File No: NA/735

May 2000

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME

FULL PUBLIC REPORT

C_{12-14} Linear Glucose Amide

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Director
Chemicals Notification and Assessment

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C_{12-14} Linear Glucose Amide

1. APPLICANT

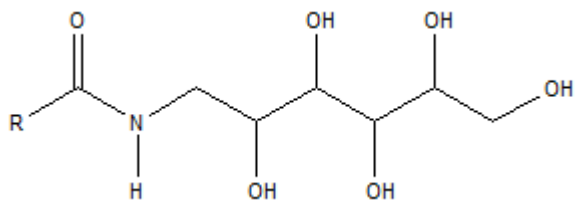
Proctor and Gamble Australia Pty Ltd of 99 Phillip St PARRAMATTA NSW 2150 has submitted a standard notification statement in support of their application for an assessment certificate for C_{12-14} Linear Glucose Amide.

No claims for exempt information were made.

2. IDENTITY OF THE CHEMICAL

Chemical Name:	D-glucitol, 1-deoxy-1-(methylamino)-, N-C_{10-16} acyl derivatives
Chemical Abstracts Service (CAS) Registry No.:	173145-38-5
Other Names:	C_{12-14} linear glucose amide GS-base E-4194.01
Marketing Name:	component of Dawn dishwashing liquid
Molecular Formula:	$C_{18}H_{39}O_6N$ (based on C_{12} acyl group) the chain length distribution is C_{10}, 0.4 %; C_{12}, 73.6 %; C_{14} 24.9 %; C_{16} 0.5 %
Molecular Weight:	365 (based on C_{12} acyl group)

Structural Formula:



Method of Detection and Determination:	UV/Visible spectroscopy Infrared spectroscopy ^{1}H nmr spectroscopy	
Spectral Data:	UV/Vis	218 nm under alkaline conditions no maxima observed between 210 and 900 nm under acidic or neutral conditions
	IR	3375 (br), 2935, 2860, 1625, 1448, 1415 1355, 1085, 1030, 895 cm^{-1}
	^{1}H nmr	4.86 (singlet), 3.96 (multiplet), 3.80 – 3.55 (multiplet), 3.47 – 3.33 (multiplet), 3.13 (singlet), 2.96 (singlet), 2.52 – 2.33 (multiplet), 1.60 (quintet), 1.33 (singlet), 0.90 (triplet) ppm

The notified chemical is a mixture of linear glucose amides with the general structural formula depicted above. The major component is the dodecyl derivative (around 74 % by weight), although there is also a significant presence of the tetradecyl analogue (25 %), and smaller presence of the hexadecyl and decyl analogue (each present at around 0.5 %). The molecular weight of 357 g/mole is the (weight averaged) molecular weight of the principal components.

The compounds contain two distinct portions, the hydrophilic glucose amide "head group", and the hydrophobic linear alkyl chain. Consequently, the compounds are non-ionic surfactants.

3. PHYSICAL AND CHEMICAL PROPERTIES

Appearance at 20°C and 101.3 kPa:	yellow solid
Melting Point:	76.5 – 113.3°C Method OECD 102
Specific Gravity:	1.14 at 20°C Method OECD 109
Vapour Pressure:	1.4 Pa at 25°C Method OECD 104
Surface Tension:	31.7 mN/m at 20°C Method OECD 115
Water Solubility:	140 mg/L at 20°C (see comments below) Method OECD 105
Particle Size:	not relevant as notified chemical is only imported in solution
Partition Co-efficient	log P_{ow} = 2.3 (from the ratio of water and n-octanol

(n-octanol/water):	solubility; the test substance is surface active, see comments below) Method OECD 117
Hydrolysis as a Function of pH:	< 10 % hydrolysis after 5 days at 50°C at pH 4, 7 and 9
Adsorption/Desorption:	not determined, estimated Log K_{oc} = 1.96 (see comments below)
Dissociation Constant:	the notified chemical contains no acidic or basic functional groups
Flash Point:	none – notified chemical not volatile
Flammability Limits:	not flammable; not readily combustible
Autoignition Temperature:	no autoignition observed below the melting point of the test substance
Explosive Properties:	not explosive by thermal stress, shock or friction
Reactivity/Stability:	not oxidising; expected to be stable under normal environmental conditions

3.1 Comments on Physico-Chemical Properties

The vapour pressure was determined using the static manometric technique, and the vapour pressure data determined at 24.8, 31.1 and 37.7°C used to interpolate the vapour pressure at 25°C, providing the result tabulated above. The relatively low vapour pressure at ambient temperature is expected for a solid compound of relatively high molecular weight.

Water solubility was determined using the flask method. In this procedure saturated solutions were prepared by stirring an excess of the compound with a known volume of water for periods of 24, 48 and 72 hours in a water bath at 30°C. After these periods each was then allowed to equilibrate at 20°C for 24 hours, and the concentration of the test material in the aqueous phase then determined using High Performance Liquid Chromatography (HPLC). The chromatograms showed two distinct peaks, with areas approximately in the ratio 3:1, corresponding to the two major components present in the new material. Each test was performed in duplicate, and at 20°C the mean concentration in the solution prepared after 24 hours stirring was 139 mg/L, while that in the solution prepared after 72 hours was 132 mg/L. These very similar values indicate that they reflect the true water solubility of the material, and the mean of all six separate determinations gave a water solubility of 140 ± 10 mg/L at 20°C. This moderate water solubility is primarily conferred on the chemical by the hydroxy groups on the glucoside moiety in the "head group" of the molecules. The pH of the aqueous solutions was nearly neutral, 6.7 after 24 hours and 7.3 after 72 hours stirring, which is in accord with the absence of acidic or basic groups within the molecules.

A test report was submitted describing the hydrolytic degradation of the compound over a 5 day test period at 50°C in buffer solutions of pH 4, 7 and 9. The concentration of the test

material in the solutions was determined using HPLC, and after the five day test period more than 90 % of the original material remained non-degraded in the test media. Under the lower temperatures encountered in the aqueous and terrestrial environmental compartments the degree of degradation would be substantially less. Consequently, it was concluded that the amide group is not susceptible to hydrolysis in the usual environmental pH region where $4 < pH < 9$.

