of the notified chemical alone)**:** 65.1% (range 55 - 75%)

5. INDUSTRIAL USE

FAT 45'164/A will be imported as a powder component of Cibacron Red LS-6G. It will be used as a cellulose textile dye using exhaust dye methods. It will be used in dyehouses only in several city and country locations mainly in NSW and Victoria, but possibly in Tasmania and South Australia as well.

6. OCCUPATIONAL EXPOSURE

FAT *45'* 164/A will be imported as a high percentage component (>70%) of a formulation known as Cibacron Red LS-6G, in ready-to-use packages. It will be imported in 30 kg fibreboard boxes lined with polythene antistatically lined drums. The notified chemical will be distributed to dye houses for use.

Up to 12 employees per dyehouse site may potentially be exposed to FAT *45'* 164/A but eight (per site) of these workers will only be using the dye in closed systems. The four batch operators who weigh out the dye powder, usually under local exhaust ventilation, and add it to the blending vessel have the most potential for exposure. At the most, any one batch operator may weigh out approximately 2.0 kg five times during the day on 125 days per year. It is estimated that a "worst case" exposure to these workers of 0.00 17 mg/kglbw/day.

The steps involved in the textile dye process are weighing, addition of the dye to the blending vessels and transferring of the dye mixture to the dyeing equipment. The dye is dissolved in a vat before it is pumped to a tank from which it is dispensed to the dye machine. These processes all occur in closed systems. The fabric is fed into the dye machine and following dyeing the fabric is washed free of unfixed dye and dried. Exposure of workers during the dyeing process is not expected.

Repacking also may be performed at the warehouse prior to distribution as samples of trial packs. This is performed in a booth where down flow air is drawn away from the operators. A maximum of 100 kg of dye is anticipated to be repacked by two personnel for several hours each year.
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7. PUBLIC EXPOSURE

The public will not be exposed to the dye during the dyeing procedures which will only be performed in commercial dye houses using closed systems. The public may contact retail fabrics such as clothing and sheeting, which contain the dye at an estimated level of 0.5% of the weight of the cellulose.

8. ENVIRONMENTAL EXPOSURE

. Release

Spills that occur during transport or handling will be cleaned up according to the MSDS and consigned to secure landfill or incinerated.

· **Use**

The notifier has provided a fixation profile which indicates that the substance exhibits 82% fixation to the substrate when the exhaust dying method is used. Unfixed dye residues will be discharged with waste water to the dyehouse effluent systems.

The notified chemical is expected to replace other reactive dyestuffs in the market place, the latter, it is claimed, usually representing older technology with inherently lower rates of . benefits. (60 - 75%).

. **Fate**

The bulk (82% fixation) of the dye will become chemically bound to the fibre and in this state is not expected to impact on the environment. Some minor losses to the environment might occur from ventilation of dusts to air or through spills at the warehouse, during transit, or at the dyehouse. The major route for environmental release has been identified as unfixed dye which has been washed from the treated fabric.

These unfixed residues (18%) will enter the aquatic environment after discharge from the dyehouse and subsequent treatment at sewage treatment plants. The exact fate of the dye residues is unclear, due to uncertainties relating to the degree of sorption onto sediments. The two options are considered below.

Option 1: Dye remains in solution
The dye's very low P_{ow} (calculated), high water solubility, relatively low hydrolytic stability and lack of surface activity, indicate that it is likely to remain in solution and not be absorbed to the sludge in significant quantities. Furthermore, reactive dyes in general have been found not to adsorb to sludge in model systems (1).

Option 2: Dye is partitioned to sediment
Preliminary studies (2) have shown that dyes with similar molecular structures to the notified chemical can be sorbed onto sludge. The mechanism by which this occurs is still uncertain but it has been demonstrated that, as the pH of the solution decreases from alkaline levels, the degree of sorption to sediments is increased.

After entering the sewage works, unfixed residues may be removed through degradation (chemical or biological) or sorption to sludge. In view of the high water solubility, it is likely that a significant proportion of the dye will remain in the aquatic compartment. Any dye partitioned to the sediment will be removed with the sludge during treatment at the dyehouse and sewage works. While azo dyes are generally stable under aerobic conditions, they are susceptible to reductive degradation under the anaerobic conditions characteristic of sediment (3).

