File No: NA/857

January 2001

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME

FULL PUBLIC REPORT

Phosphonate LR1

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Director
Chemicals Notification and Assessment

FULL PUBLIC REPORT

Phosphonate LR1

1. APPLICANT

Unilever Australia Ltd of 219 North Rocks Road, NORTH ROCKS, NSW 2151 (ACN 004 050 828) has submitted a standard notification statement in support of their application for an assessment certificate for Phosphonate LR1.

2. IDENTITY OF THE CHEMICAL

The chemical name, trade name, CAS number, molecular and structural formulae, molecular weight, details of the chemical composition and details of exact import volume and customers have been exempted from publication in the Full Public Report and the Summary Report.

Marketing name: Phosphonate LR1

3. PHYSICAL AND CHEMICAL PROPERTIES

Phosphonate LR1 is not isolated in manufacture. The commercial form of the substance contains a number of impurities, including less substituted derivatives and inorganic salts (20-40%). Unless specified, the physico-chemical properties are that of the commercial form of Phosphonate LR1.

Appearance at 20°C & 101.3 kPa:	Pale yellow white powder.
Melting Point:	>425 °C.
Density:	0.475-0.9 gm/cc (bulk density, 0.5 – 0.65 gm/cc).
Vapour Pressure:	Not provided.
Water Solubility:	~100 g/L at 20 °C.
pH:	11.6-12.8 @ 1% and 25 °C.
Partition Co-efficient (n-octanol/water):	log P_{ow} <0 at 20°C (determined by shake flask method).
Hydrolysis as a Function of pH:	Does not readily hydrolyse.

Adsorption/Desorption:	Not provided.
Particle size:	207 μm (mean particle size with 10 μm standard deviation).
Dissociation Constant:	PK_{a1}=13.1 PK_{a2}=9.9 PK_{a3}=7.9 PK_{a4}=6.4 PK_{a5}=5.1 PK_{a6}=2.9
Flash Point:	425 °C.
Flammability Limits:	Non-flammable.
Autoignition Temperature:	>440°C (Not auto-flammable).
Explosive Properties:	Not explosive.
Reactivity/Stability:	Not an oxidising substance.

3.1 Comments on Physico-Chemical Properties

Most of the physico-chemical data provided with the notification is taken from the EU HEDSET data sheet, originating from unpublished Monsanto corporation reports (Monsanto, 1979).

The new chemical is an ionic salt, and the vapour pressure is expected to be very low.

The water solubility for a multi functional ionic compound of this nature is expected to be very high, and a solubility of around 100 g/L appears reasonable.

The material contains carbon-phosphorous bonds which may be susceptible to hydrolysis at high pH, but this is unlikely to be significant under the usual environmental conditions where 4<pH<9.

The chemical is an ionic salt containing no large hydrophobic regions. Consequently it would be expected to have a very low oil/water partition coefficient and would not partition into the oil phase. The notifier provided a report (Michael and Kaley, 1979) on the experimental determination of Pow for a number of related phosphonate compounds, which are all alkali or alkaline earth salts of the parent acid. The method used was similar to the shake flask method of OECD TG 117, although this was not indicated in the report. Log Pow was between –4.7 and –3.35 for the compounds tested, in keeping with the very high water solubility of these salts. The notified material, a mixed salt of the parent acid, is expected to have similar Log Pow values. The notifier provided a report (Rhemrev-Boom, 1992) on the determination of Log Pow for the notified chemical using the molecular fragmentation method of Hansch and Leo (1979), giving an extremely low estimate for Log Pow of –10.76.

Due to its anionic character, the notified chemical is expected to have little affinity for the organic component of soils and sediments. However, neutral Ca and Mg chelates, formed in environmental waters, are expected to be adsorbed into sediments.

Under environmental pH, the molecule will be anionic. The compound has acidity constants associated with the phosphonic acid functionalities, which lie over a very broad range pKa <2.9 to pKa =13.1 (Gledhill and Feijtel, 1992). No comment on the pKa for the amine groups was offered but these are expected to be appreciably basic and protonated under environmental conditions. In respect of this, it was indicated in the notification that the pH of a 1% solution of the compound at 25 °C is between 11.6 and 12.8.

4. PURITY OF THE CHEMICAL

Below 50% (for commercial form of modified chemical).

5. USE, VOLUME AND FORMULATION

The notified chemical will not be manufactured in Australia. It will be used as a stabilizer in a laundry detergent formulation and imported as a minor component (<10%) of the detergent formulation. The detergent formulation will be imported in the form of ready-to-use detergent tablets, which are added to the wash water in washing machines. Each tablet weighs around 40 grams and would contain < 4 g of the notified chemical.

The product tablets are packed, in pairs, in cellophane sachets contained in cardboard cartons (12 sachets per carton). A sealable plastic net pouch is also supplied for dispensing the tablets in the washing machine. Five to 10 cartons are packed in an outer cardboard case depending on the pack size.

Less than 100 tonnes of the notified chemical will be imported per year.

6. OCCUPATIONAL EXPOSURE

The notified chemical will be imported as a component of a ready-to-sell product.

The finished product will arrive by ship in containers, hand stacked with cases containing the cartons. The containers will be unloaded and the cases will be stacked on pallets and delivered to the notifier's warehouse. They will subsequently be transported to customer warehouses in various states for distribution to retail stores. Transport, warehouse and store workers will handle the packaged product and will be exposed to the chemical only if packaging is breached. The workers involved in unloading the product will be wearing personal protective equipment to avoid exposure to the chemical resulting from product leaks or spills. The notifier states that the respirable fraction of the notified chemical is expected to be small as it forms less than 10% of the product. Inhalation exposure is likely to be minimal.

At retail stores, the product will be removed from the outer cases and put on shelves as

individual units. Storekeepers will handle the packed product and could only become exposed to the product or the notified chemical if the cardboard wrapping and sachets were torn.

7. PUBLIC EXPOSURE

During transport and storage, the opportunity for exposure of the general public to the notified chemical will be low. The finished laundry detergent, containing <10% of the notified chemical, will be sold to the public, so there may be frequent exposure during routine use. Detergent tablets are packaged so that if opened and used as instructed, dermal contact with the detergent tablet need not occur.

There may be dermal exposure to the detergent formulation in solution, as well as accidental ocular and oral exposure. Inhalation exposure is unlikely due to the tablet form of the laundry detergent.

The notified chemical reportedly does not fix to laundered clothing.

8. ENVIRONMENTAL EXPOSURE

8.1 Release

Less than 100 tonnes of the new chemical may be introduced in laundry detergents each year, and almost all can be expected to be released to metropolitan sewer systems after use. A very small quantity (not specified) of the detergent formulation may remain in association with packaging, to be discarded with domestic garbage and placed into landfill. If it is assumed that 1% of the detergent is discarded in this manner, then up to 1 tonne of the new chemical may be placed into landfill with domestic garbage each year. Disposal would be nationwide and very diffuse.

8.2 Fate

The new chemical is not easily biodegradable, and a variety of tests on the parent acid of the notified chemical and other related phosphonates were carried out in order to establish likely rates of removal of released material from the environment. Summary reports were provided, but the tests and the results are summarised below.

Test	Concentration of Free Acid (FA) in test medium/Test duration	Result	Reference
Semi Continuous Activated Sludge (SCAS) Test	50 mg/L/ 14 weeks	Not degraded biologically and not adsorbed onto activated sludge.	Fletcher and Pover (1981)
Primary	2 mg/L of ^{14}C in	14.4% degraded in	Heidolph *et al*

Degradation in River Water	FA/60 days	sunlight; 5.2% degraded in the dark.	(1983a)
Adsorption on River Solids	0.05-5 mg/L of ^{14}C labelled FA/8 days	Very significant partitioning of the chemical to river sediments.	Michael *et al* (1980)

The Semi Continuous Activated Sludge (SCAS) test conducted in a reactor incubated with sewage sludge bacteria and operating on domestic sewage showed no removal of the compound (50 mg/L) over a 14-week period either through biological or adsorption processes. The chemical was monitored via periodic measurements of the dissolved organic carbon (DOC) in the liquor, and results indicated that the compound would pass through conventional activated sludge stages of sewage treatment plants. However, it is interesting that none of the compound was apparently adsorbed onto the sludge, and it is assumed that the sewage used had very low divalent metal content, precluding formation of neutral complexes, which would be expected to associate with sludge.

The definitive study on the degradation of related phosphonates in river water used 2 mg/L ^{14}C labelled compounds in sterile Meremac river water. Measurements of the evolved $^{14}CO_2$ revealed that over the 60-day period, only 0.2% of the compound had degraded in dark conditions, while 4.1% had degraded in sunlight. Results suggest that the compound is susceptible to slow photolytic degradation in water. When natural eutrophic lake water was substituted for the sterile river water, significantly higher degradation was observed over the 60-day period, with 5.2% degradation in the dark and 14.4% in sunlight. These results suggest that biological degradation also occurs, probably associated with the presence of algae in the water, which utilise the released phosphorus from the test compound.

The phosphonate compounds were found to partition strongly to river sediments, probably through the mechanism of complexing with Ca^{2+} and Mg^{2+} ions present in the water and as surface ions on the sediment minerals. In both hard and soft water containing between 0.05 and 5 mg/L of test compounds and standard sediment (US Nat. Bureau of Standards), the partitioning to the sediment (the concentration in sediment/concentration in water) was always in excess of 300. Although details of the water hardness were not included in the summary report, the partitioning was always stronger in the hard water (due to complexing with ambient Ca^{2+}) than in the soft water. Also although the partitioning study was conducted over an eight-day period, the equilibrium was established within the first two days.

