File No: STD/1016

4 July 2002

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

FULL PUBLIC REPORT

PHOSPHONATE LR2

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

For the purposes of subsection 78(1) of the Act, this Full Public Report may be inspected at:

Library
National Occupational Health and Safety Commission
25 Constitution Avenue
CANBERRA ACT 2600
AUSTRALIA

To arrange an appointment contact the Librarian on TEL + 61 2 6279 1161 or + 61 2 6279 1163.

This Full Public Report is available for viewing and downloading from the NICNAS website or available on request, free of charge, by contacting NICNAS. For requests and enquiries please contact the NICNAS Administration Coordinator at:

Street Address:	334 - 336 Illawarra Road MARRICKVILLE NSW 2204, AUSTRALIA.
Postal Address:	GPO Box 58, SYDNEY NSW 2001, AUSTRALIA.
TEL:	+ 61 2 8577 8800
FAX	+ 61 2 9577 8888.
Website:	www.nicnas.gov.au

Director
Chemicals Notification and Assessment

TABLE OF CONTENTS

FULL PUBLIC REPORT

PHOSPHONATE LR2

1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT
Unilever Australasia
219 North Rocks Road
North Rocks NSW 2151

NOTIFICATION CATEGORY
Standard: Chemical other than polymer (more than 1 tonne per year).

EXEMPT INFORMATION (SECTION 75 OF THE ACT)
Data items and details claimed exempt from publication:
Chemical name
Other names
CAS number
Molecular formula
Structural formula
Molecular weight
Purity
Identity of hazardous impurities
Non hazardous impurities
Identity of additives/adjuvants
Detailed use (use cannot be exempt)
Identity of manufacturing sites

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
No variations of data requirements are claimed.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
The notified chemical has been used overseas (Europe).
Listed on TSCA Inventory

2. IDENTITY OF CHEMICAL

MARKETING NAME
Phosphonate LR2

3. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS
The notified chemical will be imported as an ingredient of a finished product (detergent formulation). However, the notified chemical may be imported in its commercial form at some time in the future.

MAXIMUM INTRODUCTION VOLUME OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS

Year	*1*	*2*	*3*	*4*	*5*

Tonnes	<10	<10	<10	<10	<10

USE
The notified chemical is intended for use as an ingredient for inclusion in cleaning formulations, which will be a floor cleaning product and a surface cleaning spray. Two products are proposed, a liquid gel for cleaning floors and a spray for surfaces such as kitchen benches.

Application

The notified chemical will be mopped up from floors cleaned with the product in dilute form or wiped from cleaned surfaces sprayed with the consumer product with sponge clothes or similar.

The liquid product will be applied as a dilution of 60 mL in 5-10 L of water and applied using a mop. The spray product will be applied to surfaces such as kitchen benches, via several pump activations, wiped with a cloth or sponge.

4. PROCESS AND RELEASE INFORMATION

4.1. Distribution, Transport and Storage

PORT OF ENTRY
Not reported

TRANSPORTATION AND PACKAGING
The notified chemical will be imported by ship and then transported by truck to the importer's warehouse prior to distribution to customer (eg. supermarket) warehouses. The products will be pre-packaged in 750 mL plastic bottles (liquid gel product) and 500 mL plastic bottles (spray product).

4.2. Operation Description

The notifier indicated that no release of the chemical is anticipated during formulation should the notified chemical be imported for use as a raw material in detergent manufacturing in the future. To conserve materials, it is normal practice to reuse all machine purges by incorporating them into the next batch of compatible products.

4.3. Occupational exposure

The notified chemical will be imported in the form of an ingredient in a finished product (<1% notified chemical in liquid detergents). In the future, the commercial form of the notified chemical (<30%) packed in 200 L drums or 1000 L Schutz may be imported for local formulation. It will be directly coupled to process equipment on load cells, for automatic dosing to the process stream. Handling would be via a closed system. However, skin contamination may result if spills occur. For the operation of changing containers, workers will be protected by local exhaust ventilation and wearing personal protective equipment (rubber gloves and protective clothing). All machine purges will be incorporated into the next batch of compatible product, so that no waste will be produced.

Workers in the warehousing and transport supply chain and at retail premises will handle the notified chemical. These workers are unlikely to be exposed to the product except in cases of spills. At retail stores, the product will be removed from the outer cases and put on shelves as individual units. Store-keepers will handle the packed product and could only become exposed to the product or the notified chemical in cases of a packaging breach.

Cleaners may be exposed to the cleaning products by dermal and/or ocular contamination. Given that the products are to be diluted before use (<0.01% notified chemical), exposure to the notified chemical in the diluted cleaning products will be minimal.

4.4. Release

RELEASE OF CHEMICAL AT SITE
No release is anticipated at customer warehouses expect in the event of an accident or spill. The small size of the containers would ensure such releases would be small.

RELEASE OF CHEMICAL FROM USE
Ultimately all of the notified chemical will be released into the sewer during use of the detergent products. The liquid detergents are applied using a mop and bucket, and it is expected that after cleaning, the contents of the bucket will be disposed of into the sewer. Similarly, the spray products are applied to surfaces such as kitchen benches, which are wiped clean with a cloth or sponge. It is expected that the cleaning cloths will be rinsed under the tap following cleaning with the contents being washed down the sink and ultimately into the sewer.

4.5. Disposal

The MSDS recommends disposal of the notified chemical by incineration or recycling.

4.6. Public exposure

It is expected that during transport, formulation, and storage, exposure of the general public to the notified chemical will be low. During an event of transport spill or leak, the spilled or leaked detergent, the product would be cleaned up and repacked in the warehouse facility for safe handling.

The end-use products will be extensively used by the consumer for surface cleaning. The liquid product will be applied as a dilution (<0.01% notified chemical) in a bucket and applied with a mop to clean the floor. The used mop contents are squeezed into the bucket and disposed of to sewer. The spray product will be applied to surfaces such as kitchen benches via several pump activations, and wiped with a cloth or sponge that is washed out in a sink. During application, there may be dermal and inhalation exposure to the liquid and spray formulations, as well as accidental ocular and oral exposure. Exposure from touching cleaned surfaces is expected to be negligible.

5. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20°C AND 101.3 kPa	Yellow to amber liquid with no odour
MELTING/FREEZING POINT	Crystallizes at -14°C
METHOD	OECD TG 102 Melting Point/Melting Range.
Remarks	Full test reports not provided. 25% active acid solution.
DENSITY	1330 kg/m^3
METHOD	OECD TG 109 Density of Liquids and Solids.
Remarks	Full test reports not provided.
VAPOUR PRESSURE	Not determined
Remarks	For the solid, the vapour pressure is expected to be very low due to the salt form, and difficult to measure owing to the presence of residual water as an impurity. For the solution, the vapour pressure will be high to reflect the presence of water as a solvent.
WATER SOLUBILITY	Not determined
Remarks	The notified chemical has a high solubility in water. However, the water solubility has not been formally measured. The product is sold commercially as an aqueous solution at 24-26% active acid, corresponding to 30-32% as the notified chemical.
HYDROLYSIS AS A FUNCTION OF PH	Not determined

Remarks	The notified chemical is expected to be stable to hydrolysis with a long lifetime in water. No hydrolysis was observed in dark controls in the photodegradation study (section 7.1.2).

PARTITION COEFFICIENT (n-octanol/water) Pow = 4.5 x 10^{-4} to 2 x 10^{-5}.

METHOD	In house method (Environmental Sciences ES-79-M-15), Monsanto.
Remarks	The octanol/water partition coefficients of five phosphonates having a range of structures, were measured by equilibrating aqueous solutions of the radio-labelled compounds with n-octanol, and then determining the concentration in each phase using liquid scintillation counting. As a quality control indicator, P was measured at two concentrations, 100 and 1000 mg/L of notified chemical. There was good agreement between the measured values at each concentration, indicating the results were accurate. The P values indicate the five phosphonates are very hydrophilic and have a poor affinity for lipids.
TEST FACILITY	MIC Environmental Sciences, Monsanto (1979).