Due to the surface active nature of the material, it was not possible to accurately estimate the value of the n-octanol/water partition coefficient partition coefficient from comparison of the retention time of the material on a C18 column with those of a series of standard compounds. Accordingly, this parameter was determined as the ratio of the solubility of the compound in n-octanol to that in distilled water. The solubility of the material in n-octanol was determined by stirring an excess of the solid material with 25 mL of n-octanol at room temperature overnight, following which the concentration in the octanol phase was determined as 24.9 g/L using HPLC. The partition coefficient was then estimated as 24900/140 = 180, giving Log P_{ow} = 2.3.

It should be noted that this estimate is a "composite" value reflecting contributions to P_{ow} (and Log P_{ow}) from the individual components.

Adsorption/desorption data was not presented in the submission, but the value of Log K_{oc} may be estimated from the value for Log P_{ow} using Quantitative Structure Activity Relationships (QSAR). While no QSAR specific for the present class glucose amides is available, the European Commission (1994) have provided a list of general equations which are appropriate for certain broad classes of compound. The equation which appears most appropriate for the present material is that given for "predominantly hydrophobic" compounds, and is –

Log K_{oc} = 0.81 X Log P_{ow} + 0.10.

Using the estimated value for Log P_{ow} = 2.3, this gives an estimated value for Log K_{oc} of 1.96. This is a modest value for this parameter indicating low affinity for the organic component of soils and sediments, and the compound could be expected to be relatively mobile in these media. However, the constituent compounds of the new material are surface active and this property may make predictions of adsorption/desorption behaviour based on values for Log K_{oc} unreliable.

The compound contains no acidic or basic functionalities, and so dissociation constant data are not appropriate for this material.

The material is surface active, and the surface tension at 20°C for a solution containing 100 mg/L of test material was determined as 33.6 mN/m (distilled water 73.6 mN/m). This is to be expected for molecules containing both a hydrophilic head group and a large alkyl chain.

4. PURITY OF THE CHEMICAL

Degree of Purity: 93 %

Hazardous Impurities: none

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

Chemical name:	d-glucitol, 1-deoxy-1-(methylamino)-
Synonyms:	N-methylglucamine
Weight percentage:	2 %
CAS No.:	6284-40-8

Chemical name:	sodium soap
Weight percentage:	3 %
CAS No.:	not applicable

Additives/Adjuvants:

Chemical name:	soaps
CAS No.:	not applicable
Weight percentage:	2.5 %

Chemical name:	ethanol
CAS No.:	64-17-5
Weight percentage:	11 %

Chemical name:	1,2-propanediol
Synonym:	propylene glycol
CAS No.:	57-55-6
Weight percentage:	6 %

Chemical name:	methanol
CAS No.:	67-56-1
Weight percentage:	0.75 %

Chemical name:	water
CAS No.:	7732-18-5
Weight percentage:	23 %

5. USE, VOLUME AND FORMULATION

The notified chemical will be a component at 51 % in the product GAS-2EM Surfactant,

which contains the additives tabulated above. This product will not be introduced to Australia, and the notified chemical will only be introduced at a concentration of 1.43 % in a finished dishwashing liquid, which will be marketed as Dawn. No reformulation will occur in Australia.

The expected import volume is 27 tonnes of notified chemical per annum.

6. OCCUPATIONAL EXPOSURE

The notified chemical will be imported as a component of a finished dishwashing liquid. It will be imported in 60 mL sample bottles and 434 mL bottles for retail sale. No reformulation or repackaging will be carried out.

Transport and Storage
The bottles containing the notified chemical will be imported in cardboard cartons, each containing 12 bottles. The cartons will be packed in containers. Waterside and transport workers will transfer the containers to a bunded area in the notifier's warehouse, from which the product will be distributed. The cardboard cartons would then be transferred by road to retailers' warehouses and finally to individual stores. No exposure of workers would be expected during these operations except in case of an accident involving damage to the packaging.

The notifier estimates that 50 waterside and transport workers will handle the product containing the notified chemical, 12 times per year for 8 hours per time. Also up to 30 warehouse workers will handle the product containing the notified chemical, 100 times per year, for 4 hours per time.

Retail
A large number of retail workers will be involved in handling the product containing the notified chemical, in supermarkets, pharmacies and department/variety stores. The workers would generally handle the plastic bottles while opening cartons and stacking shelves, and while processing purchases. No exposure of these workers would be expected except where there is a spill of the product which has to be cleaned up.

The notifier estimates that approximately 10 000 workers will handle the notified chemical, 100 times per year, for 1 hour per day.

7. PUBLIC EXPOSURE

Public exposure to the notified chemical is expected to be widespread as the product containing the notified chemical will be sold to the public. Exposure will primarily occur via the dermal route, with the possibility of accidental ocular and oral exposure. The notified chemical is present at a concentration of 1.43 % in the imported product, which will normally be used diluted further. The use of rubber gloves may further reduce the public exposure.

8. ENVIRONMENTAL EXPOSURE

8.1 Release

The use pattern of the dishwashing liquid is such that all the new compound will be released to the sewer system. Very little of the dishwashing concentrate is likely to be left in the bottles, since these are usually washed out with water prior to disposal of the empty bottles. Any residual left in the bottles would be disposed of to landfill with general domestic waste.

Once released to the sewer system, some of the notified chemical may become associated with sewer sludges and sediments. These sediments and sludges are often disposed of into landfill, although there is an increasing trend to use these materials as soil conditioners. Consequently, there is a possibility that some of the chemical may be released to the soil compartment. However, as discussed further below, the low estimated value for Log K_{oc} indicates the chemical may be relatively mobile in this media.

8.2 Fate

Almost all the notified chemical is expected to be released to the water compartment through the sewer system. Although the molecules contain a large hydrocarbon moiety which could be expected to have affinity for the organic component of sewer sludges and sediments, the apparently modest value for Log K_{oc} (estimated as 1.96), and the moderate water solubility indicate that overall the affinity of the compounds constituting the notified material for these media will be small. Consequently, while some of the new compound may become loosely associated with sewage sediments and sludge, it is likely to be mobile in these media and to re-enter the aqueous compartment. In respect of this point, the Continuous Activated Sludge study (Potoms, 1996) indicated that up to 27 % of the material may become associated with sewage sludge during the sewage treatment process. In any case, whether the compounds are in either an aqueous or soil environment, they are likely to be effectively degraded through biodegradation (see below), and are unlikely to persist in either compartment.

It is likely that the majority of released compound would remain in the aqueous compartment and, as mentioned above (see section on physico-chemical properties), is expected to be stable to hydrolytic degradation. Consequently, the major routes for degradation and elimination of the compound in the environment are expected to be primarily biological (bacteriological) processes.