The results of two other tests were provided. The first (Heidolph *et al*, 1983b) was concerned with potential mobilisation of metals from sediments through complex formation and solubilisation with the new compound. The second (Heidolph *et al*, 1983c) concerned removal of the related compounds from the water column through precipitation with flocculants such as ferric sulphate, alum and lime, commonly used during the later stages of sewage treatment processes. These studies will not be commented on in detail, but the results of the first indicated that heavy metals in sediments (eg Cu^{2+}, Zn^{2+} and Cd^{2+} etc) are not significantly mobilised by the concentrations of notified chemical likely to enter natural waters. The second study indicated as expected, that the compounds are precipitated into "chemical sludge" when solutions are treated with lime, ferric or ferrous sulphate or

aluminium salts. This is due to the formation of essentially insoluble metal complexes, which precipitate and become incorporated into metal hydroxide sludge.

Overall, most of the new compound (up to 100 tonnes per annum) will be discharged to the national sewage system where some could be expected to become associated with sewer sediments. The remainder would pass to sewage treatment plants where it is unlikely that much would be removed, since the material is not amenable to either biodegradation or adsorption to activated sludge in these systems. However, since flocculants such as lime and/or ferric sulphate are often used subsequent to activated sludge treatment[1], the compound may be removed into "chemical sludge", with very little released into receiving waters. In sewage sludge, the chemical is expected to be strongly complexed to Ca^{2+}, Mg^{2+} and Fe^{3+} ions.

Material disposed directly into landfill, as residues in packing material (estimated as up to 1 tonne per annum), would also become associated with soils and clays through complex formation with divalent and trivalent metal ions (eg Ca^{2+}, Mg^{2+} and Fe^{3+}) usually present in soil pore water.

The sludge from sewage treatment plants and from periodic cleaning of sewer mains is usually placed into landfill, used as a soil supplement or incinerated. In soil or landfill, the compound is expected to be slowly degraded through abiotic processes (eg photolysis), although some biological degradation may also occur. There is insufficient data to estimate the half-life of the notified chemical and related compounds in landfill and soil. Incineration of sludge containing the notified chemical would destroy it with production of nitrogen and carbon oxides while the phosphorus content would become assimilated into furnace ash or slag.

The high water solubility of the chemical and its apparently ready removal from the water column into aquatic sediments precludes bioaccumulation in water column dwelling organisms (Connell, 1990). However, since the compound readily partitions to sediments there may be a possibility for bioaccumulation in sediment dwelling organisms, but no supporting data on this possibility is available.

9. EVALUATION OF TOXICOLOGICAL DATA

Toxicological studies for the notified chemical (NC) are not complete. The notifier has provided additional toxicological studies on two closely related chemicals (free acid, FA and the sodium salt, SS) in support of the notification. The rationale presented for submitting these studies is that, in an aqueous environment, a salt will dissociate and therefore, regardless of the species added (free acid (FA) or salt (NC)), the chemical species available in the aqueous biological system (for example, the rat stomach at pH ~4) will be identical. These additional studies are accepted as surrogate studies for the assessment of notified chemical.

Eye and skin irritation tests have not been carried out on the notified chemical. The free acid in commercial form produced moderate irritant effects. The notifier states that, considering

[1] These reagents are usually added to reduce ortho-phosphate levels in the released sewage effluent, and also to reduce residual BOD.

the pH of the free acid (~1.3), the notified chemical, an alkaline metal salt, may be expected to have less aggressive effect.

For most studies it is not clearly mentioned if good laboratory practice (GLP) guidelines were followed. Some of the studies submitted were conducted before these guidelines were developed (1979).

9.1 Acute Toxicity

Summary of the acute toxicity of FA, SS and NC

Test	*Species*	*Outcome*	*Reference*
Acute oral toxicity (NC)	Mice	LD_{50} 14.3 g/kg	SEAC Toxicology Group, 1979a
Acute oral toxicity (NC)	Rat	LD_{50} >5.0 g/kg	Hazelton Laboratories Europe, 1979b
Acute oral toxicity (FA)	Mice	LD_{50} 4.1 g/kg	SEAC Toxicology Group, 1976
Acute oral toxicity (FA)	Rats	LD_{50} 4.1 g/kg	SEAC Toxicology Group, 1977a
Acute intraperitoneal toxicity (FA)	Mice	LD_{50} 0.30 g/kg	SEAC Toxicology Group, 1977b
Acute percutaneous toxicity (FA)	Rabbits	Non-toxic by skin absorption	Younger Laboratories, 1968a
Skin irritation (intradermal injection test; Komplex)	Rabbit	Slight to moderate	SEAC Toxicology Group, 1980a
Skin irritation (intradermal injection test, Calcium analogue – Drais Product)	Rabbit	Slight to moderate	SEAC Toxicology Group, 1980b
Skin irritation – covered patch test (FA)	Rabbit	Slight to moderate	SEAC Toxicology Group, 1977c
Skin irritation - intradermal injection test (FA)	Rabbits	Slight to moderate	SEAC Toxicology Group, 1977d
Skin irritation – intradermal injection test (SS)	Rabbit	Slight to moderate	SEAC Toxicology Group, 1980c
Skin irritation – covered patch test (SS)	Rabbit	Slight irritant	SEAC Toxicology Group, 1980d
Eye irritation (FA)	Rabbit	Moderate	Younger Laboratories, 1968b
Eye irritation (SS)	Rabbit	Slight irritant	SEAC Toxicology Group, 1980e
Skin sensitisation (NC)	Guinea pig	Weak-sensitiser	SEAC Toxicology Group, 1981a

Skin sensitisation (NC)	Guinea pig	Non-sensitiser	SEAC Toxicology Group, 1984a
Skin sensitisation (FA)	Guinea pig	Non-sensitiser	SEAC Toxicology Group, 1977e
Skin sensitisation (SS)	Guinea pig	Non-sensitiser	SEAC Toxicology Group, 1981b
Skin sensitisation (SS)	Guinea pig	Sensitiser	SEAC Toxicology Group, 1983
Skin sensitisation (SS)	Guinea pig	Weak sensitiser	SEAC Toxicology Group, 1982a

9.1.1 Oral Toxicity

9.1.1.1 Oral toxicity of NC (SEAC toxicology Group, 1979a)

Species/strain:	Mice (strain not specified)
Number/sex of animals:	3/sex/group
Observation period:	21 days
Method of administration:	Oral (gavage): 0, 10.0, 12.6, 15.9, 20.0 g/kg body weight.
Test method:	Similar to OECD TG 401
Mortality:	6/6 in 20.0 mg/kg bw group and 3/6 in 15.9 mg/kg bw group.
Clinical observations:	All mice from the highest dose group (20 g/kg) died within 18 hours after treatment. Mice from other dose groups were somnolent, hypothermic and showed signs of stress and had diarrhoea. All mice recovered within 18 hours after treatment.
Morphological findings:	Irritation of pyloric region (with petechia present), duodenum and ilium. Intracranial haemorrhaging and congestion. Autopsy revealed the stomach and intestines were distended with white creamy fluid.
LD_{50}*:*	15.9 (14.3-17.6) g/kg bw.
Result:	The notified chemical was of very low acute oral toxicity in mice.

9.1.1.2 Oral toxicity of NC (Hazelton Laboratories Europe, 1979b)

Species/strain:	Sprague-Dawley CD rats

Number/sex of animals:	2/sex/group, except top dose (5/sex)
Observation period:	14 days
Method of administration:	Oral (gavage): 0.25, 0.5, 1.0, 2.0 and 5.0 g/kg body weight.
Test method:	Similar to OECD TG 401
Mortality:	None
Clinical observations:	All animals appeared normal during the observation period.
Morphological findings:	No necropsies were performed.
LD_{50}:	>5.0 g/kg.
Comment:	The test had no control group.
Result:	The notified chemical was of very low acute oral toxicity in rats

9.1.1.3 Oral toxicity of FA (SEAC Technology Group, 1976)

Species/strain:	Mice (strain not specified)
Number/sex of animals:	3/sex/group
Observation period:	21 days
Method of administration:	Oral (gavage): 0, 1.80, 2.70, 4.05 and 6.08 g/kg body weight.
Test method:	Similar to OECD TG 401
Mortality:	6/6 in the 6.08 g/kg bw group and 1/6 in the 4.05 g/kg bw group.
Clinical observations:	In the first hour most animals exhibited hypothermia, exertion tremors and stark fur. Some were comatose and exhibited dyspnea and cynosis.
Morphological findings:	Autopsy of dead animals revealed gaseous/fluid distension of the stomach and bleaching of stomach and intestines.
Comment:	LD_{50} values and confidence limits were estimated by Weil's method (Weil, 1952).
LD_{50}:	4.6 (4.1-5.3) g/kg.

Result:	FA was of very low acute oral toxicity in mice.

9.1.1.4 Oral toxicity of FA (SEAC Technology Group, 1977a)

Species/strain:	Colworth rats
Number/sex of animals:	3/sex/group.
Observation period:	21 days
Method of administration:	Oral (gavage): 0, 3.0, 4.5, 6.75 and 10.13 g/kg body weight.
Test method:	Similar to OECD TG 401
Mortality:	6/6 in the 6.75 and 10.13 g/kg bw groups and 2/6 in the 4.50 g/kg bw group.
Clinical observations:	Most rats dosed 6.75 g/kg bw FA and above were prostrate showing signs of stress, In the first hour most animals exhibited hypothermia, dyspnea and stark fur. Some animals exhibited somnolence and clonic spasms. All rats dosed 6.75 g/kg bw FA and above died within 30 min to 42 hours after treatment.
Morphological findings:	Autopsy of dead animals revealed haemorrhages in the stomach and small intestines and flaccid small and large intestines, with fluid distension present.
Comment:	LD50 values and confidence limits were estimated by Weil's method.
LD_{50}*:*	4.8 (4.1-5.7) g/kg.
Result:	FA was of very low acute oral toxicity in rats.