ADSORPTION/DESORPTION Not determined

Remarks See Section 7.1 for the results of partitioning studies.

DISSOCIATION CONSTANT Not determined

Remarks See Section 7.1

PARTICLE SIZE
Remarks Notified chemical prepared only in solution

FLASH POINT Not Flammable

FLAMMABILITY LIMITS Not Flammable

AUTOIGNITION TEMPERATURE Does not autoignite

EXPLOSIVE PROPERTIES Not explosive

REACTIVITY
Remarks Not an oxidising substance

6. TOXICOLOGICAL INVESTIGATIONS

The notifier submitted a series of studies carried out on surrogates/closely related substances (free acid and the calcium/sodium salt of the notified chemical). The structures of the closely related substances are identical except for the way in which phosphonic acid groups are either neutralised or remain unchanged. These additional studies are accepted as surrogate studies for the assessment of the notified chemical.

Studies on closely related substances include acute toxicity in mice and rats, acute intraperitoneal toxicity in mice, acute percutaneous toxicity in rabbits, dermal and eye irritation in rabbits, skin sensitisation in guinea pigs, a sub-scute (3-week and 13-week) oral study, chronic 2-year feeding study in rats, bacterial mutation assay, cytotoxicity in Chinese hamsters, and a review of genotoxicity, bio-distribution, and subchronic and chronic toxicity in rats.

Studies on the notified chemical include dermal irritation study in rabbits (intra-dermal and covered patch), eye irritation study in rabbits and skin sensitisation in guinea pigs.

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).

6.1 Acute Toxicity

Summary of the acute toxicity of the notified chemical, free acid and the calcium/sodium salt

Test	Species	Outcome	*Reference*
Acute oral toxicity (calcium/sodium salt)	Mice	LD_{50} 14.3 g/kg	SEAC Toxicology Group, 1979a
Acute oral toxicity (calcium/sodium salt)	Rat	LD_{50} >5.0 g/kg	SEAC Toxicology Group, 1979b
Acute oral toxicity (free acid)	Mice	LD_{50} 4.1 g/kg	SEAC Toxicology Group, 1976
Acute oral toxicity (free acid)	Rats	LD_{50} 4.1 g/kg	SEAC Toxicology Group, 1977a
Acute intraperitoneal toxicity (free acid)	Mice	LD_{50} 0.30 g/kg	SEAC Toxicology Group, 1977b
Acute percutaneous toxicity (free acid)	Rabbits	Non-toxic by skin absorption	Younger Laboratories, 1968a
Skin irritation – intradermal injection test (notified chemical)	Rabbit	Slight to moderate	SEAC Toxicology Group, 1980a
Skin irritation – covered patch test (notified chemical)	Rabbit	Slight irritant	SEAC Toxicology Group, 1980b
Skin irritation (intradermal injection test; Komplex)	Rabbit	Slight to moderate	SEAC Toxicology Group, 1980c
Skin irritation (intradermal injection test, Calcium analogue – Draise Product)	Rabbit	Slight to moderate	SEAC Toxicology Group, 1980d
Skin irritation – covered patch test (free acid)	Rabbit	Slight to moderate	SEAC Toxicology Group, 1977c
Skin irritation - intradermal injection test (free acid)	Rabbits	Slight to moderate	SEAC Toxicology Group, 1977d
Eye irritation (notified chemical)	Rabbit	Slight irritant	SEAC Toxicology Group, 1980e

Eye irritation (free acid)	Rabbit	Moderate	Younger Laboratories, 1968b
Skin sensitisation (notified chemical)	Guinea pig	Non-sensitiser	SEAC Toxicology Group, 1981a
Skin sensitisation (notified chemical)	Guinea pig	Sensitiser	SEAC Toxicology Group, 1983
Skin sensitisation (notified chemical)	Guinea pig	Weak sensitiser	SEAC Toxicology Group, 1982
Skin sensitisation (calcium/sodium salt)	Guinea pig	Weak-sensitiser	SEAC Toxicology Group, 1981b
Skin sensitisation (calcium sodium salt)	Guinea pig	Non-sensitiser	SEAC Toxicology Group, 1984
Skin sensitisation (free acid)	Guinea pig	Non-sensitiser	SEAC Toxicology Group, 1977e

6.1.1 Oral Toxicity

6.1.1.1 Oral toxicity of the calcium/sodium salt (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 calcium/sodium salt was of very low acute oral toxicity in mice.

6.1.1.2 Oral toxicity of calcium/sodium salt (SEAC Toxicology Group, 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 calcium/sodium salt was of very low acute oral toxicity in rats

6.1.1.3 Oral toxicity of free acid (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:	The free acid was of very low acute oral toxicity in mice.

6.1.1.4 Oral toxicity of free acid (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 free acid 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 free acid 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:	The free acid was of very low acute oral toxicity in rats.

6.1.2 Dermal Toxicity

6.1.2.1 Intraperitoneal toxicity of free acid (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.

6.1.2.2 Acute percutaneous toxicity of free acid (Younger Laboratories, 1968a)

Species/strain:	New Zealand White rabbits.
Number/sex of animals:	3/sex
Observation period:	24 hours
Method of administration:	The free acid 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:	The free acid was of low toxicity in rabbits by the dermal route.

6.1.3 Skin Irritation

6.1.3.1 Skin irritation with the notified chemical (intradermal injection) (SEAC Technology, 1980a)

Species/strain:	New Zealand White rabbits
Number/sex of animals:	8 (sex not specified)
Observation period:	24 and 48 hours
Method of administration:	The notified chemical (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%), the notified chemical produced a moderate irritation effect. It was slightly less irritating than the free acid.

6.1.3.2 Skin irritation with the notified chemical (SEAC Technology, 1980b)

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 notified chemical (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. Free acid, calcium analogue 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 notified chemical produced only marginal effects, and was similar in irritancy to other analogues.

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

6.1.3.3 Skin irritation with Komplex (Intradermal injection) (SEAC Technology, 1980c)

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 (free acid (90% ai), notified chemical (33% ai), calcium analogue (38% ai), calcium/sodium salt (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: 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.

Komplex produced slight effects 24 hours after injection similar to the notified chemical and the calcium analogue.

Calcium analogue and free acid caused the highest responses.

Result: The notified chemical caused irritant reactions in rabbits.

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

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 (Free acid (90% ai), Calcium

analogue ex URLPS (38% ai), calcium/sodium salt 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:	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. The calcium/sodium salt produced a moderate response at the three top concentrations, but at the lowest concentration (0.1%) showed marginal effects.
Result:	The calcium/sodium salt was a moderate skin irritant in rabbits.

6.1.3.5 Skin irritation with free acid (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 free acid 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: Free acid was slightly irritating to the skin of rabbits

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

6.1.3.6 Skin irritation with free acid (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: Free acid (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% free acid 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.

6.1.4 Eye Irritation

6.1.4.1 Eye irritation with the notified chemical (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 notified chemical 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: The notified chemical was slightly irritating to the eyes of rabbits.

6.1.4.2 Eye irritation with free acid (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 free acid 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: The free acid was considered to be a moderate eye irritant in rabbits under conditions of the test.

6.1.5 Skin Sensitisation

6.1.5.1 Skin Sensitisation test with the notified chemical (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
Notified chemical, 0.83% in physiological saline
Notified chemical, 0.83% in a 1:1 mixture of FCA and saline

day 7 — A 2x4 cm filter paper patch was saturated with 33% notified chemical 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% notified chemical in distilled water. Patches of filter paper were saturated with 33% notified chemical 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: Notified chemical was not sensitising to the skin of guinea pigs.