The dye was tested for its biodegradability in accordance with OECD Guideline 301B (modified Sturm test) (4). The result, 8% degradation in 28 days, shows that the dye is not ready biodegradable. Inherent biodegradability is unclear.

The bioaccumulation potential of Fat 45' 164/A was not investigated because of the very low partition coefficient (log P_{ow} = < -10, calculated) and lipid solubility (< 0.1 mg/kg). Hydrophilic dyes with log P_{ow} < 3 have been shown not to bioaccumulate (3). Also, no bioaccumulation of the pigment is expected since its large molecular size is likely to inhibit membrane permeability and prevent uptake during exposure (5,6).

9. EVALUATION OF TOXICOLOGICAL DATA

9.1 Acute Toxicity

Table 1 Summary of the acute toxicity of FAT 45'164/A

Test	Species	Outcome	Reference
Acute oral toxicity	Rat	LD_{50} > 2000 mg/kg	(7)
Acute dermal toxicity	Rat	LD_{50} > 2000 mg/kg	(8)
Skin Irritation	Rabbit	Non -irritant	(9)
Eye irritation	Rabbit	Moderate irritant	(10)
Skin sensitisation	Guinea pig	Weak sensitiser	(11)

9.1.1 Oral Toxicity (7)

The study was carried out in accordance with the *OECD Guide-lines for testing of Chemicals No: 401*.

Sprague Dawley rats (5 per sex) were administered by gavage a single dose of 2000 mg/kg of FAT 45'164/A dissolved in water. Animals were observed for a period of 14 days after which necropsy was performed.

All animals produced red stools and urine from 1 hour after dosing until day 2. No other clinical signs, deaths, macroscopic changes or changes in body weight were observed in any animal.

It was concluded that the oral LD_{50} of FAT 45'164/A was > 2000 mg/kg.

9.1.2 Dermal Toxicity (8)

The study was carried out in accordance with the *OECD Guide-lines for testing of Chemicals No: 402*.

Sprague Dawley rats (5 per sex) were administered a single dose of FAT 45'164/A by dermal application.

On day one of the procedure the test substance was applied evenly to a portion of a shaved skin area. This was covered by a semi-occlusive dressing. FAT 45'164/A was diluted in water to a paste of concentration 43.39 % (w/v). Animals received a dose of 2000 mg/kg of FAT 45'164/A. Twenty four hours after application the skin was washed and dried. The animals were then observed for a period of 14 days after which necropsy was performed.

No mortality or macroscopic abnormalities were observed during the study. Superficial eschars were observed from days 3 to 11.

It was concluded that the dermal LD_{50} of FAT 45'164/A to rats was > 2000 mg/kg.

9.1.3 Skin Irritation (9)

The study was carried out in accordance with the *OECD Guide-lines for testing of Chemicals No: 404.*

New Zealand White rabbits (three males) were administered a single dose of 0.5 g of FAT 45'164/A moistened with water by dermal application.

On day one of the procedure the test substance was applied to a portion of the shaved area and covered with a semi-occlusive dressing. The test substance remained on the skin for four hours after which time it was removed with lukewarm tap water. An excess of test substance was wiped away with a water moistened gauze pad. Animals were then observed at 1, 24, 48 and 72 hours after removal of the dressing.

All animals showed skin discolouration which persisted during the study and was said to make reading of the erythema imprecise but possible. Erythema and oedema were absent from all animals. Body weights were normal and no other clinical symptoms were observed.

FAT 45'164/A was concluded to be a non-irritant to the skin under the conditions of this study.

9.1.4 Eye Irritation (10)

The study was carried out in accordance with the *OECD Guide-lines for testing of Chemicals No: 405.*

New Zealand White rabbits (three males) were administered a single dose of 74 mg FAT 45'164/A in a 17% w/w water suspension into the conjunctival sac of one eye. The other eye remained untreated and was used as a control. Animals were observed at 1, 24, 48 and 72 hours and 7 and 14 days after administration of the test substance.