9.1.2 Dermal Toxicity

9.1.2.1 Intraperitoneal toxicity of FA (SEAC Toxicology Group, 1977b)

Species/strain:	Mice (strain not specified)
Number/sex of animals:	3/sex/group.
Observation period:	21 days
Method of administration:	Intraperitoneal injection: 0, 0.30, 0.45, 0.68 and 1.02 g/kg body weight.

Test method:	Not stated
Mortality:	5/6 in the 1.02 g/kg bw group; 4/6 in the 0.45 and 0.68 g/kg bw groups and 1/6 in the 0.3 g/kg bw group.
Clinical observations:	In the first 2 hours, most animals exhibited hypothermia, cynosis, somnolence, laboured breathing and clonic spasma.
Morphological findings:	Autopsy of dead animals revealed white 'fatty' particles in the abdominal cavity and fluid distension of stomach and small intestines. Livers of some mice appeared bleached and they had pale kidneys.
Comment:	LD_{50} values and confidence limits were estimated by Weil's method.
LD_{50}:	0.45 (0.30-0.68) g/kg.

9.1.2.2 Acute percutaneous toxicity of FA (Younger Laboratories, 1968a)

Species/strain:	New Zealand White rabbits.
Number/sex of animals:	3/sex
Observation period:	24 hours
Method of administration:	FA was applied as a 25% suspension in corn oil (0.50, 0.79, 1.26, 2.00, 3.16 and 5.01 g/kg body weight) to the shaved, non-abraded skin of rabbits.
Test method:	Similar to OECD TG 402.
Mortality:	None
Clinical observations:	No real symptoms of acute systemic toxicity were noted.
LD_{50}:	>5 g/kg.
Result:	FA was of low toxicity in rabbits by the dermal route.

9.1.3 Skin Irritation

9.1.3.1 Skin irritation with Komplex (Intradermal injection) (SEAC Technology, 1980a)

The notifier provided skin irritation study using Komplex , containing 20.1% active ingredient. However, the identity of this active ingredient (ai) was not apparent from the test report. The test substance was injected intradermally to determine the full irritant potential of

the substance, independent of its ability to penetrate the stratum corneum. For comparison purpose, a number of related chemicals (FA (90% ai), SS (33% ai), Calcium analogue (38% ai), NC (36% ai) and sodium lauryl sulphate (SLS)) were also tested by this method.

Species/strain:	New Zealand White rabbits
Number/sex of animals:	8 (sex not specified)
Observation period:	24 and 48 hours
Method of administration:	Intradermal injection: 0.1 ml of 0.1, 0.5, 1.0 and 2% ai.
Test method:	Not stated
Comment:	The pH of NC solution was 12.8. Results were expressed as the diameter of the reaction area and necrotic centres, and the size and description of macroscopic reactions caused by the test substance, 24 and 48 hours after injection. The appearance of each reaction was graded on a scale of faint pink to deep pink. The colour of necrotic sites was also noted. Calcium analogue and FA caused the highest responses.
Result:	NC caused significant irritant reaction in rabbits.

9.1.3.2 Skin irritation with Calcium analogue – Drais Product (Intradermal injection)(SEAC Technology, 1980b)

The test substance, Calcium analogue-Drais product (containing 19% ai)(actual identity not given) was injected intradermally to determine the full irritant potential of the substance, independent of its ability to penetrate the stratum corneum. For comparison purpose, a number of related chemicals (FA (90% ai), Calcium analogue ex URLPS (38% ai), NC and sodium lauryl sulphate (SLS)) were also tested by this method.

Species/strain:	New Zealand White rabbits.
Number/sex of animals:	8 (sex not specified).
Observation period:	24 and 48 hours.
Method of administration:	Intradermal injections: 0.1 mL of 0.1, 0.5, 1.0 and 2% ai.
Test method:	Not stated
Comment:	The pH of the NC solution was 12.7. Results are expressed as the diameter of the reaction area

	and necrotic centres, and the size and description of macroscopic reactions caused by the test substance, 24 and 48 hours after injection. The appearance of each reaction was graded on a scale of faint pink to deep pink. The colour of necrotic sites was also noted. NC produced a moderate response at the three top concentrations, but at the lowest concentration (0.1%) showed marginal effects.
Result:	NC was a moderate skin irritant in rabbits.

9.1.3.3 Skin irritation with FA (SEAC Technology, 1977c)

Species/strain:	New Zealand White rabbits
Number/sex of animals:	10 (sex not specified)
Observation period:	24, 48 and 72 hours
Method of administration:	0.2 g of FA powder was applied under a 2.5 cm^2 gauze pad moistened with 0.5 mL of distilled water to one intact skin site on each animal and held under semi-occlusive dressing. After four hours, treatment sites were washed using water to remove any residual test substance.
Test method:	Not stated

The treated sites were scored for erythema, oedema, cracking and scaling on an 8-point anchored ordinate scale, ranging from 'a' (very slight) to 'h' (severe). The scores were then converted to corresponding numerical scores that were used to calculate the total irritation score per rabbit.

From the description of effects corresponding to the numerical scores used in the study, it was noted that these score points correlated to Draize scores in the following way: Score points 1 and 2 corresponded to Draize score 1, score points 3 and 4 corresponded to Draize score 2, score points 6 and 8 corresponded to Draize score 3 and score points 10 and 12 corresponded to Draize score of 4. For the purpose of this report, the score points in the study were converted to Draize points and analysed as shown in the following table.

Draize scores:

Time after	***Animal No.***									
treatment (days)	***1***	***2***	***3***	***4***	***5***	***6***	***7***	***8***	***9***	***10***
Erythema										
1	1	1	0	0	0	1	0	1	1	0
2	1	1	0	0	0	0	0	1	0	0
3	0	0	0	0	0	0	0	1	0	0
Oedema										
1	0	1	0	0	0	0	0	0	1	0
2	0	1	0	0	0	0	0	0	0	0
3	0	1	0	0	0	0	0	0	0	0

[a] see Attachment 1 for Draize scales

Result: FA was slightly irritating to the skin of rabbits

Comment: Ethylenediamine tetra-acetic acid (EDTA) and sodium lauryl sulphate (SLS) were also tested as controls. FA had similar response to that of EDTA and was less irritant than 1.0% SLS.

9.1.3.4 Skin irritation with FA (intradermal injection) (SEAC Technology, 1977d)

Species/strain: New Zealand White rabbits

Number/sex of animals: 10 (sex not specified)

Observation period: 24 hours

Method of administration: FA (0.05, 0.1, 0.5 and 1% in saline) was injected intradermally at different sites on shaven skin of rabbits to determine the full irritant potential of the substance, independent of its ability to penetrate the stratum corneum.

Test method: Not stated

Comment: Mean diameter and appearance of irritation reaction were used as a measure of irritation of the test sample.

Result: 1% FA was slightly to moderately irritant to rabbit skin after intradermal injection. The level of irritation produced by FA was significantly less than that produced by SLS (0.02%) that was also tested as a positive control.

9.1.3.5 Skin irritation with SS (intradermal injection) (SEAC Technology, 1980c)

Species/strain:	New Zealand White rabbits
Number/sex of animals:	8 (sex not specified)
Observation period:	24 and 48 hours
Method of administration:	SS (0.08, 0.4, 0.8 and 1.53% active ingredient in saline) was injected intradermally at different sites on shaven skin of rabbits to determine the full irritant potential of the substance, independent of its ability to penetrate the stratum corneum.
Test method:	Not stated
Comment:	Mean diameter and appearance of irritation reaction were used as a measure of irritation of the test sample.
Result:	At highest concentration (1.53%) SS produced a moderate irritation effect. It was slightly less irritating than FA.

9.1.3.6 Skin irritation with SS (SEAC Technology, 1980d)

Species/strain:	New Zealand White rabbits
Number/sex of animals:	8 (sex not specified)
Observation period:	24, 48 and 72 hours
Method of administration:	0.5 mL of SS (33% ai) was applied under a 2.5 cm^2 gauze pad moistened with 0.5 mL of distilled water to one intact skin site on each animal and held under occlusive dressing (zinc oxide plaster). After four hours, treatment sites were wiped clean of excess material. FA, 2044 and SLS (1% and 10%) were also tested in a similar way as standards for comparison.
Test method:	Not stated

The treated sites were scored for erythema, oedema, cracking and scaling on an 8-point anchored ordinate scale, ranging from 'a' (very slight) to 'h' (severe). The scores were then converted to corresponding numerical scores that were used to calculate the total irritation score per rabbit.

From the description of effects corresponding to the numerical scores used in the study, it was noted that these score points correlated to Draize scores in the following way: Score points 1 and 2 corresponded to Draize score 1, score points 3 and 4 corresponded to Draize

score 2, score points 6 and 8 corresponded to Draize score 3 and score points 10 and 12 corresponded to Draize score of 4. For the purpose of this report, the score points in the study were converted to Draize points and analysed as shown in the following table.

Draize scores:

Time after	***Animal No.***							
treatment (days)	***1***	***2***	***3***	***4***	***5***	***6***	***7***	***8***
Erythema								
1	1	0	0	0	0	0	0	0
2	0	1	0	0	0	0	0	0
3	1	0	0	0	0	0	0	0
Oedema								
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

[a] see Attachment 1 for Draize scales

Comment: The experimental SS produced only marginal effects, and was similar in irritancy to other analogues.