6.1.5.2 Skin Sensitisation test with the notified chemical (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
Notified chemical, 0.5% in physiological saline
Notified chemical, 0.5% in a 1:1 mixture of FCA and saline

day 7 — A 2x4 cm filter paper patch was saturated with 25% notified chemical 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% notified chemical in distilled water. Patches of filter paper were saturated with 25% notified chemical 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: The notified chemical was a skin sensitiser in guinea pigs

6.1.5.3 Skin Sensitisation test with the notified chemical (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
Notified chemical, 1% in physiological saline
Notified chemical, 1% in a 1:1 mixture of FCA and saline

day 7 — A 2x4 cm filter paper patch was saturated with notified chemical (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 notified chemical. Patches of filter paper were saturated with notified chemical 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: The notified chemical was a weak skin sensitiser in guinea pigs.

6.1.5.4 Skin Sensitisation test with the calcium/sodium salt (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
Calcium/sodium salt, 2.5% in physiological saline
Calcium/sodium salt, 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% calcium/sodium salt in distilled water. Patches of filter paper were saturated with 10% calcium/sodium salt 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 calcium/sodium salt was weakly sensitising to the skin of guinea pigs

6.1.5.5 Skin Sensitisation test with the calcium/sodium salt (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
Calcium/sodium salt, 0.25% in physiological saline
Calcium/sodium salt, 0.25% in a 1:1 mixture of FCA and saline

day 6-7 20% calcium/sodium salt 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% calcium/sodium salt in distilled water. Patches of filter paper were saturated with 5% calcium/sodium salt

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 (5%)	0/10**	0/10	0/4	0/4
Challenge 2 (5%)	0/10	0/10	¼	0/4

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

Result: The calcium/sodium salt was not sensitising to the skin of guinea pigs.

6.1.5.6 Skin Sensitisation test with free acid (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
Free acid, 1% in physiological saline
Free acid, 1% in a 1:1 mixture of FCA and saline

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

Control group: <u>Treated controls</u>: 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.

<u>Untreated controls:</u> 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% free acid. 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% free acid.

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: The free acid was not sensitising to the skin of guinea pigs in this assay.

6.2 Repeated Dose Toxicity

6.2.1 Sub-acute oral toxicity study (3 wks) with free acid (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% free acid 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% free acid) 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. The free acid 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% free acid. The caecal and colonic contents of most of the rats fed 0.5% free acid were slightly pale in colour.

Histopathology:
Histopathological observations revealed liver, spleen and kidneys were affected by free acid. In males from 2% free acid 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% free acid.

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% free acid 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% free acid were considered not to represent toxic changes induced by free acid, but to be attributable to reduced food intake. However, several effects of treatment directly attributable to the ingestion of free acid 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 free acid 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.

6.2.2 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% free acid; 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:
The free acid was toxic at higher concentration levels. Rats fed 2% free acid 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% free acid, 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% free acid. 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% free acid 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% free acid. 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% free acid. 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 free acid or in controls.

Histopathology:
Changes attributable to the ingestion of the free acid 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% free acid. 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% free acid-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 free acid 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% free acid (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%.

6.2.3 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 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 the free acid

Groups of ten male Sprague-Dawley rats were given ten daily gavage doses of labelled free acid at dose levels of 4, 16 or 64 mg/kg/day. Rats were also dosed with labelled free acid 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 free acid 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 free acid 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.

6.3 Genotoxicity

6.3.1 Salmonella

6.3.1.1 ***Salmonella typhimurium*** Reverse Mutation Assay with the calcium/sodium salt (Monsanto Research Corporation, 1981)

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 calcium/sodium salt was non mutagenic under the conditions of the test.

6.3.1.2 ***Salmonella typhimurium*** Reverse Mutation Assay with the free acid (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 the free acid were inconclusive in this assay.

6.3.1.3 *Salmonella typhimurium* Reverse Mutation Assay with the free acid (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: The free acid was non mutagenic under the conditions of the test.

6.3.2 *In Vivo* Cytogenetic Assay in the Bone Marrow Cells of the Chinese Hamster with free acid (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 free acid (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 2.5N 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 the free acid 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: The free acid was non clastogenic under the conditions of the test

6.3.3 Published study (Calvin et al, 1988).

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

The free acid 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 free acid per plate was used. The ability of the free acid 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. The free acid 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.

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

7. ENVIRONMENT

7.1. Environmental fate

Several full or summary test reports were included in the notification dossier, which describe tests carried out on the parent acid or other phosphonates having a range of structures, to determine the fate and likely rates of removal of the material from the environment. These tests have already been assessed in relation to NA/857. The data are summarised in the tables below.

7.1.1. Inherent biodegradability

TEST SUBSTANCE	Parent acid
METHOD	Semi-Continuous Activated Sludge (SCAS) Test. In-house method (Unilever Research Port Sunlight Laboratories)
Inoculum	Bacteria from domestic sewage
Exposure Period	14 weeks
Auxiliary Solvent	None
Analytical Monitoring	Dissolved Organic Carbon (DOC)
Remarks - Method	A test was performed to determine the inherent and ultimate biodegradability of free acid. Bacteria in a SCAS unit operating on domestic sewage sludge were acclimatised to the test substance over 14 weeks. At intervals during the test period, DOC measurements were taken from the test units and compared to a control unit. Higher DOC levels in the dosed units compared to the control were attributed to un-degraded test material.

RESULTS	An excess of organic carbon was measured in the dosing unit compared to the control of an amount equivalent to the amount added at the start of the test.
CONCLUSION	The test substance was not ultimately biodegraded over the 14-week period. Evidence indicated it was not adsorbed onto activated sludge and therefore it was concluded that the test material would pass through conventional sewage treatment facilities and enter surface waters.
TEST FACILITY	Unilever Research Port Sunlight Laboratories, Merseyside England (1981).

7.1.2. Primary Degradation in River Water

TEST SUBSTANCE	Phosphonate Products
METHOD	Biological and photo-degradation in River Water. In-house method (Monsanto Europe, Belgium)
Exposure Period	60 and 138 days
Analytical Monitoring	Percentage $^{14}CO_2$ evolution
Remarks - Method	To determine the rate of primary degradation, a preliminary study was performed exposing Phosphonate products at concentrations of 5-10 mg/L in Meramec River water to dark, artificially illuminated, and sunlight illuminated conditions over a period of 138 days, and then determining the amount of degradation. To determine the rate of ultimate degradation, a definitive study was conducted exposing Phosphonates dissolved in Meramec River water and eutrophic lake water to the same lighting conditions over a period of 60 days.
RESULTS	In the preliminary test, between 81 and 100% degradation was achieved under sunlight conditions, while degradation was lower (up to 55% with one outlier at 76%) under dark and under artificial light conditions. Orthophosphate accumulated in the dark, but not under artificial light, presumably due to algal utilization. In the definitive test, significantly less degradation was observed. It was thought degradation was lower because the test was conducted over a shorter period.
CONCLUSION	Results indicated both slow biological and photochemical degradation of Phosphonate products.
TEST FACILITY	Monsanto Europe SA Avenue De Tervuren 270 B-1150 Brussels Belgium (1983a).

7.1.3. Adsorption on River Solids

TEST SUBSTANCE	Phosphonates
METHOD	Sediment/water partition coefficients of phosphonates. In-house method (Monsanto Europe, Belgium)
Exposure Period	8 days
Analytical Monitoring	Liquid Scintillation Counting
Remarks - Method	The sediment/water partition coefficients (K = Cs/Cw) were measured for five phosphonates, in order to determine how completely these products are removed from water by sediment. The method involved equilibrating aqueous solutions of radio-labelled Phosphonate products with river sediment (10 g). Experiments were conducted in highly purified and synthetic hard water (average 211 mg/L as $CaCO_3$) at initial concentrations of 0.05, 0.10, 1.0 and 5.0 mg/L of ^{14}C-labelled products. The solutions were then shaken and centrifuged, and the concentrations remaining in solution measured directly at 24-hour intervals by liquid

scintillation counting. The Cs was calculated by difference.