The notifier has indicated that the new compound is biodegradable, and in the notification provided a summary table of results for a large number of studies. Copies of a selection of the original reports were also provided in the submission, and the nature of, and results of these tests are summarised in the following table and discussion.

Biodegradation

Test material	***Test Method***	***Results** – See notes below.*
Aerobic degradation		
C10-16 Glucose amide	CO_2 Evolution Test (Modified Sturm Test) OECD 301 B	98 % DOC removed
C12 Glucose amide	Continuous Activated	99.9 % of parent removed;

	Sludge Test OECD 303 A	62.4 % of ^{14}C removed as either CO_2 or into waste sludge.
Anaerobic degradation		
C12 Glucose amide	Anaerobic Biodegradation ECOTOC protocol (European Centre for Ecotoxicology of Chemicals, 1988)	21.4-70.8 % biodegradation after 90 days.
C12 Glucose amide	Anaerobic Sludge Adaptation	Acclimatisation of anaerobic digester sludge to inputs of test material
C12 Glucose amide	Anaerobic Sludge Die-away Test	$T_{1/2}$ = 1.9 hours for primary degradation

Aerobic Conditions

The CO_2 evolution test (Neven, 1991b) was performed in nutrient media inoculated with sewage microorganisms obtained from a sewage treatment plant. The level of dissolved organic carbon (DOC), together with the amount of carbon dioxide evolution was monitored over a 34 day test period. The test vessels were maintained at room temperature, ie at temperatures between 20 and 25ºC. One test was conducted with test material added to give 10 mg/L of added organic carbon, and the second with 20 mg/L. A reference test was also run containing diethylene glycol, together with a blank containing no added test or reference material.

The degree of biodegradation for the media originally containing the notified chemical at a level of 10 mg/L was 98 % after 34 days as estimated through analysis for DOC, while that for the 20 mg/L test was 99 %. The reference material had been degraded 83 % under the same conditions. The degree of degradation as estimated from the evolution of CO_2 was 86 % for the 10 mg/L test, 89 % for the 20 mg/L test and 91 % for the diethylene glycol reference.

The results of these tests indicate that the notified chemical is biodegradable under aerobic conditions. However, the CO_2 evolution curves included in the test report indicated that 10 % degradation was achieved after approximately 4 days, but that after 14 days the degree of degradation was between 50 and 60 %. Accordingly, while the results indicate that the compound is inherently biodegradable, the material may not be classified as being readily biodegradable according to the protocols of OECD TG 301 B. It should also be noted that the reference compound diethylene glycol required around 12 days “lag” time prior to the onset of appreciable degradation and did not satisfy the criteria for ready biodegradability. While 10 % degradation was achieved after 12 days, the 60 % point was not reached until incubation had continued for 26 days.

In connection with this test, a separate study (Neven, 1991a) (see section on Environmental Effects below) indicated that the new compound is moderately toxic to sewage bacteria, and inhibits bacterial respiration at exposure concentrations > 10 mg/L.

The Continuous Activated Sludge study (Potoms, 1996) was conducted in order to simulate degradation of the compound in the aerated chambers of a sewage treatment plant, and to

identify and quantify the intermediate products of degradation. The test was conducted in accordance with the protocols of OECD TG 303 A, whereby ^{14}C labelled test compound is introduced with either raw or synthetic sewage at a constant rate into an aerated vessel containing activated sludge. For the present test the hydraulic retention time of liquor in the aerated reactor was around 10 hours, the sludge retention time around 10 days, and the temperature was always between 20 and 25°C. The evolved CO_2 was collected and assayed for ^{14}C content, as was the waste activated sludge itself and the treated effluent. A mass balance developed on the basis of the ^{14}C activity in the gas, effluent and sludge components was able to indicate the overall fate of the test material. In addition, radio thin layer chromatography (Rad-TLC) analysis of the reactor effluent may be used to identify some of the intermediates products resulting from the degradation process.

In the present case the test was run over a 29 day period with influent concentration of the test compound of 325 µg/L (average), and indicated that on average 35.5 % of the labelled carbon content of the notified chemical was mineralised in the activated sludge unit to carbon dioxide, while 26.9 % became assimilated (in some form) with the waste sludge and the remainder (around 33 %) was discharged as soluble degradation compounds in the liquid effluent.

The Rad-TLC analysis of the liquid effluent indicated that the major soluble degradation products present were N-succinoyl-N-methyl glucamide ($HOOC(CH_2)_2CONHCH_3$) and N-adipoyl-N-methyl glucamide ($HOOC(CH_2)_4CONHCH_3$). Such compounds could be expected during the progressive biodegradation of C12 glucose amide. This analysis also indicated that only around 0.1 % of the original C12 glucose amide remained in the effluent stream. This indicated that overall 99.9 % of the original material had been destroyed during the treatment process.

Anaerobic Conditions

Aqueous Anaerobic Biodegradation
A series of tests for anaerobic degradation (Wierinck, 1994a) were run on the compound according to ECETOC protocols (ECETOC, 1988). In these trials the test substance, together with mineral supplements, was introduced into a sealed vessel containing inoculum derived from working anaerobic sludge digesters at sewage treatment plants, and left to ferment. For each trial a parallel control test containing the inoculum, but no test material was run. The amount of evolved methane and carbon dioxide was monitored over a 90 day test period using manometric techniques. Seven individual tests were performed using inocula derived from five different domestic sewage treatment plants, and two inocula derived from industrial waste treatment plants. The two industrial inocula were provided from a plant acclimatised to treating starch waste, and another acclimatised to treatment of protein rich waste water.

The results of the individual tests were not uniform, and the degree of biodegradation for the inocula derived from the five domestic sewage plants varied between 21.4 and 70.8 % after the 90 day test period. However, it is significant that for both inocula acclimatised to treating either starch rich or protein rich effluent, there was no biodegradation. In fact, the test substance had an inhibitory effect on the bacterial metabolism in both cases, with less gas production than the respective controls.

It may be concluded that while the alkyl glucose amide may be susceptible to aqueous phase anaerobic degradation, the degree to which this occurs depends on the nature of, and previous

environments of the anaerobic bacteria. It is likely that for effective anaerobic degradation, the bacterial cultures require some time to adapt to the glucose amide substrates. For bacterial cultures which have become highly specialised, and acclimatised to operate on particular and well defined types of waste streams (eg starch or protein rich), the notified chemical appears to be toxic.