There was obvious swelling of the eyelids and nictitating membranes with the lids partially or half closed in all three animals at 1 hour. This had reduced to slight swelling in 2 animals by 24 hours and in all animals by 72 hours, but was absent by day 7 in all animals. Slight redness of the blood vessels of the conjunctivae was evident in all animals for 72 hours and one animal at 7 days. This was absent from all at day 14. The reflex of the pupil was normal in all animals. Circumcorneal injections were evident in the treated eye of all rabbits for 72 hours. It was still present in two animals by day 7 but was absent by day 14. The cornea appeared normal in all animals at all time points. Discolouration of the cornea was present at the 1 hour observation period and was said to make corneal opacity readings imprecise but possible.

No clinical signs of systemic toxicity were observed.

FAT 45'164/A was concluded to be a moderate irritant to the eye of the rabbit under the conditions of the study.

9.1.5 Skin Sensitisation (11)

The study was carried out in accordance with the *OECD Guide-lines for testing of Chemicals No: 406*.

The test used was the guinea-pig maximisation test of Magnusson and Kligman (12).

Pretest

To determine the dose level for intradermal injection in the main study, 0.1 ml of a 10%, 5%, and 1% solution of FAT 45'164/A in water were injected into the clipped dorsal region of four Hartley strain guinea-pigs (2 male and 2 female). The resulting dermal reactions were assessed 24 and 48 hours later.

Oedema was present at 5% and 10% and although it was not possible to score erythema due to skin staining a maximum score of 3 was assumed due to the oedema. No oedema was present after the 1% application and no score was given for erythema. After 48 hours the readings

gave the same results. A concentration of 1% FAT 45'164/A was selected for intradermal induction.

To determine the dose level for topical induction in the main study 0.5 ml of a 18% and 35% w/w paste of FAT 45'164/A in water was applied to the clipped and shaved flanks of four guinea pigs. Filter paper saturated with the test substance was applied to the skin under occlusive bandage. The dressings were removed after 48 hours. Assessments were made 1 hour after removal of the bandage. No oedema was observed in any animal at any dose level. Erythema could not be observed due to skin discolouration. A 35 % solution of FAT 45'164/A was chosen for induction study.

To determine the dose level for challenge in the main study 0.5 ml of a 18% and 35% w/w paste of FAT 45'164/A in water was applied to the clipped and shaved flanks of four guinea pigs. Filter paper saturated with the test substance was applied to the skin under occlusive bandage. The dressings were removed after 24 hrs. Assessments were made 24 and 48 hrs after removal of the bandage. No oedema was observed in any animal at any dose level. Erythema could not be observed due to skin discolouration. Treated skin from 2 animals was taken for cutaneous biopsy at 24 hrs and from 2 animals at 48 hrs. The 48 hr biopsy revealed that the skin of one animal treated with 35% test substance showed signs of moderate irritation. All other biopsies gave results within the normal range. An 18 % solution of FAT 45'164/A was chosen for the challenge study, being the maximum non irritant concentration.

Induction Study

Thirty female guinea-pigs of the Hartley strain (20 test and 10 control animals) were used.

On day 1 three pairs of intra-dermal injections (0.1 ml) were made into the clipped scapular region of each guinea-pig. The injected solutions were:

Freund's Complete Adjuvant (50:50) with physiological saline.
FAT 45'164/A diluted to 1% with water,
FAT 45'164/A diluted to 2% in water and emulsified in a 50:50 mixture with Freund's Complete Adjuvant in physiological saline (final concentration 1%).

Control animals received the same treatment but without the test substance.

On day 8 the scapular region was clipped and shaved and treated with 10% sodium-lauryl-sulfate (SLS) in paraffin to enhance a mild inflammatory reaction. On day 9 the same areas as the injection sites were treated with an occlusive epidermal application of 0.5 ml of 35% paste of FAT 45'164/A w/w in water in the same manner as described above for topical application. This was the maximum concentration that would form a paste. The bandage remained in place for 48 hours. Control animals were similarly treated but without the use of the test substance. The sites were evaluated 24 and 48 hours after removal of the bandages.

Challenge Study

11 days after the epidermal induction application, the test and control animals were challenged topically with FAT 45'164/A. Filter paper was saturated with 0.5 ml of an 18% w/w suspension of FAT 45'164/A in water, or with water only. The test substance and water vehicle were applied to the left and right flanks respectively of each guinea pig. The techniques used were the same as those described above. The bandages remained for 24 hours and assessment was made of the skin reactions at 24 and 48 hours after removal of the bandages.