RESULTS

At the three lowest test concentrations, K was greater than 100 for all five products after 1 day, indicating they had rapidly partitioned into the sediment. Partitioning was slower at the 5 mg/L level. However, after 8 days, all K values were 300 or greater. Equilibrium was attained within 2-4 days at the lower concentrations (0.5-1.0 mg/L), while it was frequently greater than 8 days for the 5 mg/L level. The latter may be due to saturation of adsorption sites. Partitioning of the parent acid was generally higher in the hard water than in the soft water at the lower concentrations, but was lower in the hard water than in the soft water at the highest concentrations (5 mg/L). The former suggests complexing with free Ca^{2+} and Mg^{2+} ions in the hard water. Details of the composition of the hard and soft water were not included.

CONCLUSION

The test substances are expected to partition rapidly into sediment and suspended particulate matter.

TEST FACILITY

Monsanto Europe SA Avenue De Tervuren 270 B-1150 Brussels Belgium (1980).

7.1.4. Mobilisation of Metals

TEST SUBSTANCE

Phosphonate products

METHOD

Two studies were summarised in an extract from a report, which address the potential for Phosphonate products to mobilise metals from sediment. The first study examined the potential of 5 Phosphonate products at concentrations of 1, 10, 50, and 100 mg/L to mobilize Ca^{2+}, Mg^{2+}, Zn^{2+}, Fe^{2+}, Cd^{2+}, Cr^{3+}, Cu^{2+}, and Pb^{2+} from Mississippi River sediments over a 200-hour period. Because Phosphonate product levels in the former study were in excess of anticipated environmental levels, a second study was performed to examine the same 5 Phosphonate products using lower concentrations (0.05, 0.1, 1.0, and 5.0 mg/L), and a standard Reference Material Sediment in both soft and hard water.

RESULTS

Results of the first study indicated Cd, Pb, Ca and Mg were not mobilised by any concentration while the other metal ions were mobilised depending on the Phosphonate compound and the concentration in solution, with Fe, Cr, Zn and Cu being most frequently mobilised. Results of the second study indicate no metals mobilised except for moderate amounts of Fe, Zn and Cr. The extent of mobilisation in hard and soft water was typically less than 0.5 and 0.2 mg/L, respectively per metal.

CONCLUSION

Phosphonates are able to mobilise and form complexes with some metal ions depending on the type of product and their concentrations. It is likely that the chelates formed would partition into soils or sediment and not be available to biological species.

TEST FACILITY

Monsanto Europe SA Avenue De Tervuren 270 B-1150 Brussels Belgium (1983b).

7.1.5. Waste Water Treatability and Water Purification

TEST SUBSTANCE

Phosphonate products

METHOD

A study was conducted to determine whether the products could be removed from waste-water and potential drinking water by flocculation and chlorination. Products at concentrations of 0.1, 1.0, and 5 mg/L in

	hard and soft water were exposed to various flocculants including lime, alum, ferrous and ferric sulfates, and a synthetic cationic polymer (Nalco 8102). Phosphonate Products at concentrations of 1 mg/L were exposed to chlorination.
RESULTS	Results of the study indicated that the synthetic polymer was not effective, except in combination with other flocculants. The remaining flocculants were able to remove >95% of low concentrations of Phosphonates from both hard and soft water. At 5 mg/L Phosphonate, removal rates were lower, with lime removing 20-67% and alum removing 44-98%. The Phosphonate products underwent substantial chlorination, although the identity and fate of the chlorinated products was not identified.
CONCLUSION	The phosphonates appear to be fairly easily removed by ordinary waste-water treatment methods.
TEST FACILITY	Monsanto Europe SA Avenue De Tervuren 270 B-1150 Brussels Belgium (1983c).

7.1.6. Bioaccumulation

The bioconcentration factors were calculated for five phosphonates using the measured n-octanol/water partition coefficients described in Section 6. The calculated bioconcentration factors ranged from 0.02 to 0.04, indicating that these products should not bioaccumulate in aquatic organisms.

7.1.7. Photodegradation

TEST SUBSTANCE	Phosphonates
METHOD	Photochemical Transformation of Phosphonate product in Aqueous Solution
Light source and Spectrum	Natural sunlight
Relative Intensity	Variable.
Exposure Period	17 days
Remarks - Method	The photolysis of aqueous solutions of five phosphonates in sunlight was determined in combination with four metal ions (ferric, chromic, zinc and cupric). Solutions containing 10 mg/L of product in active acid form were prepared. The appropriate volume of stock solution of salt was then added to make up equi-molar mixtures and adjusted to the required pH values. Solutions were placed in sealed quartz tubes for exposure to sunlight. Initial and final orthophosphate concentrations were determined by UV-visible spectrometry.
RESULTS	
Remarks - Results	The results indicated direct photolysis of phosphonates is not highly significant (<15%). However, all of the products underwent sensitised photolysis in the presence of ferric nitrate with conversion of phosphonates to orthophosphates ranging from 44 to 83% at pH7 after 17 days. Except with one Phosphonate, other metal ions did not have a significant photo-sensitizing effect.
CONCLUSION	Direct photolysis of phosphonates is not expected to be significant. Indirect photolysis is a potential mode of removal of products from the environment, however, photolysis may be precluded because studies indicate these products have a relatively short time in the natural water column owing to their rapid removal by adsorption onto natural sediments.

TEST FACILITY	Solutia Services International, Belgium (undated).

7.2. Ecotoxicological investigations

7.2.1. Acute toxicity to fish

TEST SUBSTANCE	Free acid
METHOD	Acute Toxicity Test in Goldfish.
Species	*Carassius auratus*
Exposure Period	48 hours
Water Hardness	300 ± 20 mg $CaCO_3$/L
Remarks – Method	A preliminary test was performed against groups of 3 fish, which were exposed to concentrations of 1, 10, 50, 100, 500, 1000, and 5000 mg/L of test substance. Solutions were changed twice daily to maintain concentrations. The number of dead fish was recorded at 24 and 48 hours.
RESULTS	No fish exposed to concentrations of 1-500 mg/L died during the 48 hour test period, while all fish exposed to 1000 and 5000 mg/L died within less than an hour. No definitive test was performed in light of the low acute toxicity of the test substance.
CONCLUSION	The test substance is not expected to present an acute hazard to fish.
TEST FACILITY	SEAC Toxicology Group, Unilever Research Colworth, Bedford, England (1979e).

7.2.2. Acute/chronic toxicity to aquatic invertebrates

TEST SUBSTANCE	Free acid
METHOD	Acute Toxicity Test in Daphnia magna - static test conditions.
Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	None
Water Hardness	265 mg $CaCO_3$/L (hard water) 41 mg $CaCO_3$/L (soft water)
Remarks - Method	An acute toxicity test was performed against 10 juvenile *Daphnia* (4 reps per concentration range) 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 free acid. The second test carried out in soft water using nominal test concentrations of 0 (control), 50, 100, 250 and 500 mg/L of test substance.
RESULTS	
LC50	>500 mg/L at 48 hours (hard water) 100 to 250 mg/L (soft water).
NOEC (or LOEC)	Not reported.
Remarks - Results	In hard water, no immobilisation of daphnia occurred over the 48 hour test period, when exposed to test concentrations of 300 mg/L and below. Only 2.5% mortality was observed after 48 hours in the 350 mg/L solution, and 7.5% mortality occurred in both the 400 and 500 mg/L solutions. In the soft water, 10% of the *Daphnia* were immobilised after 48 hours exposure to concentrations of 100 mg/L solution, and 87.5% and 100% of animals were immobilised in the 250 and 500 mg/L solutions respectively.
CONCLUSION	The test substance is not toxic to Daphnia (Mensink *et al* 1995). The

results showed the test compound to be appreciably more toxic in water with low calcium content.