Aqueous Anaerobic Sludge Adaptation Test
A separate study aimed at producing samples of digester inoculum which had been adjusted to cope with the notified chemical was conducted (Wierinck, 1994b). Samples of sludge adjusted in this manner were used in subsequent ^{14}C die-away test, discussed below.

Essentially, this procedure consisted of progressively feeding the test compound into a digester which had been previously inoculated with sludge from a working anaerobic digester. The test compound was continuously fed into the test vessel with artificial sewage over a 20 day period until it was present at a (nominal) final level of 50 mg/L of organic carbon. Following this, the digester was operated for a further 100 days under a similar regime except that mixed liquor (ie sludge and supernatant) was withdrawn daily at a rate to simulate a sludge retention time of 20 days. A control experiment was run in an identical manner, except that no test compound was added to the sewage influent. The evolved bio-gases (primarily methane and carbon dioxide) were collected continuously, and fluctuations in the generation of evolved gases were monitored in order to ascertain the effects of the test compound on the biological activity of the bacteria.

The results of this test indicated that after about two months, the sewage digester sludge had adapted to the presence of the new compound, and the digestion was proceeding well. In fact, it was observed that after around 60 days operation, the digester into which the new compound was being introduced (at a rate equivalent to 0.5 grams of chemical oxygen demand per litre per day) was producing slightly more bio-gas than the control reactor.

It may therefore be concluded that anaerobic digester sludge may be acclimatised to accommodate the new compound, which is degraded in this media. Further, in this particular experiment no toxic effects resulting from introduction of the chemical were observed. In respect of this, the toxic effects noted above in digesters inoculated with sludge previously acclimatised to treating waste streams containing high starch or protein contents waste were possibly due to the "over specialised " nature of these bacterial populations.

^{14}C Die-away Test
This test (Nuck & Federle, 1994) was conducted in order to determine the rate of biodegradation and to establish the mechanism for anaerobic degradation. The test compound was ^{14}C labelled C12 glucose amide, and this was introduced into a digestion vessel inoculated with sludge which had previously been adjusted to the chemical in the manner indicated in the discussion above. The test was run in parallel with an abiotic control (ie an identical test set up without digester inoculum), and the rate of mineralisation (ie conversion to methane and carbon dioxide) determined by monitoring the difference in radioactivity level between the test digester and the control. The digester was maintained at 35°C, and samples taken from the digester were periodically analysed for parent compound and metabolites using Rad-TLC.

After 5 hours incubation at 35°C the Rad-TLC results indicated that around 90 % of the parent C12 glucosamine had disappeared from the test vessel, while almost all remained in

the abiotic control vessel. The half life for disappearance of the parent compound was 1.9 hours (indicating relatively fast degradation), and the Rad-TLC identified N-methyl glucamine ($C_6H_{13}O_5NHCH_3$) as the primary metabolite. This compound was subsequently mineralised to CO_2 and CH_4, but at a much slower rate than the primary degradation. Mineralisation of this metabolite was significantly slower, with a half life of 13.3 hours.

The results of this test support those of the previously discussed work, and indicate the new compound is susceptible to biodegradation under anaerobic conditions.

However, it should be noted that the chemical nature of the metabolites found in this study (eg N-methyl glucamine) are quite different from those produced during the study on aerobic degradation (eg N-succinoyl-N-methyl glucamide – see discussion above). This reflects the large differences in the catabolic pathways between anaerobic and aerobic bacteria. Both types of metabolite are relatively water soluble, and should not partition significantly to sediments.

Bioaccumulation

Lyman et al (1982) give a number of QSARs from which the Bioaccumulation Factor (BCF) may be estimated from values of Log P_{ow}. Their relation 5-2, which is –

Log BCF = 0.76 X Log P_{ow} – 0.23,

together with the determined value for Log P_{ow} of 2.2 provides an estimate of for the bioaccumulation factor of around 28. This is a very low value indicating little potential for bioaccumulation. The modest value for Log P_{ow} and reasonably high water solubility are in accord with this conclusion.

9. EVALUATION OF TOXICOLOGICAL DATA

9.1 Absorption, Distribution and Elimination

Reports on absorption, distribution and elimination tests on analogues of the notified chemical (C_{12} glucose amide, C_{18} glucose amide) in rats when applied by the oral and dermal routes were provided by the notifier.

9.1.1 Oral Application (Powers et al., 1991b)

Test Material:	C_{12} Glucose amide, C_{18} Glucose amide, ^{14}C labelled
Species/strain:	rat/Sprague-Dawley
Number/sex of animals:	12 males per compound
Observation period:	8 hours; sacrifices at 2 hour intervals
Method of administration:	gavage, dose levels 1736 mg/kg (C_{12}) and 3830 mg/kg (C_{18})

Test method:	as per predetermined protocol
Gross Pathology:	the stomachs were distended with food, a white precipitate and gas for the duration of the study at 2 hours post dose, the entire small intestine was filled with a pale yellow fluid with a small amount of white material in the upper quarter; at 4 hours the white material was present along the full length of the small intestine and had entered the caecum in the C_{18} dosed animals; by the 8 hour observation the small intestines appeared normal and white material was present in the caeca; no formed faeces in the large intestine appeared to contain white material
Radioanalytical Results:	absorption of radioactivity increased through the experiment with the highest tissue radioactivity levels being found at 8 hour after dosing all tissues sampled with the exception of the testes showed parallel increases in radioactivity, with consistently higher levels than whole blood; the liver showed the highest level, followed by kidney, plasma and bone marrow; the bone marrow had a 2 fold increase over whole blood for C_{12}, and a 3.5 fold increase for C_{18}; the testes showed consistently lower levels than whole blood no radioactivity balance was performed in this study
Result:	the analogue chemicals were absorbed from the digestive system and these chemicals or metabolites were widely distributed throughout the tissues after 8 hours

9.1.2 Dermal Application (Powers et al., 1991a)

Test Material:	C_{12} Glucose amide, ^{14}C labelled
Species/strain:	rat/Sprague-Dawley
Number/sex of animals:	4/male
Observation period:	72 hours
Method of administration:	semi-occluded dose cell applied for 72 hours, test material dissolved in absolute ethanol; dose level 9.9 mg/kg; skin area 7.63 cm^2
Test method:	as per predetermined protocol
Comment:	the dose cell for one animal became unattached by 72 hours; based on the differences in radioactive distribution for this

	animal, it was concluded that this animal had ingested test material; the radiochemical data for another animal indicated that seepage from the dose cell and subsequent ingestion had occurred; the results were therefore based on the remaining two animals
Radioanalytical Results:	a radioactive material balance of 95 % (± 5 %) was found
	at the end of 72 hours, 94.4 % of the dosed radioactivity was found in the dose cell and skin wash, 0.27 % was found in the urine and cage wash, 0.19 % was found in the examined tissues and the carcass, 0.1 % was found in the faeces and gastrointestinal tract wash and 0.02 % was found in the expired carbon dioxide
	very low levels of radioactivity were found in all tissues examined at 72 hours; the highest level was in the femur, followed by the carcass, whole blood, adipose tissue and bone marrow
Result:	the analogue chemical was absorbed through the skin to the extent of 0.5 % of the applied dose during 72 hours; the principal route of elimination was through urine