Due to the staining of the skin by the test substance making erythema readings impossible, histopathological examinations of the skin was performed for all animals; half were performed at 24 hours and half at 48 hours.

Results

After intradermal and epidermal induction administration, the skin of all animals was tinted thus making erythema readings impossible. No oedema was observed.

Following challenge, a positive reaction was observed in one out of the 20 treated animals but no control animals. Erythema was impossible to read due to skin staining. No oedema was noted in any animal. However, histological evaluation gave a positive cell-mediated delayed hypersensitivity in one animal when examined at 24 hours. Histopathology revealed slight to moderate non-specific inflammation in the other treated and control animals.

Body weights were not affected during the study and no toxic symptoms were observed in any animals.

Five per cent (1/20) of the treated animals exhibited delayed hypersensitivity, which resulted in FAT 45'164/A being classified as a weak sensitiser according to the scale of Magnusson and Kligman.

In conclusion FAT 45'164/A is considered to be a weak skin sensitiser in the guinea pig.

9.2 Repeated Dose Toxicity (13)

Groups of 10 Sprague-Dawley rats (5 of each sex) were treated orally by gavage, once daily, 7 days a week for 4 weeks. Animals received 0, 150, 600 or 1000 mg/kg/day of FAT 45'164/A dissolved in distilled water at 5 ml/kg. These are described below as groups control, LD, MD, and HD. On day 29 male animals were sampled and necropsied and on day 30 females were also sampled and necropsied. In a recovery study 5 animals of each sex were treated with 0 or 1000 mg/kg and allowed a 14 day recovery period prior to necropsy being performed.

No changes in body weights or food consumption were observed that could be attributed to treatment. No treatment-related deaths or changes in organ weights were observed among any treatment group.

Two small changes in the blood clinical chemistry of the animals was observed. The level of platelets increased from a control value of 1003 to 1280 in the high treatment group of males. The prothrombin time in females increased but at the lowest treatment group only so was probably unrelated to the treatment substance.

Changes in the haematology of animals treated with FAT 45'164/A included the following: coloured blood sera was observed in group MD and HD animals at week four but not at week 6. Total mean bilirubin levels had increased 2.8 fold in males at week 3, 4.1 fold in males at week 4 and 2.1 fold in females at week 4 ($p < 0.001$). These had returned to control values by week 6.

The blood protein levels had decreased from 65 g/L to 60 g/L ($p < 0.01$) in HD males and in HD females from 63 to 58 g/L ($p < 0.05$). This was associated with a decrease in albumin from 37 g/L to 34 g/L in HD males only ($P < 0.001$). Creatinine levels were increased slightly from 4.8 mg/L to 5.6 mg/L in HD males ($p < 0.05$) but was within the historical range of normal values. The triglyceride levels in some females increased from 0.57 g/L in controls to 1.37 g/L in the HD group($p < 0.05$) with considerable variability. Levels were still high in the 6 week samples with a value of 0.99 g/L in the HD group compared to 0.60 g/L of controls.

In males, small decreases in sodium in MD and HD groups and increases in calcium (MD & HD) and phosphorus (LD & HD) were also observed.

The following changes were observed in the urine chemistry. Dose related colouration of the urine from orange to red was observed in all treated groups. In some HD animals the

colouration made estimations of the levels of protein, glucose, ketone, urobilinogen, bilirubin and blood difficult.

Five males (MD & HD) and one female (HD) had additional casts in the urine compared to controls. Half of the HD males had yeast in the urine which was absent from control animals but this was considered as incidental by the study director. Pink coloured epithelial cells were observed in one LD female and eight HD females. This observation was also made in three HD males.

A slight increase in the specific gravity of the urine of HD males was observed.

Small changes in organ weights, as well as macroscopic and microscopic variations were noted. The absolute kidney weights of MD males were increased. Because neither HD males nor females were affected this was not considered to be of toxicological significance. The absolute weight of the adrenals of HD males was significantly lower than controls (0.058 g compared to 0.074 g for controls), as were the kidneys (3.29 g compared to 3.72g). The liver to body weight ratio was slightly elevated in the HD females (3.19 g compared to 3.00 g).