TEST FACILITY	SEAC Toxicology Group, Unilever Research Colworth, Bedford, England (1981e).

7.2.3. Algal growth inhibition test (1)

TEST SUBSTANCE	Free acid
METHOD	Acute Toxicity Test in Alga.
Species	*Chlorella vulgaris*
Exposure Period	72 hours
Auxiliary Solvent	None
Water Hardness	Equimolar solutions of Ca^{2+}
Analytical Monitoring	Biomass growth by absorbance at 440 nm.
Remarks - Method	The acute tests against Algae 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. Each solution was made up containing molar concentration of calcium ions equal to the molar concentration of the test compound. *Chlorella* were incubated for 72 hours at 24-25ºC in Bolds Basal medium containing the various concentrations of test material, and the growth in algal biomass monitored by absorbance at 440 nm.
RESULTS	No inhibition of algal growth was observed for the test medium containing nominal concentrations of 300 mg/L and less of test material. Up to 47% inhibition was recorded after 72 hours incubation in 500 mg/L medium. The effects of the test substance on algae were algistatic rather than algicidal because when the algae were removed from the medium containing nominal 500 mg/L of compound and placed in clean medium, they regained normal growth behaviour.
CONCLUSION	The results of this study indicate that the EC_{50} for free acid against *Chlorella* in equimolar Ca^{2+} is >500 mg/L. Hence the substance is not toxic under the test conditions described.
TEST FACILITY	SEAC Toxicology Group, Unilever Research Colworth, Bedford, England (1982b).

7.2.4. Algal growth inhibition test (2)

TEST SUBSTANCE	DTPMP [analogue] (The test substance has an additional $N(CH_2)_2$ and $–C–PO_3^–H_2$ unit compared to the parent acid).
METHOD	Modified OECD TG 201 Alga, Growth Inhibition Test
Species	*Selenastrum capricornutum*
Exposure Period	96 hours
Auxiliary Solvent	None
Water Hardness	Not reported
Remarks - Method	A modified OECD 201 test was performed to determine the effects of adjusting the free metal ion concentrations on the growth of green algae exposed to DTPMP. It was hypothesised that if the free ion concentrations were constant, DTPMP would not inhibit algal growth. Test media were prepared with OECD standard media, and with increased concentrations of iron, cobalt, copper, and zinc, in amounts enough to obtain constant free ion concentrations with increasing test substance concentrations. The test substance concentrations were: 0, 0.30, 1.0, 3.0 and 10 mg (a.i.)/L. Fe concentrations were not adjusted in line with Co, Cu, and Zn due to the higher expected stability constant for Fe, assumed to be –25.

RESULTS	The growth of green algae was inhibited even at the lowest concentrations when exposed to OECD standard Fe additions, with the EC_{50}<1.0 mg/L (lowest EC_{50} = EbC_{50} <0.3 mg/L). There was also a demonstrated decrease in Cu, Co, Fe and Zn free ion concentration in the growth media with increasing test substance concentrations. When the concentrations of these ions were increased in the medium in order to maintain the same free ion concentrations as in the OECD standard medium, no growth inhibition was observed even at the highest DTPMP concentration, which gave an NOEC>10 mg/L.
CONCLUSION	Growth inhibition of green algae following DTPMP exposure in standard OECD 201 test medium is not caused by its intrinsic toxicity, but by the test substance complexing essential elements required for algal growth. The addition of Fe to the test medium has a crucial effect on the free ion concentrations of the essential elements when its complex stability constant is higher than the other elements.
TEST FACILITY	TNO Nutrition and Food Research Institute, The Netherlands (1996).

7.2.5. Algal growth inhibition test 3

TEST SUBSTANCE	Free acid
METHOD	The Response of Algae to free acid (Procedures mostly followed Miller *et al*. 1978).
Species	*Selenastrum capricornutum, Microcystis aeruginosa, Anabaena flos-aquae, Navicula pelliculosa.*
Exposure Period	14 days
Auxiliary Solvent	None
Water Hardness	Not reported
Analytical Monitoring	Analysis of Test concentrations by persulfate/UV oxidation for colorimetric determination of phosphonates. Culture growth monitoring with a Coulter Counter on days 2, 4, 7, 9, or 10, 11 or 12, and 14.
Remarks - Method	A three-phase, long-term (14 day) test study was performed to determine the effects of free acid on different algal species and when varying nutrient concentrations. In Phase 1 of the test, the effects of free acid on the green algae, *Selenastrum capricornutum,* was evaluated at four different concentrations of nutrient media to determine whether nutrient availability played a role in the response. The nutrient media consisted of various salts of Na, Mg, Ca, K, B, Mn, Mo, Zn, Co, Cu, and Fe. The nutrient salt concentrations were varied to achieve concentrations equivalent to 0.5X, 1X, 2X, and 3X levels specified in the method used (i.e. Miller *et al.* 1978). In Phase 2 of the test, the responses of three additional algae species to free acid were evaluated at two different nutrient concentrations (1X and 2X), to compare the responses of algae from different taxonomic groups. Algae were exposed in triplicate to nominal concentrations of 0, 0.1, 0.56, 1.0, 5.6 and 10 mg/L. In Phase 3 of the test, *S. capricornutum* was exposed to 5.6 mg/L of ^{14}C-free acid in 3X medium for 14 days to evaluate utilisation of free acid as a nutrient.
RESULTS	In the Phase 1 test, the EC_{50} values of *Selenastrum capricornutum* exposed to the test substance were <0.1 mg/L on Day 4, in all media concentrations. On Day 14, the EC_{50} values increased to between 3.9 and 7.9 mg/L, with the media with the highest nutrient levels having the highest EC_{50} value, suggesting an interaction (complexing) between the free acid and the nutrient medium. In Phase 2 of the test, the response of the three additional algal species to free acid showed them to be less sensitive to free acid than *S. capricornutum.* The EC_{50} values on Day 4 ranged from 0.56 to >10 mg/L, while the EC_{50} values on Day 14 were >10 mg/L for all but one species and test concentration suggesting an

analytical error occurred in the latter. In Phase 3 of the test, the radioactivity levels of free acid in the medium decreased rapidly over the test period. Some of the loss was ascribed to uptake by algae and conversion of the labelled moiety to CO_2, which could have been lost directly to the atmosphere in addition to being taken up by the algae.

CONCLUSION

The test results show an interaction between free acid and the nutrient media, which suggest a nutrient limitation effect rather than to direct toxic effects to algae. Initially algal growth is inhibited, however, by day 14, higher concentrations of test substance is required to inhibit growth. In general, the higher nutrient concentrations tended to reduce the magnitude of the effects as well as raise the threshold concentration at which effects occurred. This form of growth response, i.e. rapid growth followed by a plateau or gentle increase, is consistent with a nutrient-limited system. Tests on other phosphonate compounds show similar results (Gledhill and Feijtel 1992). It is thought that initially the phosphonates chelate some of the essential micronutrient required for algal growth in the test media. However, over time, some of the phosphonates degrade or metabolise to release nutrients and additional phosphorus which results in algal growth stimulation when present at low concentrations (eg. Gledhill and Feijtel 1992 p282).

TEST FACILITY

SRI International, Menlo Park, CA (1984).

8. RISK ASSESSMENT

8.1. Environment

8.1.1 Environment- exposure assessment

Usage patterns indicate that ultimately all of the imported volume (less than 10 tonnes) of the notified chemical could be released into the sewer in a diffuse manner during use of the detergent products. Worst case Predicted Environmental Concentration (PEC) in sewage effluent is estimated to be around 2.9 μg/L. This value assumes all of the annual import volume is used over 365 days, and that each individual of a population of 19 million contributes 150 L of water to the sewer each day. The concentration in effluent would be reduced once released into the receiving waters by an amount depending on whether it is released into the ocean or into a river.