Result: SS was slightly irritating to the skin of rabbits

9.1.4 Eye Irritation

9.1.4.1 Eye irritation with FA (Younger Laboratories, 1968b)

Species/strain: New Zealand White rabbit

Number/sex of animals: 3 (2 males, 1 female)

Observation period: 1 to 7 days after treatment

Method of administration: 100 mg of finely ground FA powder were placed into the conjunctival sac of the right eye of each rabbit. The eyes were examined for signs of irritation after 1, 24, 48, 72, 120 and 168 hours. The treated eyes were rinsed with warm isotonic saline 24 hours after treatment later. After the reading, fluorescein (concentration not stated) was instilled to detect corneal damage.

Test method: Not stated

Comment: Treated eyes were graded according to the severity of erythema, oedema, discharge, corneal opacity and iridial reactions on an arbitrary scale of 0 to 110.

Moderate discharge and erythema, mild oedema and mild corneal cloudiness developed in all treated eyes after 1 hour. Iris clarity improved overnight and discharge had nearly ceased within 3 days. A slight degree of redness and oedema remained after 7 days.

Result: FA was considered to be a moderate eye irritant in rabbits under conditions of the test.

9.1.4.2 Eye irritation with SS (SEAC Technology Group, 1980e)

Species/strain: New Zealand White rabbits.

Number/sex of animals: 6 (sex not specified).

Observation period: 24, 48 and 72 hours.

Method of administration: 50 mg (in 0.1 mL) of SS were placed into the conjunctival sac of the right eye of each rabbit. The eyes were examined for signs of irritation after 24, 48 and 72 hours. The treated eyes were rinsed with warm isotonic saline 24 hours after treatment later. After the reading, fluorescein (concentration not stated) was instilled to detect corneal damage.

Test method: Modified Federal Hazardous Substances Labelling Act (UK) method. Similar to OECD TG 405.

Draize scores of unirrigated eyes:

	Time after instillation									
Animal	***15 minutes***		***1 day***		***2 days***		***3 days***		***7 days***	
Cornea	***o***	***a***	***o***	***a***	***o***	***a***	***o***	***a***	***o***	***a***
1	NE	NE	0.5	1	NE	NE				
2	NE	NE	0.5	1	NE	NE				
3	NE	NE	0.5	1	NE	NE				
4	NE	NE	0	0	NE	NE				
5	NE	NE	0	0	NE	NE				
6	NE	NE	0.5	1	NE	NE				

	15 minutes	***1 day***	***2 days***	***3 days***	***7 days***
Iris					
1	NE	0	0		
2	NE	0	0		
3	NE	0	0		
4	NE	0	NE		
5	NE	0	NE		
6	NE	0	0		

Conjunctiva	***r***	***c***	***d***	***r***	***c***	***d***	***r***	***c***	***d***
1	1	0	NE	0	0	0	0	0	0
2	1	0	NE	1	0	0	0	0	0
3	1	1	NE	1	0	0	0	0	0
4	0	0	NE	0	0	0	NE	NE	NE
5	1	0	NE	0	0	0	NE	NE	NE
6	1	0	NE	1	0	0	0	0	0

[1] see Attachment 1 for Draize scales
O = opacity, a = area, r = redness, c = chemosis, d = discharge, NE = Not estimated

Result: SS was slightly irritating to the eyes of rabbits.

9.1.5 Skin Sensitisation

9.1.5.1 Skin Sensitisation test with NC (SEAC Toxicology Group, 1981a)

Species/strain: Guinea pig/ Dunkin-Hartley

Number of animals: 10 (6 males, 4 females)

Induction procedure:

Test group:
day 0

Three pairs of intradermal injections (0.1 mL) were made on the shoulder region:

FCA diluted 1:1 with physiological saline
NC, 2.5% in physiological saline
NC, 2.5% in a 1:1 mixture of FCA and saline

day 7

A 2x4 cm filter paper patch was saturated with 40% NC in distilled water and placed over the shaved area and covered by impermeable polythene adhesive tape.

Control group:

Treated controls: During the induction phase control animals (4/same sex) were treated in the same way as test animals except that the test substance was omitted from the intradermal injections and topical applications.

Untreated controls: At every challenge in the test, 4 previously untreated animals of the same sex and weighing approximately the same as the test animals at that challenge are treated in exactly the same way as the test animals.

Challenge procedure:

day 21

The test and control animals were challenged topically two weeks after topical induction using 10% NC in distilled water. Patches of filter paper were saturated with 10% NC solution and placed on shaved flanks for 24 hours.

Further challenges were made at weekly intervals as required.

Test method:

OECD TG 406, Magnusson and Kligman Guinea Pig Maximisation Test (Magnusson and Kligman, 1970).

Challenge outcome:

	Test animals		***Control animals***	
Challenge Concentration	***24 hours****	***48 hours****	***24 hours***	***48 hours***
Challenge 1 (10%)	1/10**	1/10	0/4	0/4
Challenge 2 (10%)	0/10	1/10	0/4	0/4

*Time after patch removal
** number of animals exhibiting positive response (scattered erythema, faint pink)

Result:	The notified chemical was weakly sensitising to the skin of guinea pigs

9.1.5.2 Skin Sensitisation test with NC (SEAC Toxicology Group, 1984a)

Species/strain:	Guinea pig (strain not specified)
Number of animals:	10 (6 males, 4 females)
Induction procedure:	
Test group: day 0	Three pairs of intradermal injections (injection volume not specified) were made on the shoulder region: FCA diluted 1:1 with physiological saline NC, 0.25% in physiological saline NC, 0.25% in a 1:1 mixture of FCA and saline
day 6-7	20% NC applied to the shoulder injection sites under occlusion.
Control group:	Treated controls: During the induction phase control animals (4/same sex) were treated in the same way as test animals except that the test substance was omitted from the intradermal injections and topical applications. Untreated controls: At every challenge in the test, 4 previously untreated animals of the same sex and weighing approximately the same as the test animals at that challenge are treated in exactly the same way as the test animals.
Challenge procedure:	
day 21	The test and control animals were challenged topically two weeks after topical induction using 5% NC in distilled water. Patches of filter paper were saturated with 5% NC solution and placed on shaved flanks for 24 hours. Further challenges were made at weekly intervals as required.
Test method:	OECD TG 406, Magnusson and Kligman Guinea Pig Maximisation Test.

Challenge outcome:

Challenge Concentration	Test animals		Control animals	
	*24 hours**	*48 hours**	*24 hours*	*48 hours*
Challenge 1 (5%)	0/10**	0/10	0/4	0/4
Challenge 2 (5%)	0/10	0/10	1/4	0/4

* Time after patch removal
** number of animals exhibiting positive response (faint erythema, usually confluent)

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

9.1.5.3 Skin Sensitisation test with FA (SEAC Toxicology Group, 1977e)

Species/strain: Guinea pigs (strain not specified)

Number of animals: 10 (6 males, 4 females)

Induction procedure:

Test group:
day 0 Three pairs of intradermal injections (0.1 mL) were made on the shoulder region:

FCA diluted 1:1 with physiological saline
FA, 1% in physiological saline
FA, 1% in a 1:1 mixture of FCA and saline

day 7 A 2x4 cm filter paper patch was saturated with 5% FA and placed over the shaved area and covered by impermeable polythene adhesive tape.

Control group: Treated controls: During the induction phase control animals (4/same sex) were treated in the same way as test animals except that the test substance was omitted from the intradermal injections and topical applications.

Untreated controls: At every challenge in the test, 4 previously untreated animals of the same sex and weighing approximately the same as the test animals at that challenge are treated in exactly the same way as the test animals.

Challenge procedure:

day 21 The test and control animals were challenged topically two weeks after topical induction using 2% FA. Patches of filter

paper were saturated with 2% FA and placed on shaved flanks for 24 hours.

A third challenge was made with 1% and 2% FA.

Test method: OECD TG 406, Magnusson and Kligman Guinea Pig Maximisation Test.

Challenge outcome:

	Test animals		***Control animals***	
Challenge Concentration	***24 hours****	***48 hours****	***24 hours***	***48 hours***
Challenge 1 (2%)	0/10**	0/10	0/4	0/4
Challenge 2 (2%)	0/10	0/10	0/4	0/4
Challenge 3 (1% and 2%)	0/10	0/10	0/4	0/4

* Time after patch removal
** number of animals exhibiting positive response (scattered, mild erythema, faint pink)

Result: FA was not sensitising to the skin of guinea pigs in this assay.

9.1.5.4 Skin Sensitisation test with SS (SEAC Toxicology Group, 1981b)

Species/strain: Guinea pig/ Dunkin-Hartley

Number of animals: 10 (6 males, 4 females)

Induction procedure:

Test group:
day 0 — Three pairs of intradermal injections (0.1 mL) were made on the shoulder region:

FCA diluted 1:1 with physiological saline
SS, 0.83% in physiological saline
SS, 0.83% in a 1:1 mixture of FCA and saline

day 7 — A 2x4 cm filter paper patch was saturated with 33% SS and placed over the shaved area and covered by impermeable polythene adhesive tape.

Control group: Treated controls: During the induction phase control animals (4/same sex) were treated in the same way as test animals except that the test substance was omitted from the intradermal injections and topical applications.

Untreated controls: At every challenge in the test, 4 previously untreated animals of the same sex and weighing approximately the same as the test animals at that challenge are treated in exactly the same way as the test animals.