9.2 Acute Toxicity

The acute toxicity test reports provided by the notifier related to the substances E4194.01 and E4086.01 (for the oral toxicity and skin sensitisation studies); both were described as C_{10-16} glucose amide. E4194.01 has been identified as the notified chemical. For the acute oral toxicity study and the skin sensitisation study, a formulation containing 44.7 % active ingredient was used; for the other studies a formulation containing 98.8 % active ingredient was used. The genotoxicity studies recorded here were carried out using the test article SS0001.01, which is elsewhere identified as being the same as E4086.01 (C_{10-16} Glucose Amide, 45 % pure).

Summary of the acute toxicity of C_{10-16} glucose amide

Test	*Species*	*Outcome*	*Reference*
acute oral toxicity	rat	LD_{50} > 2000 mg/kg	(Decker et al., 1991a)
acute dermal toxicity	rabbit	LD_{50} > 2000 mg/kg	(Baldrick & McRae, 1991)
skin irritation	rabbit	slight irritant	(Decker et al., 1991b)
eye irritation	rabbit	severe irritant	(Liggett & McRae, 1991)
skin sensitisation	guinea pig	not sensitising	(Ullmann et al., 1991)

9.2.1 Oral Toxicity (Decker et al., 1991a)

Test Material:	E4086.01
Species/strain:	rat/HanIbm: WIST (SPF)
Number/sex of animals:	5/sex/dose
Observation period:	15 days
Method of administration:	gavage, 44.7 % active ingredient, dose levels 2000 mg/kg (900 mg/kg active ingredient) and 4444 mg/kg (2000 mg/kg active ingredient)
Test method:	OECD TG 401
Mortality:	no deaths occurred during the study
Clinical observations:	three males in the 900 mg/kg active ingredient group appeared slightly sedated on day 1; one male in the 900 mg/kg active ingredient group and one animal of each sex in the 2000 mg/kg active ingredient group had ruffled fur within the first 2 days body weight loss was observed for one female of the 2000 mg/kg active ingredient group between days 8 and 15; during the same period retarded body weight gain was observed for two females of this group and one female of the 900 mg/kg active ingredient group
Morphological findings:	no gross abnormalities were observed on day 15
LD_{50}:	> 2000 mg/kg active ingredient
Result:	E4086.01 was of very low acute oral toxicity in rats

9.2.2 Dermal Toxicity (Baldrick & McRae, 1991)

Test Material:	E4194.01 (notified chemical, 98.8 % pure)
Species/strain:	rabbit/New Zealand white
Number/sex of animals:	5/sex
Observation period:	15 days
Method of administration:	semi-occlusive patch; dose level 2000 mg/kg; test material moistened with water; 24 hour exposure

Test method:	According to EC Directive 84/449/EEC
Mortality:	no deaths occurred during the study
Clinical observations:	reduced appetite and thin looking appearance for 4 males, diarrhoea and lethargy for 2 male, nasal exudate for 2 males and 1 female, ocular exudate for 1 male and dark urine for one male; all were considered to be common findings for this strain of rabbit and therefore not treatment related minor bodyweight losses were seen for 3 males during week 1 and 1 male and 1 female during week 2; other bodyweight gains were generally low
Morphological findings:	pale kidneys and congested lungs for 1 male, congestion at the tips of the papillae for both kidneys for 1 female; focal scabbing or scabs at the treatment site for 2 males and 2 females
Dermal responses:	well defined erythema and well defined oedema (slight in one case) were observed following removal of dressings; necrotic foci at the dose site for 3 animals the reactions were maintained, often accompanied by hyperkeratinisation, throughout the study, or developed to necrosis with well defined oedema (6 animals); necrotic reactions were still present in 3 animals at study termination

Draize scores (Draize, 1959):

Time after treatment (days)	***Animal #***									
	1	***2***	***3***	***4***	***5***	***6***	***7***	***8***	***9***	***10***
Erythema										
2	2A*	2	2	2A	2	2A	2	2	2	2
3	B	2	2	B	2C	2A	2C	2	2C	2C
4	B	2	2	B	2C	2A	2C	2	2C	2C
5	B	2C	2C	B	2C	2AC	2C	2C	2C	2C
6	B	2C	2C	B	B	2AC	B	2C	2C	2C
7	B	2C	2C	B	B	2AC	B	2C	2C	2C
8	B	B	2C	B	B	2AC	B	2C	2C	B
9	B	B	2C	B	B	2AC	B	2C	1C	B
10	B	B	1C	B	B	2AC	B	1C	1C	B
11	B	B	1C	B	B	2AC	B	1C	1C	B
12	B	B	1C	B	B	2AC	B	1C	1C	B
13	B	B	1C	B	B	2C	B	1C	0C	B
14	B	2D	0	2D	B	1	B	0	0D	2D
15	B	1	0	1	B	1	B	0	0D	1
Oedema										
2	2	2	2	1	2	2	2	2	2	2
3	2	2	2	2	2	2	2	2	2	2
4	2	2	2	2	2	2	2	2	2	2
5	2	2	2	2	2	2	2	2	2	2
6	2	2	2	2	2	2	2	2	2	2
7	2	2	2	2	2	2	2	2	1	2
8	2	2	2	2	2	2	2	2	1	2
9	2	2	1	2	2	2	2	2	1	2
10	2	1	1	2	2	2	2	1	0	2
11	1	1	1	2	2	1	2	0	0	2
12	1	1	1	2	2	1	2	0	0	2
13	1	1	1	1	2	1	2	0	0	2
14	1	1	0	1	1	1	1	0	0	1
15	1	0	0	0	1	0	1	0	0	0

* see Attachment 1 for Draize scales

A necrotic foci
B necrosis; unable to assess for erythema
C hyperkeratinisation
D desquamation

LD_{50}: > 2000 mg/kg

Result: the notified chemical was of low dermal toxicity in rats

9.2.3 Inhalation Toxicity

No inhalation toxicity data were presented by the notifier. The notified chemical has a low vapour pressure (1.4 Pa at 25°C) and will only be imported in solution at a concentration of 1.43 %. It is therefore unlikely to present an inhalation hazard either as a vapour or as an aerosol.