The stomach contained treatment related dark coloured areas in MD & HD animals of both sex. This was associated with inflammation changes, mainly of the lamina propria of the stomach, which was observed in all treatment groups at 4 weeks. Such inflammation was also noted in one control female animal. Red coloured pigment was noted in the stomach mucosa in many treated animals . After the recovery period, neither pigment nor inflammation were observed in any treated animal.

A slight increase in the vacuolation of the adrenal cortex was observed in 3 HD males and one control male animal. No accompanying increase in adrenal weight was observed and the significance of this result is unknown. The study author suggested that this may be a stress related change as this is usually associated with an increase in the production of cortical hormones.

In conclusion, administration of FAT 45'164/A results in the colouration of the urine, faeces, body and sera of the rat, as well as a range of other clinical chemistry changes. The stomach appears to be a target organ for toxicity. Some changes were noted in animals of all treatment groups.

9.3 Genotoxicity

9.3.1 Salmonella typhimurium and Escherichia coli Reverse Mutation Assay (14)

The study was carried out in accordance with the *OECD Guide-lines for testing of Chemicals No: 471.*

FAT 45'164/A was tested in the reverse mutation assay on *S. typhimurium* strains TA98, TA100, TA1535 and TA1537, and *E. coli* strain WP2*uvrA* in the presence and absence of rat liver microsomal S9 activation. The test substance was evaluated using liquid preincubation and plate incorporation conditions and all tests were performed in triplicate.

Based on the results of a preliminary study the concentrations selected for the liquid preincubation study and the plate incorporation study for all strains with and without S9 mix were 0, 16.7, 50.00, 167, 500.0, 1670, or 5000 µg FAT 45'164/A dissolved in water. No toxicity was evident at any of these doses but the plates became intensely coloured at Ú 1670 µg/plate and the background was difficult to score at 5000 µg per plate. Positive controls used in the absence of activation were sodium azide for TA 1535 and TA 100, 9-aminoacridine for TA 1537, 2-nitrofluorene for TA 98 and ENNG for WP2*uvrA*. 2-Anthramine was used as the control for all strains in the presence of S9.

There were no increases in the numbers of revertant bacteria colonies under any of the conditions employed in this study. All positive control substances produced marked increases in the number of revertant colonies within the anticipated range.

In conclusion, FAT 45'164/A was not mutagenic to *S. typhimurium* or *E. coli* under the conditions of these experiments.

9.3.3 Chromosomal Aberrations in Chinese Hamster Ovary Cells (15)

The study was carried out in accordance with the *OECD Guide-lines for testing of Chemicals No: 473*.

FAT 45'164/A was investigated for its potential to cause chromosomal aberrations *in vitro* in CHO-K1-BH4 cell line from the Chinese hamster.

The culture medium (water) were used as the negative control; nitrosoguanidine without S9 mix (10.2 µM final concentration) and N-nitrosodimethylamine with S9 mix (13.5 mM final concentration) dissolved in medium were the positive controls utilised. 5-bromo-2'-deoxyuridine, a thymidine analog, was used as a chromosome marker to determine the mitotic delay caused by FAT 45'164/A. Induced rat liver was used to make the S9 mix.

Cytotoxicity experiments

Two cytotoxicity experiments using 1-2000 µg/ml FAT 45'164/A were performed in order to determine the toxicity of the test substance to the cells.

In one experiment, cells were treated for 6 hours prior to being washed with saline and given fresh medium, in the presence or absence of S9 mix. Cells were then incubated for a further 28 hours. In a second experiment, the cells were treated with FAT 45'164/A without metabolic activation for 24 hours. Colcemid was added to each flask for 2-3 hours prior to harvest to arrest the cells in metaphase in each experiment.

All cell cultures survived treatment in the cytotoxicity test. No significant increases (> 50%) compared to controls in the average proliferation time occurred at any dose level of FAT 45'164/A. A maximum depression of 43% of the mitotic index occurred. No significant changes to the osmolarity or pH of the medium of any treated cultures occurred. As a result of this study, 200, 1000 and 2000 µg/ml (the limit of solubility) were selected for the aberration study.