Challenge procedure:

day 21

The test and control animals were challenged topically two weeks after topical induction using 33% SS in distilled water. Patches of filter paper were saturated with 33% SS solution and placed on shaved flanks for 24 hours.

Further challenges were made at weekly intervals as required.

Test method: OECD TG 406, Magnusson and Kligman Guinea Pig Maximisation Test.

Challenge outcome:

	Test animals		***Control animals***	
Challenge Concentration	***24 hours****	***48 hours****	***24 hours***	***48 hours***
Challenge 1 (33%)	0/10**	0/10	0/4	0/4
Challenge 2 (33%)	0/10	0/10	0/4	0/4
Challenge 3 (33%)	0/10	0/10	0/10	0/10

* time after patch removal ** number of animals exhibiting erythema

Comment: In the first challenge, the response was similar to that observed in treated controls. In the second challenge minimal erythema was observed and, under the conditions of the test, is not considered a positive response.

Result: SS was not sensitising to the skin of guinea pigs.

9.1.5.5 Skin Sensitisation test with SS (SEAC Toxicology, 1983)

Species/strain: Guinea pigs/Albino Dunkin/Hartley

Number of animals: 10 (6 males, 4 females)

Induction procedure:

Test group: day 0

Three pairs of intradermal injections (0.1 mL) were made on the shoulder region:
FCA diluted 1:1 with physiological saline
SS, 0.5% in physiological saline
SS, 0.5% in a 1:1 mixture of FCA and saline

day 7

A 2x4 cm filter paper patch was saturated with 25% SS and placed over the shaved area and covered by impermeable polythene adhesive tape.

Control group:

Treated controls: During the induction phase control animals (4/same sex) were treated in the same way as test animals except that the test substance was omitted from the intradermal injections and topical applications.

Untreated controls: At every challenge in the test, 4 previously untreated animals of the same sex and weighing approximately the same as the test animals at that challenge are treated in exactly the same way as the test animals.

Challenge procedure:

day 21

The test and control animals were challenged topically two weeks after topical induction using 25% SS in distilled water. Patches of filter paper were saturated with 25% SS solution and placed on shaved flanks for 24 hours.

Further challenges were made at weekly intervals as required.

Test method:

OECD TG 406, Magnusson and Kligman Guinea Pig Maximisation Test.

Challenge outcome:

	Test animals		***Control animals***	
Challenge Concentration	***24 hours****	***48 hours****	***24 hours***	***48 hours***
Challenge 1 (25%)	6/9**	4/9	0/4	0/4
Challenge 2 (25%)	4/8	4/8	0/3	0/3

* time after patch removal.
** number of animals exhibiting positive response (faint erythema, usually confluent).

Comment:

The response to the challenge ranged from faint to moderate erythema. In challenge 1, the response in 2 animals was borderline in terms of a positive response.

Result:

SS was a skin sensitiser in guinea pigs

9.1.5.6 Skin Sensitisation test with SS (SEAC Toxicology Group, 1982a)

Species/strain:	Guinea pigs (Albino Dunkin/Hartley)
Number of animals:	10 (6 males, 4 females)
Induction procedure:	
Test group: day 0	Three pairs of intradermal injections (0.1 mL) were made on the shoulder region: FCA diluted 1:1 with physiological saline SS, 1% in physiological saline SS, 1% in a 1:1 mixture of FCA and saline
day 7	A 2x4 cm filter paper patch was saturated with SS (described as 'neat' in test report; concentration of active ingredient not stated) and placed over the shaved area and covered by impermeable polythene adhesive tape.
Control group:	Treated controls: During the induction phase control animals (4/same sex) were treated in the same way as test animals except that the test substance was omitted from the intradermal injections and topical applications. Untreated controls: At every challenge in the test, 4 previously untreated animals of the same sex and weighing approximately the same as the test animals at that challenge are treated in exactly the same way as the test animals.
Challenge procedure:	
day 21	The test and control animals were challenged topically two weeks after topical induction using neat SS. Patches of filter paper were saturated with SS and placed on shaved flanks for 24 hours. Further challenges were made at weekly intervals as required.
Test method:	OECD TG 406, Magnusson and Kligman Guinea Pig Maximisation Test.

	Challenge outcome:			
	Test animals		***Control animals***	
Challenge Concentration	***24 hours****	***48 hours****	***24 hours***	***48 hours***
Challenge 1	3/9**	2/9	0/4	0/4
Challenge 2	2/9	1/9	0/4	0/4

* time after patch removal
** number of animals exhibiting positive response (faint erythema, usually confluent).

Result: SS was a weak skin sensitiser in guinea pigs.

9.2 Repeated Dose Toxicity

9.2.1 Sub-acute oral toxicity study (3 wks) with FA (SEAC Toxicology Group, 1979c)

Species/strain: Colworth Wistar rats

Number/sex of animals: 8/sex/group

Method of administration: Oral (dietary)

Dose/Study duration: 0.1, 0.2. 0.5, 1.0 and 2.0% FA in purified diet for three weeks (equivalent to a mean dose of 0.124, 0.244, 0.599, 1.208 and 2.285 g/kg/day in males and 0.121, 0.246, 0.630, 1.266 and 2.343 g/kg/day in females). A control group received the purified diet alone.

Test method: OECD TG 407

Clinical observations:
All rats survived to the end of the trial and showed no toxicity symptoms, signs of distress or decrease in activity. Significantly reduced food and water intake and body weight gains were observed in the highest dose group (2% FA) rats. Male rats in this group drank significantly less water than control rats.

Clinical chemistry/Haematology
Significantly lower levels of plasma magnesium, protein, cholesterol and glucose were noted in the highest dose group (both sexes). Male rats had significantly higher levels of plasma alanine transaminase. FA had no effect on haematological parameters.

Pathology
Relative organ weights (brain, kidney, heart, adrenal, spleen and testes) were significantly higher in the high dose group compared to controls. Pathological changes observed included depleted abdominal fat depots in high dose group. Pallor of the contents of the caecum or colon was seen in all rats fed 1% and 2% FA. The caecal and colonic contents of most of the rats fed 0.5% FA were slightly pale in colour.

Histopathology:
Histopathological observations revealed liver, spleen and kidneys were affected by FA. In males from 2% FA group, liver granular neutral fat content was higher compared to that of control group. Haemosiderin content of periportal parenchymal cells was significantly reduced in both male and female rats. The incidence of low grade, periportal lymphocyte/neutrophil infiltration was slightly greater in this group.

Accelerated haemopoietic activity was found in the spleens of both male and female rats from the high dose group, with myeloid hyperplasia in male rats and megakaryotic hyperplasia in female rats observed. The haemosiderin content of red pulp macrophages was significantly reduced in both male and female rats of this group and at 0.5 and 1% FA.

In kidneys, tubular necrobiosis was observed in outer medulla in the high dose group. Nuclear pyknosis, cellular swelling and increased cytoplasmic acidophilia were conspicuous in affected tubules. Absence of nephrocalcinosis (intratubular mineral deposition) in female rats fed 1 and 2% FA was considered to be treatment related. Haemosiderin was absent in the proximal convoluted tubules at 0.5, 1.0 and 2.0% and present at very low levels at 0.1 and 0.2%.

Comment:
Some of the effects observed in experimental rats receiving 2% FA were considered not to represent toxic changes induced by FA, but to be attributable to reduced food intake. However, several effects of treatment directly attributable to the ingestion of FA were observed at all levels of the chemical tested. Most significant were those indicative of reduced tissue levels of minerals and probably reflect the chelating ability of ingested FA and reduced mineral absorption from the intestine. For example, the absence or reduction of haemosiderin in the red pulp of the spleen, peripheral parenchyma in the liver and the proximal convoluted tubules of the kidney is ascribed to chelation by the test chemical.

In addition, evidence of liver toxicity was suggested by the increase in neutral fat content, supported by the increase of plasma alanine transaminase. Nephrotoxicity was indicated by tubular necrobiosis with the lesions observed similar to those observed with other chelating agents.

Result:
A no observed adverse effect level (NOAEL) could not be established, as effects of treatment were noted at all dietary levels in kidney. The low observed adverse effect level (LOAEL) was 121 mg/kg/day.

9.2.2 Sub-chronic oral toxicity study (90 days) (SEAC Toxicology Group, 1984b)

Species/strain:	Colworth Wistar rats
Number/sex of animals:	10/sex/group
Method of administration:	Oral (dietary)
Dose/Study duration:	0.05, 0.1, 0.2, 0.5 and 2.0% FA; 90 days. Two control groups, one fed purified diet and the other, purified diet

deficient in iron, were also included.

Test method: OECD TG 408

Clinical observations:
FA was toxic at higher concentration levels. Rats fed 2% FA developed some degree of paralysis that was apparent by their shuffling gait and reduced muscle tone, particularly in hind limbs and tail. Significantly reduced body weight gain and food and water intake were observed in both male and female rats of this group. Three rats from this dose group were killed on humane grounds before completion of study. All rats, apart from those fed 2% FA, survived to the end of the study and showed no toxic symptoms, signs of distress or decrease in activity.

Prior to necropsy, whole body radiographs were taken of animals from all groups. Skeletal abnormalities were observed in rats dosed at 2%, with the males more severely affected. In affected rats, the skeleton was smaller and had lower radio-opacity, with the most affected areas being the thorax, spine, scapulae and long bones of the limbs. These features were also observed in the rats humanely killed during the study.