The notified chemical is not volatile, is highly water soluble, and owing to its strong chelating ability, has a high affinity to the mineral portions of soils and sediments. A test monitoring the content of DOC in activated sewage sludge exposed to the parent acid (free acid) indicated the compound was not biodegraded over a 14 week period and did not adsorb onto activated sewage sludge during the test, suggesting the notified chemical would pass through conventional activated sludge stages of sewage treatment and enter surface waters (Unilever 1981). However, another study indicated removal of the products from the water column through precipitation with flocculants such as ferric sulphate, alum and lime, which are commonly used during the later stages of sewage treatment processes. The products form insoluble metal complexes, which precipitate and become incorporated into metal hydroxide sludge (Monsanto 1983c). These studies suggest that the notified chemical would pass through sewage treatment works having only primary levels of treatment, while it would partition into sludge in treatment works with secondary and tertiary level treatments.

In the natural environment, the notified chemical is not expected to readily degrade either through biotic or abiotic mechanism, but rather is expected to partition fairly rapidly into sediments, particularly if the water is hard. In a study examining the degradation of phosphonates in river water, the compound showed only slow photolytic degradation (Monsanto 1983a). Another study examining photo-degradation of aqueous phosphonate products in natural sunlight indicated direct photolysis was not significant, while higher degradation rates occurred in the presence of ferric nitrate (Solutia undated). In the Monsanto study, the phosphonate compounds were found to partition strongly to river sediments, most likely through complexing with Ca^{2+} and Mg^{2+} and other

mineral cations in the water column and on the surfaces of suspended sediments. Hence, the phosphonate products are not expected to remain in the natural water column for long owing to their rapid removal by adsorption onto natural sediments.

8.1.2 Environment- effects assessment

No ecotoxicity tests are available for the notified chemical. However, the notifier submitted several ecotoxicology test reports for the parent acid (free acid) and for a related compound, toward green algae. The results of these tests indicate the toxicity of the phosphonate compounds is dependent on the level of Ca^{2+} and other divalent metal ions in the test water. For example, in the toxicity test towards *Daphnia,* the LC_{50} in the test conducted in soft water (41 mg $CaCO_3$/L) was between 100 and 250 mg/L compared to an LC_{50}>500 mg/L in the test conducted in hard water (265 mg $CaCO_3$/L). In the toxicity test for a structurally related compound toward algae, toxicity was mitigated when the concentrations of Fe, Co, Cu and Zn ions were adjusted to obtain a constant free ion concentration with each increasing test substance concentration. In a further series of studies performed to determine the effects of free acid on algae when the concentrations of cations in the test media were varied, the EC_{50} values were generally highest (i.e. toxicity lower) in the test media with the highest nutrient levels. These results are all consistent with the free metal cations in the test media forming strong complexes with the test substances, thereby significantly reducing their toxicity.

A predicted no effects concentration (PNEC) can be determined when at least one acute LC_{50} for each of the three trophic levels is available (fish, *Daphnia*, algae). The PNEC is calculated by taking the LC_{50} value of the most sensitive species, and dividing this value by an assessment safety factor. It is clear from the submitted studies that the most sensitive species is the freshwater algae, *Selenastrum capricornutum,* having a lowest 96 hour EC_{50} calculated for free acid of <0.1 mg/L in soft water. Therefore, using this value and a worst-case scenario safety factor of 100 (OECD), the $PNEC_{aquatic}$ is <1.0 μg/L.

8.1.3 Environment- risk chracterisation

Usage patterns indicate that all of the imported volume of notified chemical is likely to be released into the aquatic environment via sewage treatment systems predominantly through use of the detergent products. Worst case Predicted Environmental Concentration (PEC) in effluent released from the sewer is estimated to be around 2.9 μg/L, assuming no adsorption or degradation. The concentration in effluent, however, would be reduced once released into the receiving waters by an amount depending on whether it is released into the ocean or into a river. In a large coastal city it is assumed that the sewage effluent is diluted by a factor of 10 after discharge into the ocean, while a dilution factor of 3 is assumed for rural areas. This would lead to a PEC of 0.3 μg/L in coastal waters and 0.97 μg/L in inland waters.

The ecotoxicity data indicate the parent acid of the notified chemical is not harmful to fish and is only slightly toxic to *Daphnia*. However, the substance is able to inhibit the growth of algae and can be highly toxic to these organisms (Mensink *et al* 1995). The PEC/PNEC ratios for the natural aquatic environment, using algae as the most sensitive species, are 0.3 and 0.97 respectively, with the former being significantly less than one, and the latter close to one, indicating a potential for concern in the latter case. However, despite these results, the chemical is not expected to pose a significant threat to aquatic organisms, including algae, when released into the environment in small quantities.

The parent acid of the new compound, which is a strong metal sequestrant, appears to be more toxic to aquatic species than the salts of the compound. The inhibition of algal cell growth by the parent acid (free acid) is considered to result from the test substance sequestering critical micronutrient metals in the growth medium, and hence starving the algae. This phenomenon has been documented for many other chelating substances as well (eg. Schowanek *et al*. 1996). In natural aquatic environments, however, the polymer is not expected to limit nutrient availability and hence algal growth when released in small quantities, because nutrients are not expected to be restricted in these environments. In any case, the available information indicates the free acid would not exist as such in the receiving waters given that free calcium and magnesium in the water will form complexes with this compound.

Thus the calculated PECs do not take into account adsorption and removal of the notified chemical, which would occur in sewage treatment facilities or in natural water bodies. Most of the polymer will probably be removed in the sewer by precipitation and settling in clarification tanks, especially where there are facilities have secondary and tertiary levels of treatment. Even if only primary treatment is available, most of the chemical's sequestering potential should be significantly reduced by formation of insoluble metal complexes with metal cations scavenged in the nutrient-rich sewage water. In the natural aquatic environment, the chemical is also not expected to remain in the water column, but rather will partition into sediment.

Ultimately, most of the chemical will enter the soil/sediment environment either through disposal of solid waste following sewage treatment or through partitioning directly from the water column into sediments. Most sewage sludge in Australia is dried and either sent to landfill or incinerated (ash may be sold to farmers), but with sludge being increasingly used as soil conditioners and fertilisers in industries such as agriculture, horticulture and land rehabilitation (Beretka and Whitfield 1993; Sydney Water 2000). Sewage sludge contains appreciable amounts of toxic heavy metals including `arsenic, lead, cadmium, and mercury, and restricting inputs in effluent is desirable to reduce these unwanted elements (Tiller 1989), especially if sludge is being used as a soil conditioner. Thus scavenging by the notified chemical of heavy metal ions already residing in the sewer could conceivably contribute to the heavy metal load in sewage sludge (Bubb and Lester 1991). In addition, remobilising of toxic metals from sediment in natural waters could contribute to increased loads of these chemicals in waterways.

The results of a test on potential mobilisation of metals from sediments through complex formation (and solubilisation) however, indicated that heavy metals in sediments (eg Cu^{2+}, Zn^{2+} and Cd^{2+} etc) are not significantly mobilised by the low concentrations of phosphonate compounds likely to enter natural waters (Monsanto 1983b). Heavy metal contamination of soils resulting from the presence of the notified chemical in sewage sludge is also not expected to be significant given the low concentrations and anticipated nationwide use of the chemical.

In soil environments, the chemical is expected to be somewhat persistent, but would eventually undergo photo-degradation and slow mineralisation by micro-organisms. Soil biodegradation studies indicate phosphonate degrading micro-organisms are common in soils and are able to mineralise a range of phosphonates. Half-lives of some of these compounds are in the order of 40-50 days (Gledhill and Feijtel 1992).