9.2.4 Skin Irritation (Decker et al., 1991b)

Test Material: E4194.01 (notified chemical, 98.8 % pure)

Species/strain: rabbit/ChbbIbm: NZW (SPF)

Number/sex of animals: 2 male, 1 female

Observation period: 14 days

Method of administration: semi-occlusive patch, dose 0.5 g test material moistened with bi-distilled water; 4 hour exposure

Test method: OECD TG 404

Draize scores (Draize, 1959):

Time after	***Animal #***		
treatment (days)	***1***h	***2***h	***3***d
Erythema			
1	1[a]	1	1
2	1	1	1
3	1	1	1
7	1	0	1
14	0	0	0
Oedema			
1	1	0	0
2	1	0	1
3	1	0	1
7	0	0	1
14	0	0	0

[a] see Attachment 1 for Draize scales

Comment: very slight erythema and oedema persisted to day 7 and was reversible by day 14

Result: E4194.01 was a slight irritant to the skin of rabbits

9.2.5 Eye Irritation (Liggett & McRae, 1991)

Test Material: E4194.01 (notified chemical, 98.8 % pure)

Species/strain: rabbit/New Zealand White

Number/sex of animals: 3 male

Observation period: 35 days

Method of administration: 6.3 mg (for animals 1 and 2) and 4.6 mg (for animal 3) test substance was applied directly onto the corneal surface of one eye; the untreated eye was used as a control

Test method: Proctor and Gamble Protocol No. C2B-E (similar to OECD TG 405 except the quantity instilled in the eye was < 10 mg, rather than 100 mg as recommended in the Guideline)

Draize scores (Draize, 1959) of unirrigated eyes:

	Time after instillation									
Animal	***1 day***		***2 days***		***3 days***		***4 days***		***7 days***	
Cornea	***o***	***a***	***o***	***a***	***o***	***a***	***o***	***a***	***o***	***a***
1	1[1]	4	1	4	2	3	2	4	2	2
2	1	4	2	4	1	4	1	4	1	2
3	0	0	0	0	0	0	0	0	0	0
Iris										
1	0		0		1		0		0	
2	1		1		1		1		0	
3	0		0		0		0		0	

Conjunctiva	***r***	***c***	***d***	***r***	***c***	***d***	***r***	***c***	***d***	***r***	***c***	***d***	***r***	***c***	***d***
1	2	1	2	2	2	2	2	2	1	2	2	2	2	1	0
2	2	2	2	2	2	2	2	2	2	2	2	2	1	0	1
3	1	1	1	1	1	0	1	1	0	1	0	0	0	0	0

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

Mean Scores (24, 48, 72 hours):

corneal opacity	1.3, 1.3, 0
iris lesions	0.3, 1, 0
conjunctival redness	2, 2, 1
chemosis	1.7, 2, 1

	mean scores are for each animal
Comment:	in animal 3, all effects cleared by 7 days; in animal 1 all effects cleared by 14 days; in animal 2, vascularisation of the cornea (opacity grade 2) was observed at 14 days, and corneal opacity persisted to day 35; conjunctival effects cleared by day 35
Result:	E4194.01 was severely irritating to the eyes of rabbits based on the persistence of the effects

9.2.6 Skin Sensitisation (Ullmann et al., 1991)

Test Material:	E4086.01
Species/strain:	guinea pig/Ibm: GOHI (SPF)
Number of animals:	10/sex test group 5/sex control group
Induction procedure:	test animals – test material (0.3 mL, 20 % active ingredient w/v in bi-distilled water) was applied in an occluded chamber to a clipped area of the left shoulder for 6 hours; the residue was removed with lukewarm water the induction procedure was carried out three times at intervals of 1 week
Challenge procedure:	test and control animals – 12 days after the last induction exposure, test material (0.3 mL, 10 % active ingredient w/v in bi-distilled water) was applied in an occluded chamber to a clipped area of skin which had not previously been exposed to the notified chemical dermal responses were evaluated after 24 and 48 hours
Test method:	OECD TG 406
Comment:	no skin reactions were observed in either test or control animals the strain was shown to be sensitive to allergic reactions in a separate test using formaldehyde solution
Result:	E4086.01 was not sensitising to the skin of guinea pigs

9.3 Repeated Dose Toxicity

9.3.1 28 Day Oral Study (Schulze, 1991)

Test Material:	SS0001.01 (C_{10-16} Glucose Amide, 45 % pure)
Species/strain:	rat/Crl: CDF F344
Number/sex of animals:	10/sex/group
Method of administration:	gavage; dose volume 10 mL/kg/day test material in distilled water, divided into two doses administered 4 hours apart
Dose/Study duration:	0, 10, 100, 500, 1000 mg/kg/day active ingredient for 28 consecutive days
Test method:	OECD TG 407
Mortality:	5 males and 7 females receiving 1000 mg/kg/day died or were sacrificed in extremis due to treatment related causes during the first two weeks of the study; 5 animals across all treated groups died during blood collection on days 27 and 28 - these deaths were not considered treatment related

Clinical observations:

In the 500 mg/kg/day animals, wheezing, salivation, urine stains and bloody crust on the nose were observed; in the 1000 mg/kg/day animals, dyspnea and languid appearance were noted in addition to the above clinical signs. No treatment related clinical signs were seen in animals of the lower dose groups.

Reduced food consumption and corresponding decreases in body weight gain were seen in the animals of the 500 mg/kg/day and 1000 mg/kg/day groups.

Opthalmic examination revealed no treatment related findings.

Clinical chemistry/Haematology

A wide range of clinical chemistry parameters was significantly changed in the 500 mg/kg/day and 1000 mg/kg/day groups compared with the controls. These changes were considered to be generally secondary to the poor nutrition of these animals. A statistically significant decrease in triglycerides in 100 mg/kg/day males was not considered biologically relevant due to the small magnitude of the change and the lack of consistency between the sexes at this dose.

Mild anaemia was observed in the 1000 mg/kg/day animals. Incidental changes in white blood cell counts were considered consistent with an inflammatory response.

Urinalysis parameters were generally unchanged from controls, except for the observation of lower pH in the 1000 mg/kg/day males. This was considered consistent with the nutritional deficiencies which have been previously noted.