Clinical chemistry/Haematology
Plasma calcium and phosphate levels were increased and plasma magnesium and alkaline phosphatase levels were reduced in rats fed 2% FA. These changes were related to the histological changes observed in these rats. Reduced serum protein and plasma cholesterol and elevated levels of plasma creatine kinase, aspartate transaminase and lactate dehydrogenase were also noted in these rats. No significant changes in serum electrophoretic parameters were noted.

The 2% FA group had lower packed cell volume and haemoglobin concentration and higher white blood cell count when compared to those of controls. All other dose groups showed a non-dose-related increase in red blood cell count in female rats. A slight decrease in mean corpuscular volume and mean corpuscular haemoglobin was also noted in some groups of rats, which was not dose related.

Pathology:
Increased relative spleen, heart, kidney and adrenal weights were noted in rats fed 2% FA. Pallor of caecal contents, sometimes associated with a slight increase in bulk fluidity, was seen in rats fed the highest dose, and to a lesser extent in rats fed 0.5% and 0.2% FA. Fat deposition within the abdominal cavity was conceived to be low in the high dose group (visual assessment).

Severe skeletal changes were seen in the high dose rats. Femur, tibia, ribs, sternum and vertebrae were severely affected. In the worst cases, the deformation of thoracic cage was associated with a reduction in the height of the thoracic cavity. No such effects were observed in rats fed the lower levels of FA or in controls.

Histopathology:
Changes attributable to the ingestion of FA were seen in bone, with the femur, tibia, ribs, sternum and vertebrae examined microscopically. Examination revealed thickening, distortion and mis-alignment of bones.

In kidneys, presence of multinucleate cells in the outer medulla and a reduction in the haemosiderin content of the proximal convoluted tubules were seen in rats fed 2% FA. A reduction of nephrocalcinosis was observed in female rats at all doses, however, males were less affected. In female rats, there was a parallel decrease in tubular atrophy and interstitial fibrosis, for which mineral deposition is considered to be a contributory factor. The haemosiderin content in the proximal convoluted tubules was reduced in both males and females at 2%.

In the 2% FA fed group, male rats had reduced liver fat. Females at 0.5% and 2% demonstrated reduced haemosiderin content of liver parenchymal cells. Myelopoietic activity was greatest in the spleen of both male and female rats of the 2% group, with the haemosiderin content in red pulp macrophages reduced in males only at 0.5% and 2%.

Histological examination of the spinal cord revealed small numbers of degenerate fibres in a few rats, which is considered a result of severe pathological changes in the vertebrate rather than any neurological defects. A reduced haemosiderin content was observed at 2% in macrophages within the endometrium.

Comment:
The effects attributable to the ingestion of FA are considered to be directly or indirectly due to its chelating properties. This was seen in its effect on the availability of iron, leading to a reduction in haemosiderin levels, and on the availability of minerals, leading to a reduction in nephrocalcinosis.

Microscopic examination revealed reduced haemosiderin content in the liver, kidney, spleen and endometrium. Consistent with reduced iron levels, a pallor of caecum contents was observed in rats in the macroscopic examination.

Reduced mineral availability was demonstrated in skeletal observations during radiography and pathology examination. Distortion and thickening of bone was particularly noticeable in the ribs and long bones of the limbs. The effects were consistent with the shuffling movements and reduced mobility observed in animals during the study.

Result:
An overall NOAEL of 0.2% FA (equivalent to 0.17 g/kg/day), was established based on reduced levels of tissue iron (haemosiderin) at 0.5 and 2%. A NOAEL for skeletal abnormalities and occurrence of multinucleate cells in renal tubules was established at 0.5%.

9.2.3 Published Study (Calvin et al, 1988).

The notifier provided a published study on the bio-distribution, repeat dose toxicity and genotoxicity of the free acid form of the notified chemical, FA, in rats. Repeat dose toxicity (sub-chronic and chronic) and bio-distribution results of the study are summarised in this section and the genotoxicity effects are summarised in Section 9.3.

Bio-distribution of FA

Groups of ten male Sprague-Dawley rats were given ten daily gavage doses of labelled FA at dose levels of 4, 16 or 64 mg/kg/day. Rats were also dosed with labelled FA in the feed or drinking water to achieve a dose of 3.8 mg/kg/day. Ninety minutes after the tenth dose, rats were killed.

Urinary output of the labelled material was variable from day to day, with mean values that showed little effect of repeated dosing, or dose level on absorption. The mean percentages of dose present in the daily urine output were 0.7. 0.8 and 1.2% in the 4, 16 and 64 mg/kg/day dose groups, respectively. Levels of the labelled chemical in plasma, washed red blood cells and carcass (minus the gastrointestinal tract (GIT)) increased as linear functions of the daily dosage. The concentration of the label in the washed GIT increased sharply at 64 mg/kg/day relative to the concentrations at lower dose levels. The percentage of label in bone increased with daily dose and reached a maximum of approximately 50% of total carcass ^{14}C at the highest dose.

In a comparison of administration routes with a dose of 4 mg/kg/day for 10 days, gavage dosing resulted in four- to six fold increases in the bone compared to the concentrations achieved by administering the compound via the feed or drinking water. Accumulation of the label in the carcass showed a similar trend. Over 90% of ^{14}C label was found in the faeces of rats fed by any route.

Sub-chronic feeding study (13 weeks)

Sprague-Dawley rats (25/sex/group) were fed 5, 50 and 500 mg/kg/day FA in their diets for 13 weeks. After 6, 9 and 12 weeks of treatment, blood samples were taken from 5 rats in each group for haematological analysis. After 13 weeks all surviving rats were killed for detailed macroscopical and histo-pathological analysis.

In addition to these groups, five rats/sex/group were treated similarly but not subjected to any haematological or blood chemistry determinations during the course of the first 13 weeks. During the ensuing 9-week recovery period all of these rats received untreated diet. After 2 or 4 weeks of recovery, blood samples were withdrawn from the tail vein of each rat and measurements were made of haemoglobin (Hb) concentration and erythrocyte count. At the end of 9-week period all surviving rats were killed for macroscopic pathology and organ weight analysis.

No rats died during the 13-week study. Terminal body weights were similar in all treatment groups compared to those of controls. Rats in the highest dose group (500 mg/kg/day) had decreased packed cell volume (PCV) and Hb. All other haematological parameters for the treated animals were comparable to the controls.

Absolute and relative liver weights were decreased in high dose males, but not in females. Necropsy of the surviving rats did not reveal any treatment-related macroscopic pathology. The only macroscopic change considered to be treatment-related was a decrease in stainable iron (haemosiderin) in the spleens of females given 500 mg/kg/day. This reduction was not seen in the high dose males.

After 4 weeks of recovery on untreated diet, signs of anaemia in the high dose rats were absent. Organ weights had all returned to normal. Microscopic pathology of spleen and the amount of hemosiderin in the spleen, as assessed by staining, was similar in all treated and control rats.

Chronic feeding study (130 weeks)
Fischer 344 rats (50/sex/group) were fed 0, 4, 20 and 100 mg/kg/day FA in the diet for up to a maximum of 130 weeks (the feeding regimen was continued till 78-82% rats in any one group per sex died) duration of study was determined by the time taken. Moribund rats and all those surviving until the end of the treatment period were killed and a detailed necropsy performed.

During the treatment period, 285 rats died. Mortality among the treated males was lower than that among the male controls. Among females, death rate in all groups was higher than that in the control group. No significant differences in mean body weights of dosed groups versus controls occurred. There were no treatment-related changes in absolute or relative organ weights and haematological parameters in the treated rats.

Blood chemistry determinations revealed no significant differences in plasma transferases or ion concentrations. No significant changes were noted in plasma iron and total iron-binding capacity between controls and treated rats. Bone measurements in males and females revealed no treatment-related differences with respect to length, fat-free dry weight ash weight or bone minerals.

Incidence of combined pancreatic islet-cell adenomas and carcinomas in high-dose females was statistically increased relative to the incidence in female controls. However, there was no evidence of an increased incidence of islet-cell hyperplasia accompanying tumours in any treatment group. When all proliferative islet-cell alterations were combined, the incidence in high dose females was comparable to the incidence in controls. The incidence of combined pancreatic islet-cell tumours in treated males was comparable to that observed in the control males. Analysis of all other neoplastic alterations revealed no significant differences between control and treatment groups.

9.3 Genotoxicity

9.3.1 *Salmonella typhimurium* Reverse Mutation Assay

9.3.1.1 *Salmonella typhimurium* Reverse Mutation Assay with NC (Monsanto Research Corporation, 1981b)

Strains:	TA98, TA100, TA1535 and TA1537
Metabolic activation:	Microsomal fraction from liver homogenates of Aroclor-induced male Sprague-Dawley rats (S9 fraction).
Concentration range:	0, 0.001, 0.004, 0.01, 0.02, 0.04, 0.1, 0.2, 0.3, 1, 3 and 10 mg/plate in distilled water (0.001-1 mg/plate without S9 and 0.01-10 mg/plate with S9).
Test method:	Similar to OECD TG 471
Comment:	The plate incorporation assay was used. Positive controls were 9-aminoacridine (TA 1537, -S9), 2-nitrofluorene (TA 98, TA 100, -S9), 2-aminoanthracene (TA 1535, TA 1537,

+S9), sodium azide (TA 1535, -S9) and benzo(a)pyrene (TA 98, TA 100, +S9). The positive control substances confirmed the reversion properties and specificity of the strains and were within historical ranges. The negative controls were within the historical ranges.

In preliminary toxicity testing with TA100, a dose of ≥1 mg/plate was bacteriotoxic in the absence of S9, and a dose of 10 mg μg/plate was bacteriotoxic in the presence of S9.