8.2. Human health

8.2.1. Human health - effects assessment

SUMMARY OF TOXICOLOGICAL INVESTIGATIONS

Endpoint and Result	*Assessment Conclusion*
Acute oral LD_{50}>4100 mg/kg (free acid and calcium/sodium salt)	Low toxicity
Acute dermal (surrogate data- intraperitoneal and percutaneous)	Low toxicity
Skin irritation (covered patch tests- Rabbits)	Slightly irritating
Eye irritation (Rabbits)	Slightly irritating
Guinea pig, skin sensitisation (Guinea pigs)	Evidence of skin sensitisation (weak)
Rat, oral repeat dose toxicity (free acid)- 3-week 90 day	LOAEL 121 mg.kg/day NOAEL 170 mg/kg/day

Genotoxicity – bacterial reverse mutation; Mouse *in vivo* cytogenetic assay; (surrogate data)	Non genotoxic

DISCUSSION

The notified chemical, free acid and calcium/sodium analogues had very low acute oral (LD_{50} >4100 mg/kg) toxicity. Dermal toxicity was not tested. However, intraperitoneal and percutaneous toxicity studies with free acid indicated that it is of low toxicity by dermal route. No acute inhalation studies were available.

Skin irritation studies with the notified chemical (as a 33% solution) resulted in slight irritation. However, a covered patch test in rabbits with the free acid (in powder form) resulted in slight to moderate irritation. Further skin irritation tests on both chemicals (free acid and notified chemical) were conducted in rabbits by intradermal injection, with the results uncertain as the studies were to non-standard protocols and mainly for comparative purposes. The notified chemical is not classified as a skin irritant based on the available data.

The notified chemical was a slight eye irritant, whereas the free acid was a moderate eye irritant in rabbits.

The skin sensitisation tests conducted on the notified chemical showed results ranging from sensitiser to non-sensitiser. However, tests conducted on the calcium/sodium salt showed that it was non-sensitising in one study and weakly sensitising in the other. A similar study with the free acid form was negative. 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 free acid in bone indicated a high affinity for mineralising tissues, consistent with its chelating properties. Higher excretion rates through faeces indicated that the free acid was not readily absorbed in the gastrointestinal tract.

The free acid and calcium/sodium salt did not display genotoxic activity in a number of *in vitro* and *in vivo* studies carried out in a variety of biological systems.

CONCLUSION

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

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.

HEALTH HAZARD CLASSIFICATION

The notified chemical is not classified as a hazardous substance according to NOHSC *Approved Criteria for Classifying Hazardous Substances* [NOHSC, 1999]. The eye and skin irritation effects were not classifiable and did not warrant including risk phrases.

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.

8.2.2. Human health – risk characterisation

OCCUPATIONAL HEALTH AND SAFETY

The notified chemical will be initially imported as an ingredient for inclusion in cleaning formulations (<1%). Transport and storage workers can only be exposed to the products containing the notified chemical in cases of accidental spills. Therefore, the risk of adverse health effects for these works is low.

The notified chemical (<30%) may be imported in the future for local formulation. The notified chemical is found to be slightly to moderately irritating to the skin, a weak skin sensitiser and slight eye irritant. However, the risk of irritant effects due to the notified chemical is expected to be low due to the engineering controls (closed system and automated process) employed during formulation. Nevertheless, workers involved in the formulation process should wear gloves, goggles and overalls, to reduce the risk of irritation, for example if spillage occurs.

Cleaners will be using diluted cleaning products (containing <0.01% of notified chemical). Dermal and ocular contamination with the diluted products are not expected to cause skin irritation/sensitisation or eye irritation. No information was provided on the inhalation toxicity. However, given the formulation and the low amount of notified chemical in the products, inhalation exposure is not expected to be significant. The health risk to these workers due to the notified chemical is very low.

PUBLIC HEALTH

The end-use floor/surface cleaning products containing low concentration of the notified chemical will be extensively used by consumers. The notified chemical is a slight to moderate eye and skin irritant, and a weak skin sensitiser. During application, there may be dermal and inhalation exposure to the liquid and spray formulations, as well as accidental ocular and oral exposure. However, the hazard is likely to be minimal due to the very low final-use concentrations. Residue of the notified chemical remaining on the cleaned surface is negligible. Consequently, the risk from use of the notified chemical is considered to be low. There will be minimal public exposure from transport, storage, and formulation.

9. CONCLUSIONS – ASSESSMENT LEVEL OF CONCERN FOR THE ENVIRONMENT AND HUMANS

9.1 Environment

The chemical is not considered to pose a risk to the environment based on its use pattern in detergents when taking into account the low import volume and low expected exposure. The parent acid is listed on AICS and along with other similar substances is already being used in formulated detergents in a similar manner to the notified chemical.

9.2 Human health – Occupational health and safety

There is Low Concern to occupational health and safety under the conditions of the occupational settings described.

Local formulation of the cleaning products is envisaged in the future. The assessment showed that there is medium occupational health and safety concern for formulators and thus personal protection equipment is required.

9.3 Human health – public

There is negligible concern to public health when used according to the set conditions.

10. 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, 1994a).

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

11. LABEL

The label for the notified chemical provided by the notifier was in accordance with the NOHSC *National Code of Practice for the Labelling of Workplace Substances* (NOHSC, 1994b). The accuracy of the information on the label remains the responsibility of the applicant.

12. RECOMMENDATIONS

REGULATORY CONTROLS

- Use the following safety phrases for the notified chemical:
  - S24: Avoid skin contact
  - S25: Avoid contact with eyes
  - S36 Wear suitable protective clothing
  - S37 wear suitable gloves

CONTROL MEASURES

Occupational Health and Safety

- Employers should implement the following engineering controls to minimise occupational exposure to the notified chemical during formulation of the products containing the notified chemical:
  - Automated and closed systems

- Employers should implement the following safe work practices to minimise occupational exposure during handling of the notified chemical during formulation:
  - Prevent splashes and spills

- Employers should ensure that the following personal protective equipment is used by formulators to minimise occupational exposure to the notified chemical:
  - Chemical resistant gloves, goggles and protective overalls

  Guidance in selection of personal protective equipment can be obtained from Australian, Australian/New Zealand or other approved standards.

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

- If products and mixtures containing the notified chemical are classified as hazardous to health in accordance with the NOHSC *Approved Criteria for Classifying Hazardous Substances*, workplace practices and control procedures consistent with provisions of State and Territory hazardous substances legislation must be in operation.

Environment

Disposal

- The notified chemical should be disposed of by incineration or recycling.

Emergency procedures

- Spills/release of the notified chemical should be not be allowed to enter drains and water courses. Large spills should be contained and transferred to appropriate containers for disposal.

Secondary notification

The Director of Chemicals Notification and Assessment must be notified in writing within 28 days by the notifier, other importer or manufacturer:

(1) Under Sub-section 64(1) of the Act:

If the conditions of use are varied, then greater exposure of the public may occur. In such circumstances, further information may be required to assess the risks to public health.

Or

(2) Under Sub-section 64(2) of the Act:
- if any of the circumstances listed in the subsection arise.

The Director will then decide whether secondary notification is required.

13. BIBLIOGRAPHY

Beretka J and Whitfield D (1993). Survey of Industrial Process Wastes and By-Products Generated in Australia. CSIRO Australia; University of Western Sydney.

Bubb JM and Lester JN (1991). The Impact of Heavy Metals on Lowland Rivers and the Implications for Man and the Environment. *The Science of the Total Environment*, 100: 202-233.

Calvin G, Long PH, Stitzel KA, Anderson RL, Balmbra RR, Bruce RD, Bhatt A, Miller PM and Broadmeadow A (1988) [notified chemical], genotoxicity, biodistribution and subchronic and chronic toxicity in rats, Fd. Chemi.Toxicol. 26: 601-610.