Gross Pathology:

Thickened or roughened mucosa of the non-glandular region of the stomach was observed in the 500 and 1000 mg/kg/day. Thickened or dark mucosa of the glandular region of the stomach was also observed for the 1000 mg/kg/day group. The stomach weight was observed to be increased relative to the body and brain, and the thymus weight decreased relative to the body and brain in the 500 and 1000 mg/kg/day groups.

Histopathology:

A number of histopathological changes in the stomach were observed in the 500 and 1000 mg/kg/day groups. These included ballooning degeneration of the surface epithelium, acanthosis, parakeretosis, erosion, ulceration, focal haemmorhage and inflammation of the non-glandular region and an increased number of goblet cells and/or mucous on the mucosal surface in the glandular region. These changes are suggestive of test substance related irritation to the non-glandular region of the stomach.

Necrosis and/or lymphoid depletion of the thymus were observed in the 1000 mg/kg/day animals and to a lesser effect in the 500 mg/kg/day animals; the study authors considered these changes to be stress related.

Comment:

Administration of the test substance at 500 and 1000 mg/kg/day resulted in increased mortality (at 1000 mg/kg/day), decreased bodyweight and food consumption, altered clinical pathology values consistent with inflammation and nutritional deficits, and histological changes to the stomach.

Alterations to clinical chemistry parameters were not associated with microscopic changes in specific organs and tissues.

Result:

Based on the findings at 500 and 1000 mg/kg/day, a No Observed Effect Level (NOEL) of 100 mg/kg/day was established in this study.

9.3.2 13 Week Oral Study (Decker & Hoff, 1991)

Test Material:	SS0001.01 (C_{10-16} Glucose Amide, 45 % pure)
Species/strain:	rat/HanIbm: WIST (SPF)
Number/sex of animals:	10/sex/dose, with an additional 10/sex/dose allowed to recover for 28 days following the study
Method of administration:	gavage; dose volume 10 mL/kg/day test material in distilled water

Dose/Study duration:	0, 10, 50, 200, 500 mg/kg/day active ingredient for 91 consecutive days
Test method:	OECD TG 408
Mortality:	4 males and 2 females receiving 500 mg/kg/day died or were sacrificed in extremis due to treatment related causes between days 27 and 91 of the study; dosing errors led to deaths of 4 other animals across several treated groups (1 male and 1 female at 500 mg/kg/day, 1 male at 200 mg/kg/day and 1 male at 10 mg/kg/day)

Clinical observations:

There was a dose related increase in incidence and severity of respiratory problems, primarily noisy breathing, in the 50 (males only), 200 and 500 mg/kg/day groups; dyspnea and laboured respiration were observed in some 500 mg/kg/day animals. Noisy breathing persisted in three out of ten recovery group females at the end of 28 treatment free days.

Slight to moderate sedation and emaciation was found in some animals and ruffled fur was found in all animals treated with 500 mg/kg/day.

Food consumption was significantly reduced in the 500 mg/kg/day animals, during weeks 1 and 2, and 5 to 10 in males, and during weeks 1 and 2, 7 and 8 and 12 and 13 in females. Reductions in body weight gain were noted in the males of the two highest dose groups, during weeks 9, 12 and 13 for the 200 mg/kg/day group and during weeks 2 to 14 in the 500 mg/kg/day group. The body weight for the latter group returned to normal following one treatment free week.

Opthalmic examination revealed no treatment related findings.

Clinical chemistry/Haematology

Among the animals treated with 500 mg/kg/day, there were a number of significant clinical chemistry findings. For both sexes there was a decrease in chloride concentration. For the males a slight increase in alanine aminotransferase and alkaline phosphatase was observed, and for the females a slight increase in uric acid and triglyceride concentration was observed.

A slight decrease in calcium concentration was seen for the males of all treated groups, and a slight increase in total protein and globulin concentration and a decrease in albumin to globulin (A/G) ratio was seen for the females treated with 50 mg/kg/day and above.

All findings with the exception of the chloride concentration for both sexes at 500 mg/kg/day and the A/G ratio for the 500 mg/kg/day females were reversed after 28 treatment free days. The study authors concluded that the findings are likely to reflect metabolic adaptation due to an increased functional load on the liver.

A number of haematology parameters were significantly changed for the animals treated at

200 and 500 mg/kg/day. These included a slight increase in erythrocyte count for both sexes at 500 mg/kg/day, slightly increased haemoglobin concentration for the 500 mg/kg/day males, slightly increased methaemoglobin concentration for the 500 mg/kg/day females, slightly increased haemocrit for males at 200 mg/kg/day and both sexes at 500 mg/kg/day and slightly decreased mean corpuscular haemoglobin concentration for females at 200 mg/kg/day and both sexes at 500 mg/kg/day.

The study authors concluded that the changes reflect slight haemoconcentration and suggest changes in basal fluidity, and do not consider them to be of toxicological significance. The changes were found to be reversible after 28 treatment free days.

The only change in urinalysis parameters which was reported was a slight increase in overnight urinary output for both sexes at 500 mg/kg/day during week 13. This was considered to be due to increased fluid intake.

Gross Pathology:

No treatment related abnormalities were observed at necropsy.

The liver weights for females at 500 mg/kg/day, as well as the liver weight relative to body weight for females and males at 500 mg/kg/day, were significantly increased at the end of the treatment period; no significant increase was seen after the recovery period.

Histopathology:

Four premature decedents showed lung changes indicative of an accident in dosing; no histopathological indication of the cause of death was noted for the animals which died of treatment related causes.

Inflammatory changes were observed in the nasal cavity (exudate) and lungs of some animals in the 200 mg/kg/day and 500 mg/kg/day groups. These changes were considered by the study authors to be related to the general poor condition of the animals and consistent with reflux of irritating material.

Thymic atrophy (cortical) and focal haemmorhage was noted in some animals at 500 mg/kg/day and was considered by the study authors to be due to the poor condition of the animals. Thymic changes were also seen in the 28 day study in animals receiving 500 and 1000 mg/kg/day.

Stomach changes observed in the 28 day study were not seen in the 13 week study.

Comment:

Administration of the test substance at 500 mg/kg/day resulted in increased mortality and biochemical and histopathological changes which were considered due to the poor general condition of the animals. No microscopic indication of the mechanism of toxicity was observed.