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

9.3.1.2 *Salmonella typhimurium* Reverse Mutation Assay with FA (SEAC Toxicology Group, 1979d)

Strains: TA 98, TA 100 and TA 1537

Metabolic activation: Post-mitochondrial supernatant of rat liver (S9 fraction).

Concentration range: Various doses between 0.5 and 1000 μg/plate.

Test method: Similar to OECD TG 471

Comment: The plate incorporation assay was used. Positive controls were 9-aminoacridine (TA 1537, -/+S9), 2-aminofluorene (TA 98, TA 100, and TA 1537, -S9), benzo(a)pyrene (TA 98, TA 100, +S9), daunorubicin (TA 98, -/+S9), 4-nitroquinoline-N-oxide (TA 98 and TA 100, -S9).

FA was initially mutagenic at a single low dose level in TA 98, with and without metabolism. Since this is uncommon, a re-test of the sample, in TA 98 alone, over a range of low doses was conducted. In the re-test it was mutagenic without, but not with, metabolism at the lower doses. However, due to lack of growth of the bacteria, the validity of these results was not confirmed. Further tests with new strains of TA98 did not confirm a mutagenic response.

The positive control substances confirmed the reversion properties and specificity of the strains and were within historical ranges.

Result: Mutagenicity tests with FA were inconclusive in this assay.

9.3.1.3 *Salmonella typhimurium* Reverse Mutation Assay with FA (SEAC Toxicology Group, 1981c)

Strains:	TA 1535 and TA 1538
Metabolic activation:	Post-mitochondrial supernatant of rat liver (S9 fraction).
Concentration range:	0, 3.1, 6.25, 12.5, 25, 50 and 100 μg/plate.
Test method:	Similar to OECD TG 471
Comment:	The plate incorporation assay was used. Positive controls were 9-aminoanthracene (TA 1535 and TA 1538, +S9), 2-nitrofluorene (TA 1538, -/+S9), sodium azide (TA 1535, -/+S9). The positive control substances confirmed the reversion properties and specificity of the strains and were within historical ranges. In one test there was an increase in the number of revertants in strain TA 1535 at the two lowest dose levels of FA tested, without metabolism. In another test with strain TA 1538 alone, there was an increase in the number of revertants at only 25 μg/plate, with metabolism. Results were not dose-related. Due to inconsistency in the results and in the absence of a dose-related response, FA was not considered mutagenic by these tests.
Result:	FA was non mutagenic under the conditions of the test.

9.3.2 In Vivo Cytogenetic Assay in the Bone Marrow Cells of the Chinese Hamster with FA (SEAC Toxicology Group, 1981d)

Species/strain:	Chinese hamsters
Number and sex of animals:	10/sex in each group
Doses:	0, 2.3 and 4.6 g/kg body weight
Method of administration:	Oral (gavage)
Test method:	Similar to OECD TG 474
Comment:	Chinese hamsters were administered 2.3 g/kg and 4.6 g/kg bw FA (0.4 and 0.8 times the oral LD_{50} (5.7 g/kg) established in preliminary acute toxicity studies) by gavage as a 20% solution in 2.5 N sodium hydroxide. Approximately 24 hours after the last dose, the hamsters were killed, both femurs were removed from each animal and bone marrow smears were prepared. Negative control animals received 0.85% saline at a volume equivalent to the upper dose level and the positive control

	animals received 100 mg/kg bw cyclophosphamide by intraperitoneal injection. The smears were stained and examined by light microscopy. Ten metaphase divisions from each slide were scored. The number of metaphase divisions with aberrations in test animals was similar to that in negative controls. No evidence was found that FA caused chromosome damage in Chinese hamster bone marrow under the conditions of this test. Chromosome damage was found in bone marrow from hamsters treated with the positive control.
Result:	FA was non clastogenic under the conditions of the test

9.3.3 Published study (Calvin et al, 1988).

Following is the summary of the genotoxic effects of FA from the published study (Calvin et al, 1988).

FA was tested for its mutagenic potential in a series of strains of *Salmonella typhimurium*, with and without metabolic activation. A concentration range of 0.062-2.0 mg FA per plate was used. The ability of FA to induce mutation of mammalian cells in culture using the L5178Y $TK^{+/-}$ mouse lymphoma mutagenesis assay and to initiate unscheduled DNA synthesis in cultures of rat hepatocytes was also tested. FA was also used in an *in vivo* cytogenetics study that focused on its genotoxic potential as manifested by the production of chromosomal abnormalities such as deletions, exchanges, rings and breaks in bone marrow cells of treated rats.

FA did not display genotoxic activity in any of the *in vitro* and *in vivo* genotoxicity tests carried out in a variety of biological systems.

9.4 Overall Assessment of Toxicological Data

Toxicological data on the notified chemical, NC, is not complete. The notifier has submitted supplementary data consisting of toxicological studies with the free acid (FA) and the sodium salt (SS).

All three chemicals had very low acute oral (LD_{50} >4.1 g/kg) toxicity. Dermal toxicity was not tested. However, intraperitoneal and percutaneous toxicity studies with FA indicated that it is of low toxicity by dermal route. No acute inhalation studies were available.

No skin irritation studies were available for the notified chemical. However, a covered patch test in rabbits with the free acid (in powder form) resulted in slight to moderate irritation and a similar test with the sodium salt (as a 33% solution) resulted in slight irritation. Further skin irritation tests on both chemicals were conducted in rabbits by intradermal injection,

with the results uncertain as the studies were to non-standard protocols and mainly for comparative purposes. Therefore, in the absence of reliable data for the notified chemical, classification as a skin irritant is made on the basis of pH data, where a 1% solution of the notified chemical is reported to have a pH 11.6-12.8 (25 ºC).

In separate tests, FA was a moderate eye irritant in rabbits, whereas SS was a slight eye irritant.

In two Magnusson and Kligman maximisation studies in guinea pigs, the notified chemical was non-sensitising in one study and weakly sensitising in the other. A similar study with the free acid form was negative, however, in three studies with sodium salt, the results ranged from negative to sensitising. On balance, it can be concluded that the notified chemical may be a weak skin sensitiser in guinea pigs.

No repeat dose studies were conducted on the notified chemical; however, the free acid form has been extensively tested. Complete 3-week and 90-day feeding studies in rats were provided by the notifier, together with additional published 13-week and 130-week rat feeding studies. The most significant effects in all studies relate to the chelating ability of the chemical and the resulting disturbance of mineral metabolism. In the 3-week study in Wistar rats, a LOAEL of 121 mg/kg/day was established due to the absence or reduction of haemosiderin in the proximal convoluted tubules of the kidney at all doses. Similar effects were observed in the liver and spleen. Similar effects were observed in the 90-day study, with a NOAEL established at 170 mg/kg/day, based on reduced haemosiderin. In this study, the effect of reduced mineral availability was observed as severe skeletal distortion and thickening of bone. A reduction in splenic haemosiderin was also reported in the published 13-week study in Sprague-Dawley rats, however, in the published 130-week study in Fischer 344 rats, where mortality was high, no significant adverse effects were reported. In a published toxicokinetic study, localisation of FA in bone indicated a high affinity for mineralising tissues, consistent with its chelating properties. Higher excretion rates through faeces indicated that FA was not readily absorbed in the gastrointestinal tract.

FA or NC did not display genotoxic activity in a number of *in vitro* and *in vivo* studies carried out in a variety of biological systems.

The notified chemical is classified as a hazardous substance according to NOHSC *Approved Criteria for Classifying Hazardous Substances* [NOHSC, 1999] based on eye and skin irritation effects and requires the risk phrase R36/38 on Material Safety Data Sheets (MSDS) and labels affixed to containers of products containing at or greater than 20% of the notified chemical.

The risk phrase R48 is not warranted as no adverse health effects were noted at or below 50 mg/kg/day (cut-off concentration for R48 in the NOHSC *Approved Criteria)* in the 90-day oral study in rats.

10. ASSESSMENT OF ENVIRONMENTAL EFFECTS

The toxicology reports submitted were for the parent acid known as FA. The tests were not conducted to the strict OECD protocols, and although goldfish and chlorella are usually regarded as insensitive species, the test reports provided are accepted.

Test	Species	Results (Nominal)
Acute Toxicity	Goldfish (*Carrasius auratus)* In hard water (300 mg/L as $CaCO_3$).	 500 mg/L <LC50 (48 h) <1000 mg/L.
Acute Immobilisation	*Daphnia magna* In hard water (265 mg/L as $CaCO_3$). In soft water (41 mg/L as $CaCO_3$).	LC50 >500 mg/L. 250 mg/L >LC50 >100 mg/L.
Growth Inhibition	Algae (*Chlorella)* In equimolar Ca^{2+} solution.	EC50 >500 mg/L.

Fish

The test of acute toxicity to goldfish (SEAC Toxicology, 1979e) was performed in a static test at 15 ºC over a 48-hour period with twice daily replacement of the test solutions. The test solutions employed were prepared at nominal concentrations of 1, 10, 50, 100, 500, 1000 and 5000 mg/L using water with nominal hardness of 300 mg/L as $CaCO_3$ (equivalent to 120 mg/L Ca^{2+}). Three goldfish were used at each concentration. For the test solution at (nominally) 500 mg/L and all lower concentrations no deaths among the fish occurred over the 48-hour test period. Further, no comments on adverse effects to the fish exposed to these nominal concentrations were made in the report. However, for the nominally 1000 and 5000 mg/L solutions all three fish had died within 1 hour of being placed in the test media. This dramatic change in the toxic characteristics of the compound is discussed in relation to ambient Ca^{2+} levels in the conclusion below.