Gledhill WE and Feijtel TCJ (1992). Environmental Properties and Safety Assessment of Organic Phosphonates Used for Detergent and Water Treatment Applications. In: O. Hutzinger (ed), The Handbook of Environmental Chemistry Volume 3 Part F. Springer-Verlag. Pp261-285.

Magnusson B and Kligman AM (1970) Allergic contact dermatitis in the guinea pig: Identification of contact allergens. C. C. Thomas, Springfield, Illinois, USA.

Mensink BJWG, Montforts M, Wijkhuizen-Maslankiewicz L, Tibosch H and Linders JBHJ (1995). Report no. 679101022: Manual for Summarising and Evaluating the Environmental Aspects of Pesticides. National Institute of Public Health and Environmental Protection, Bilthoven, The Netherlands.

Miller WE, Greene, JC, Shiroyama T (1978). The *Selenastrum capricornutum* Purity Algal Assay Bottle Test. EPA_600/9-78-018.

*Monsanto (1979). MIC Environmental Sciences Report No. ES-79-SS-15, November 19 1979.

*Monsanto (1980). Free acid: Adsorption on River Solids. Monsanto Europe SA (Belgium), Study No. ES-79-SS-23, December 1980.

*Monsanto Research Corporation (1981). Calcium/sodium salt: Mutagenicity test in Salmonella typhimurium, Study No. 80-12-4-437-00A. USA.

*Monsanto (1983a). Free acid: Primary Degradation in River Water. Monsanto Europe SA (Belgium), Study No. ES-82-SS-101, January 1983.

*Monsanto (1983b). Free acid: Mobilisation of Metals. Monsanto Europe SA (Belgium), Study No. ES-82-SS-101, January 1983.

*Monsanto (1983c). Free acid: Waste Water Treatability and Water Purification. Monsanto Europe SA (Belgium), Study No. ES-82-SS-101, January 1983.

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

National Occupational Health and Safety Commission (1994b) National Code of Practice for the Labelling of Workplace Substances [NOHSC:2012(1994)]. Australian Government Publishing Service, Canberra.

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

*SEAC Toxicology Group (1976). Free acid: Acute Oral Toxicity in Mice, Study No. MT 1658. UK.

*SEAC Toxicology Group (1977a). Free acid: Acute Oral Toxicity in Rats, Study No. RG1042. UK.

*SEAC Toxicology Group (1977b). Free acid: Acute Intraperitoneal Toxicity in Mice, Study No. MT 1677. UK.

*SEAC Toxicology Group (1977c). Free acid: Dermal Irritation in Rabbits (Covered Patch Test), Study No. CP 77 864. UK.

*SEAC Toxicology Group (1977d). Free acid: Dermal Irritation in Rabbits (Intradermal Injection Test), Study No. INJ77.231B. UK.

*SEAC Toxicology Group (1977e). Free acid: Sensitisation Test in Guinea Pigs, Study No. SSM76.469. UK.

*SEAC Toxicology Group (1979a). Calcium/sodium salt: Acute Oral Toxicity in Mice, Study No. MT79.1996. UK.

*SEAC Toxicology Group (1979b). Calcium/sodium salt: Acute Oral Toxicity in Rats, Study No. 1988-64/8-11. UK.

*SEAC Toxicology Group (1979c). Free acid: Sub-Acute Oral Toxicity in Rats (3 Weeks), Study No. RG1029. UK.

*SEAC Toxicology Group (1979d). Free acid: Mutagenicity Test in Salmonella Typhimurium, Study No. MUTA 79/031, MUTA 79/032, MUTA 79/033, MUTA 79/047, MUTA 79/051 and MUTA 79/057.

*SEAC (1979e). Free acid: Acute Toxicity Test in Goldfish. Study No. FU 78/010, February 1979. Toxicology Group (Unilever UK).

*SEAC Toxicology Group (1980a). Notified chemical: Dermal Irritation in Rabbits (Intradermal Injection Test), Study No. INJ80.12A. UK.

*SEAC Toxicology Group (1980b). Calcium/sodium salt: Dermal Irritation in Rabbits (Intradermal Injection Test), Study No. INJ80.27. UK.

*SEAC Toxicology Group (1980c). Calcium/sodium salt: Dermal Irritation in Rabbits (Intradermal Injection Test), Study No. INJ80.27. UK.

*SEAC Toxicology Group (1980d). Notified chemical: Dermal Irritation in Rabbits, Study No. CPS80.30. UK.

*SEAC Toxicology Group (1980e). Notified chemical: Eye Irritation in Rabbits, Study No. RE80/82.

UK.

*SEAC Toxicology Group (1981a). Calcium/sodium salt: Sensitisation Test in Guinea Pigs, Study No. SSM80.327. UK.

*SEAC Toxicology Group (1981b). Notified chemical: Sensitisation Test in the Guinea Pigs, Study No. SSM80.161. UK.

*SEAC Toxicology Group (1981c). Free acid: Mutagenicity Test in Salmonella typhimurium, Study No. MUTA 81.44, MUTA 81.45 and MUTA 81.52. UK.

*SEAC Toxicology Group (1981d). Free acid: Cytogenicity Test in Chinese Hamsters, Study No. MUTC 27. UK.

*SEAC (1981e). Free acid: Acute Toxicity Test in Daphnia Magna. Study No. AT/251/02 AT/25/03, October 1981. Toxicology Group (Unilever UK).

*SEAC (1982). Free acid: Acute Toxicity Test in Algae. Study No. AL/251/02, June 1982. Toxicology Group (Unilever UK).

*SEAC Toxicology Group (1982b). Notified chemical: Sensitisation Test in the Guinea Pigs, Study No. SSM83.248. UK.

*SEAC Toxicology Group (1983). Notified chemical: Sensitisation Test in the Guinea Pigs, Study No. SSM83.077. UK.

*SEAC Toxicology Group (1984a). Calcium/sodium salt: Sensitisation Test in Guinea Pigs, Study No. SSM84.236. UK.

*SEAC Toxicology Group (1984b). Free acid: 13-Week Oral Toxicity in Rats, Study No. RG 1030. UK.

SEAC Toxicology Group (2000) Calcium/sodium salt: Safety Data Document No. D00/001. UK.

Schowanek D, McAvoy D, Versteeg D and Hanstveit A (1996). Effects of nutrient trace metal speciation on algal growth in the presence of the chelator [S,S]-EDDS. *Aquatic Toxicology* 36:253-275.

*Solutia (1999). Determination of EDTMPA in Free acid, calcium/sodium salt and notified chemical. Report Number: 62-04. Solutia Company, Newport.

*Solutia (Undated). Photochemical Transformation of [product] Phosphonates in Aqueous Solution. Report Number ES-79-SS-21. Solutia Company, Newport.

*SRI International (1984). The response of algae to Free acid. Final Report. SRI Project Number LSC-6761. 30 October 1084.

Sydney Water (2000). Annual Environment & Public Health Report 2000. Beneficial Use of Biosolids from Sewage Treatment (http://www.sydney water.com.au).

Tiller KG (1989). Heavy Metals in Soils and Their Environmental Significance. Advances in Soil Science, Volume 9, pp113-142. Springer-Verlag.

*TNO (1996). Effect of [analogue] on the growth of green alga *Selenastrum capricornutum* in relation to the free ion concentration in algal growth medium with increased iron, cobalt, copper, and zinc concentrations (OECD Guideline No. 201). Report V96.099. TNO Nutrition and Food Research Institute, The Netherlands.

*Unilever (1981). Semi-continuous Activated Sludge (SCAS) Test on Free acid. Unilever Research (UK), Study No. BD/251/01, October 1981.

*Younger Laboratories (1968a). Free acid: Acute Percutaneous Toxicity Test in Rabbits, Study No. Y-68-58. UK.

*Younger Laboratories (1968b). Free acid: Eye Irritation in Rabbits, Study No. Y-68-58. UK.

* Unpublished summary report provided by the notifier