Aberration assay

Logarithmically growing cells in fresh medium were selected for the following studies. Three experimental schedules were followed. In schedule 1 treatments were initiated in cultures in serum-free medium with and without S9 by the addition of 100 µl of control or test article dilutions to achieve concentrations of 0, 200, 1000, or 2000 µg/ml. Duplicate flasks were treated for 6 hours. Cells were then rinsed and supplied with fresh medium and left for an 18 or 42 hr period. In schedules 2 and 3 cells were treated with FAT 45'164/A for 24 hrs in a serum-containing medium at the same doses as above. Cells were harvested at the end of the treatment period (schedule 2) or after a 24 hr recovery period (schedule 3).

Colcemid was added to the cultures for the last 2-3 hrs of incubation. In an independent retest, identical conditions to the above were employed except that the treatment medium was aspirated 2 1/2 hrs prior to harvest to ensure that the chromosome analysis was not interfered with by the test article.

The main study and the independent retest indicated that there was no statistically significant increases in the frequency or proportion of cell chromosomal aberrations in treated cells

compared to that of the negative controls. The positive controls both elicited significant increases in the proportion of, and frequency of, aberrations. There was no increase in the frequency of polyploid metaphases at any dose level.

FAT 45'164/A was concluded to be nonclastogenic under the conditions of this study.

9.4 Overall Assessment of Toxicological Data

FAT 45'164/A was found to be non-toxic to rats via the oral (LD_{50} > 2000 mg/kg) and dermal (LD_{50} > 2000 mg/kg) routes. It was non irritating to the rabbit skin and a moderate irritant to the rabbit eye. The substance was a weak skin sensitiser according to the criteria of Magnusson and Kligman. Although it caused sensitisation in one out of the twenty animals tested this does not meet the Worksafe criteria of being classified as hazardous. It caused no deaths during a repeated dose study but animals treated with all doses including the lowest of 150 mg/kg exhibited some effects from the chemical. The stomach appeared to be the main target organ for toxicity. FAT 45'164/A was not genotoxic to *Salmonella typhimurium* or *Escherichia coli* in a reverse mutation assay, and was not clastogenic towards Chinese hamster ovary cells.

10. ASSESSMENT OF ENVIRONMENTAL EFFECTS

Ecotoxicity tests were performed using FAT 45' 164/A dye and the results (table 2) were provided by the notifier. These tests were performed in accordance with standard OECD test methods and at facilities complying with OECD principles of GLP.

The carp (16) and daphnia (17) studies were conducted using nominal concentrations of 1, 10, 50, 100 and 250 mg/L.

In the carp study, the measured concentration was within 91% of the nominal concentration at all times. No observations relating to the appearance of the test solutions were reported. During the first 48 hrs of the test some of the fish in the 100 and 250 mg/L solution were displaying modified swimming behaviour (fish agitated and/or at the bottom of the tank). This behaviour was not detected during the routine 72 and 96 hr observations. Only one death was noted (24 hours, 50 mg/L) which was neither time nor concentration related.

During daphnia studies, measured concentrations varied from a minimum of 80% of nominal at the commencement of the study, to a minimum of 54% at the end of the test period. After 48 hrs, phase separation was noted in test solutions which exceeded 50 mg/L, appearing as a dark red deposit and a translucent phase. At the 1 and 10 mg/L concentrations a light to dark red colouration was observed. It was noted that difficulties were encountered in mobility observations for the 100 and 250 mg/L test solutions after 48 hrs due to their opacity and colouration.

The substance is nontoxic to aquatic fauna, and is not expected to accumulate in sediment or to bioaccumulate. Slight algistatic effects (i.e. growth inhibition) were noted. However, data provided by the notifier in a previous submission for a similar chemical, indicate that this effect may not be due to toxicity but rather reductions in the quality or quantity of light transmitted to the algae in the dye solution.