Daphnia

The acute tests against daphnia (SEAC Toxicology, 1981e) were performed using static methodology in both hard and soft water over a 48-hour period. In the hard water the test solutions employed were prepared at nominal concentrations of 0 (control), 5, 150, 250, 300, 350, 400 and 500 mg/L of FA in water with hardness stated as 265 mg/L as $CaCO_3$, equivalent to 106 mg/L of Ca^{2+}. Ten juvenile daphnia were used at each concentration, and the test at each concentration was conducted using four replicates (ie 40 daphnia tested at each concentration). For the test solution at (nominally) 300 mg/L and all lower concentrations no immobilisation among the daphnia occurred over the 48-hour test period, but 2.5% mortality was observed after 48 hours in the (nominally) 350 mg/L solution and 7.5% mortality in both the 400 and 500 mg/L solutions. The data indicated that the LC50 of the test compound in water of hardness 265 mg/L as $CaCO_3$ is >500 mg/L.

The second test carried out in soft water (hardness 41 mg/L as $CaCO_3$, equivalent to 16.4 mg/L of Ca^{2+}) showed the test compound to be appreciably more toxic in water with low calcium content. The test was performed essentially as described above using nominal test concentrations of 0 (control), 50, 100, 250 and 500 mg/L. After 48 hours exposure to the nominal 100 mg/L solution, 10% of the daphnia had died (were immobilised), increasing to

87.5% and 100% in the 250 and 500 mg/L solutions respectively. The data was interpreted to show that the LC50 for daphnia in this low hardness water is between 100 and 250 mg/L.

Algae

The acute tests against algae (SEAC Toxicology, 1982b) were performed using static methodology over a 72-hour period. Nine test solutions were prepared at nominal concentrations of between 0 (control) and 500 mg/L of FA. However, in view of the increased toxicity of the compound in low Ca^{2+} water observed with both fish and daphnia, it is important to note that the report indicated that each solution was made up containing the same molar concentration of calcium ions as the test compound.

Chlorella was incubated for 72 hours at 24-25 ºC in Bolds Basal medium containing the various concentrations of test material. The growth in algal biomass was monitored by absorbance at 440 nm. No inhibition of algal growth was observed for the test medium containing nominal concentrations of 300 mg/L and less of test material, although up to 47% inhibition was recorded after 72 hours incubation in 500 mg/L medium. The results of this study were interpreted to indicate that the EC50 for FA against Chlorella in equimolar Ca^{2+} is >500 mg/L.

It is also of interest that the effects of the compound on this species appear to be algistatic rather than algicidal. Algae removed from the medium containing the nominal 500 mg/L of test compound and placed in clean medium regained normal growth behaviour.

Conclusion

It is clear that the toxicity of the test compound, the parent acid of the notified chemical, is very dependant on the level of Ca^{2+} ions in the water. These ions (as well as other divalent metal ions) form strong complexes with the parent acid, and in this form the toxicity at all three trophic levels seems to be significantly reduced. This is shown quite clearly by the difference in toxicity towards daphnia between the tests conducted in soft water (Ca^{2+} = 16.4 mg/L) with 250 mg/L >LC50 >100 mg/L compared with that in hard water (Ca^{2+} = 106 mg/L) where LC50 >500 mg/L. In the case of the gold fish test, assuming a calcium level of 120 mg/L Ca^{2+}, the toxicity increases dramatically above the test concentration of 500 mg/L, and it is concluded that the toxicity of this compound is mitigated when the mole ratio $[Ca^{2+}]/[FA]$ >2.6 (approximately). For the notified chemical the mole ratio is in fact 2.5, so that on release of the compound to receiving waters LC50 values in excess of 500 mg/L could be expected.

11. ASSESSMENT OF ENVIRONMENTAL HAZARD

Up to 100 tonnes of the notified chemical may be used in Australia each year and almost all is expected to be released to sewer. Release will be widespread and diffuse, and assuming that the detergents are used 360 days per annum, that the Australian population is 19,000,000 and that each individual contributes 150 L to raw sewage each day, the Predicted Environmental Concentration (PEC) in the sewer is estimated to be around 70 µg/L. Assuming that none of the chemical is adsorbed into sludge or removed through biological or abiotic processes, all would pass through the sewage treatment works and be discharged to receiving waters. In a large coastal city it is usually assumed that the sewage effluent is diluted by a factor of 10 after discharge to the ocean, while a dilution factor of 3 may be more

appropriate in rural areas. This leads to a PEC of 7 μg/L near coastal cities and 23 μg/L in country areas. The notifier also supplied some PEC calculations, which used more generous dilution factors and derived PECs of 2.45 μg/L and 10.8 μg/L for city and country, respectively. However these PEC estimates have not accounted for removal of the compound from the water column through association with aquatic sediments, which is a very important mechanism in respect of overall environmental fate.

Once released to the sewer, in most cases the chemical is expected to form complexes with ambient Ca and Mg in the water, and some may become associated with sewer sludge. Chemical reaching sewage treatment plants is unlikely to be removed through activated sludge treatment, but may be precipitated into chemical sludge through addition of flocculants such as lime, ferric sulphate or alum which are often added during the final stages of sewage treatment.

Sewage sludge will be placed into landfill, used as a soil supplement or incinerated, although the amount likely to be incinerated is difficult to estimate. In the worst case potentially 100 tonnes of the new chemical may be placed into landfill or incorporated into soil amendment agents each year. Although the chemical is slowly degraded through physical processes (particularly via photolysis) and slow biological processes, there is insufficient available data to predict half-life in the environment. The slow rates of the degradation processes that have been investigated, suggest that the new chemical is likely to persist in soil and sediments.

The parent acid of the new compound appears to be more toxic than the notified chemical to aquatic species. However, available information indicates that the free acid would not exist as such in environmental receiving waters, since the ambient Ca and Mg in environmental waters form complexes with this compound. Such complexes appear to be non-toxic provided there is considerable excess of metal ions present. Toxic effects to aquatic organisms are unlikely for the notified chemical.

Much of the chemical will eventually be placed into landfill or become associated with soils through application of sewage sludge to agricultural land. The available data indicates the compound will persist in these situations, and since there is no available information on the toxicity of soil bound material to soil organisms, no conclusions concerning possible environmental effects can be made.

The environmental hazard from use of the notified chemical in laundry detergents is considered to be low to aquatic organisms. The hazard to soil organisms is also likely to be acceptable.

12. ASSESSMENT OF PUBLIC AND OCCUPATIONAL HEALTH AND SAFETY EFFECTS

Based on toxicological data on the notified chemical and supplementary toxicological data on free acid and sodium salt, the notified chemical is likely to be of very low acute oral toxicity and low dermal toxicity. It is likely to be a moderate skin and eye irritant. Based on the results from a number of guinea-pig studies for both the notified chemical and the sodium salt, where the results ranged from negative to sensitising, the notified chemical may be a weak skin sensitiser.

The notified chemical is determined to be a hazardous substance according to NOHSC *Approved Criteria for Classifying Hazardous Substances* [NOHSC, 1999] based on eye and skin irritation effects and requires the risk phrase R36/38 on Material Safety Data Sheets (MSDS) and labels affixed to containers of products containing at or greater than 20% of the notified chemical.

There is no data on the repeat dose effect of the notified chemical. The free acid form has been extensively tested. The most significant effects in all studies relate to the chelating ability of the chemical resulting in disturbance of mineral metabolism and its ability to reduce haemosiderin levels in kidney, liver and spleen. A NOAEL of 170 mg/kg/day was established based on reduced haemosiderin levels.

The final product is not classified as hazardous based on the concentration of the notified chemical. Accordingly, the product MSDS indicates that it is not a hazardous substance.

Occupational health and safety
The notified chemical will be imported as a minor ingredient of ready-to-sell laundry detergent tablets. The product is packaged in robust cartons designed for international transport. No reformulation will occur in Australia.

Australian workers will be involved in the transport, storage and sale of the packed product. Exposure to the notified chemical is not expected during transport or storage as long as the packaging remains intact. Exposure after a spill would be controlled by use of the recommended practices for spillage clean up given in the MSDS supplied by the notifier. The risk of adverse health effects for transport and storage workers is considered low.

Public health
The product tablets, containing the notified chemical, will be used by the public as laundry detergent. There is potential for widespread and frequent dermal exposure to the public. However, dermal exposure is minimised by the packaging and the absence of any notified chemical residues in washed clothes.

Consequently, the potential hazard from use of the notified chemical is considered to be low. There will be minimal public exposure from transport, storage or sale of the product.

13. RECOMMENDATIONS

To minimise occupational exposure to Phosphonate LR 1 the following guidelines and precautions should be observed:

- 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 anew Zealand, 1998); all occupational footwear should conform to AS/NZS 2210 (Standards Australia/Standards New Zealand, 1994); or other internationally acceptable standards;

- Spillage of the notified chemical should be avoided. Spillages should be swept up promptly and put into containers for disposal;
- Good personal hygiene should be practised to minimise the potential for ingestion;
- A copy of the MSDS should be easily accessible to employees.

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 was provided in a format consistent with the *National Code of Practice for the Preparation of Material Safety Data Sheets* (NOHSC, 1994).

This MSDS was provided by the applicant as part of the notification statement. It is reproduced here as a matter of public record. The accuracy of this information remains the responsibility of the applicant.

15. REQUIREMENTS FOR SECONDARY NOTIFICATION

Under subsection 64(1) of the Act, secondary notification may be required if the notified chemical is imported for formulation.

Under subsection 64(2) of the Act, secondary notification of the notified chemical may be required if any of the stipulated circumstances arise. No other specific conditions are prescribed.

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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

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