Table 2. Summary of Ecotoxicity Results of FAT 45'164/A (nominal concentrations)

Species	Test	Result	Ref
Carp *Brachydanio rerio*	96 hour LC_{50} OECD 203	LC_{50} > 250 mg/L	16
Water flea, *Daphnia magna*	48 hour EC_{50} immobilisation OECD 202	EC_{50} > 250 mg/L	17
Algae *Scenedesmus subspicatus*	72 hour inhibition of growth & reproduction OECD 201	growth inhibition E_BC_{50} = 27 mg/L (95% confidence 20-37 mg/L) and E_BC_0 < 5 mg/L growth rate reduction E_RC_{50} >100 mg/L E_RC_0 = 23 mg/L	18

11. ASSESSMENT OF ENVIRONMENTAL HAZARD

As indicated above, 82% of the dye is fixed during the dyeing process, thus 18% of the applied dye could be discharged into effluents at the dyehouses where it is used. Table 3 summarises calculations used by the notifier to determine the predicted environmental concentration (PEC) for city and country operations. Although these predictions are based on worst case scenario projections, higher levels may be approached in a country dyehouse during drought conditions.

Table 3. Estimation of Predicted Environmental Concentration

Process or dilution factor	City dyehouse	Country dyehouse
Effluent concentration in all dye wash-waters	820 µg/L	820 µg/L
Dilution factor in sewage treatment plant	100:1	3:1
Concentration balance in effluent from sewage treatment plant		
No removal of dye in sludge:	8.1 µg/L	205 µg/L
50% removal of dye in sludge:	4.05 µg/L	102 µg/L
Dilution factor in receiving waters	3:1 to 10:1	3:1
Predicted environmental concentration (PEC) in receiving waters		
No removal of dye in sludge:	2-0.7 µg/L	51 µg/L
50% removal of dye in sludge:	1-0.4 µg/L	26 µg/L
Safety factor* for exposure of most sensitive aquatic organism (Algae, *Scenedesmus subspicatus*, for growth inhibition: E_BC_{50} = 27 mg/L)	13300	530

* The safety factor is the highest PEC divided by the lowest EC_{50}.

Calculations in Table 3 include the internationally accepted assumption that *50%* of the dyestuff is retained with the sludge in biological effluent treatment works. If we assume that no dyestuff is retained with the sludge during biological treatment (as shown for the study in reference 2), then the worst case PEC for the dye in receiving waters is 51 µg/L which occurs in the country dyehouse operation.

Based on this scenario, the PEG of 51 pg/I. gives a safety factor of 530 to algal species. Although the algal species tested is considered by the US EPA to be insensitive (19) the notifier has provided information for similar dyes that the growth inhibition effect of the dye on algae is a function of decreased light intensity or change in light quality reaching the algae in the coloured media. In any event, the dye's high solubility suggests that once released to the waterways, dilution would be expected to swiftly reduce the environmental concentration to undetectable levels.

The substance is not expected to reach the terrestrial compartment in any significant amounts, nor have any impact on terrestrial (soil) organisms.

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

FAT 45'164/A is a powder with a calculated partition coefficient of log P_{ow} < -14.3, low fat solubility (< 0.1 mg/100g at 37°C) and a molecular weight > 1000 suggesting that it will not easily cross biological membranes and is unlikely to accumulate in biological tissue. It has a negligible vapour pressure and < 11.90 % of the particles are less than 106 µm. The proportion of particles in the respirable range less than 10 µm is unknown, but is likely to be low. The potential for entering the lungs is low as a result of the non-dusting formulation of FAT 45'164/A.

FAT 45'164/A is likely to be of low oral and dermal toxicity in humans but may be moderately irritating to the eyes. Irritation of the skin is unlikely, but skin sensitisation is possible in a minority of people.

There is a limited amount of handling of the dye due to the use of closed systems. Local exhaust ventilation is normally used during weighing processes and down draft air systems and a booth is used when repackaging is necessary. It is expected that only 4 workers at each of 12 dye houses will be exposed directly to FAT 45'164/A during their work, and that the remaining workers will be using the dye in closed systems. Those exposed to FAT 45'164/A may make contact with the dissolved dye via skin or eye contact.

In conclusion, when used under the conditions described by the notifier, FAT 45'164/A presents a low risk to those working with the chemical. Since the chemical will be strongly bound to the textile fibres, and acute, short term repeat dose, and genotoxicity studies indicated that the formulated dye containing FAT 45'164/A has low toxicity, the chemical is unlikely to constitute a hazard to public health.