On the basis of mortality and morbidity (the only observed indicators of toxicity) at 500 mg/kg/day, the No Observed Adverse Effect Level (NOAEL) is determined at 200 mg/kg/day. Based on clinical signs and the effects on body weight gain, a NOEL of 50 mg/kg/day was established.

Result:

A NOAEL of 200 mg/kg/day and a NOEL of 50 mg/kg/day were established in this study.

9.4 Developmental Toxicity (Wirth, 1991)

Test Material:	SS0001.01
Species/Strain:	rat/Crl:CD VAF/Plus
Number/sex of animals	100 females, mated
Method of administration:	gavage, vehicle deionised water, dose level 10 mL/kg
Dose:	test material administered as a single dose on days 6 through 15 of gestation at dose levels of 0, 15, 150, 363 mg/kg/day
Clinical Observations	increased salivation was observed in all animals in the 363 mg/kg/day group and, at low incidence, in the 150 mg/kg/day group; decreased activity was observed in two animals in the 363 mg/kg/day group and material around the mouth was observed in 4 animals of this group and one animal in the 150 mg/kg/day group a significant decrease in bodyweight gain was observed in the 363 mg/kg/day group during the overall gestation time (days 0 to 20), particularly during the early treatment period (days 6 to 9)
Gross Pathology:	no significant treatment related effects were observed at necropsy
Caesarian Section Observations:	no treatment related differences were observed in the caesarian section parameters
Foetal Morphological Observations:	no statistically significant treatment related differences were observed between the incidence of foetal malformations between the treated groups and the control groups

	developmental variations were generally comparable between treated and control groups although a slight increase in unossified sternebrae #5 and #6 was observed in the 150 mg/kg/day and 363 mg/kg/day groups; no dose related trend was observed and the variations were assigned to normal biological variability
Test Method:	OECD TG 414
Comment:	oral administration of the notified chemical as an aqueous solution on days 6 through 15 of gestation at a dose level of 363 mg/kg/day produced maternal toxicity indicated by salivation, decreased activity and material around the mouth and significant depression in maternal body weight gain during the treatment period; increased salivation was also observed at low incidence at 150 mg/kg/day; no significant adverse affects on selected reproductive parameters were observed in the treated animals
Result:	the notified chemical does not appear to be a selective developmental toxicant at dose levels producing maternal toxicity; a NOEL for developmental toxicity of 363 mg/kg/day was established in this study; a NOAEL for maternal toxicity of 150 mg/kg/day was established on the basis of depression of bodyweight gain at the higher dose

9.5 Genotoxicity

9.5.1 *Salmonella typhimurium* and *Escherichia coli* Reverse Mutation Assay (San & Wagner, 1991a; San & Wagner, 1991b)

Two independent reports were generated on two separate batches of test article SS0001.01. The batches were identified as MA# T9570 and MA# TA010.

Strains:	*Salmonella typhimurium*: TA98, TA100, TA1535, TA1537, TA1538 *Escherichia coli*: WP2*uvr*A
Concentration range:	0, 0.5, 1.5, 4.5, 15, 45, 150, 450, 1500, 2250 μg/plate (active ingredient) concentrations were tested in triplicate, in the presence and absence of metabolic activation; each batch was tested in two repeat experiments appropriate strain specific positive control reference substances were used

Metabolic Activation System:	rat liver S9 fraction from animals pretreated with Aroclor 1254
Test method:	OECD TG 471 (plate incorporation method)
Comment:	MA# T9570: in the first experiment with concentrations of 45 μg/plate and above, toxicity as indicated by a moderate reduction in background lawn became apparent for all salmonella strains at 450 μg/plate with or without S9; repeat experiments on this sample using lower concentration ranges were performed MA# TA010: maximum concentrations of 450 μg/plate were used for the salmonella strains no substantial increase in the number of revertant colonies or indication of clear dose response was observed for either sample; due to a dosing error, only a single assay was evaluated the positive and vehicle controls responded as expected, indicating that the test system responded appropriately in any of the experiments
Result:	SS0001.01 was not considered mutagenic in the bacterial strains tested in the absence or presence of metabolic activation provided by rat liver S9 fraction

9.5.2 Mouse Lymphoma Forward Mutation Assay (Bigger & Clarke, 1991)

Test Material:	SS0001.01 (C_{10-16} Glucose Amide, 45 % pure)
Cells:	mouse lymphoma L5178Y
Doses:	initial experiment: 0, 2.3, 4.5, 14, 23, 29, 36 μg/mL, in the absence of S9 0, 2.3, 4.5, 14, 23, 29, 36, 43, 50 μg/mL, in the presence of S9 repeat experiment: 0, 2.3, 4.5, 9.0, 13, 18, 22, 27, 32 μg/mL, in the absence of S9 0, 13, 18, 22, 27, 32, 36, 38, 41, 43, 45 μg/mL, in the presence of S9
Metabolic Activation System:	rat liver S9 fraction from animals pretreated with Aroclor 1254 and Arochlor 1242 (2:1 mixture)
Treatment Regime:	cell culture was treated with test material in the presence or absence of metabolic activation for 4 hours; the cells were

	washed and resuspended in fresh medium; a fixed number of cells was then suspended in selection medium to selectively recover only TK-/- mutants; they were then seeded into dishes and colonies allowed to grow for 10 to 12 days
Test method:	OECD TG 476
Positive controls	ethyl methanesulphonate 0.5, 0.25 μL/mL (for cells treated without metabolic activation) 7,12-dimethylbenz(a)anthrene 2.5, 5.0 μg/mL (for cells treated with metabolic activation)
Comment:	in an initial range-finding test, 100 % toxicity was observed at and above 450 μg/mL the mutant frequencies observed at the TK locus were below the minimum criteria with and without metabolic activation and the compound was considered to be non-mutagenic the solvent and positive controls fulfilled the requirements for a valid test
Result:	SS0001.01 did not induce forward mutations in mouse lymphoma L5178Y cells *in vitro* with or without metabolic activation

9.5.3 Chromosomal Aberrations in Chinese Hamster Ovary (CHO) Cells *In Vitro* (Putman & Morris, 1991)

Test Material:	SS0001.01 (C_{10-16} Glucose Amide, 45 % pure)
Cells:	Chinese Hamster Ovary (CHO)
Doses:	two independent assays were performed using the following concentrations 1.7, 3.3, 6.5, 13, 25, 50, 100 μg/mL
Metabolic Activation System:	rat liver S9 fraction from animals pretreated with Aroclor 1254
Treatment Regime:	where metabolic activation was used, test material or positive controls were added to cell cultures in serum free medium for 6 hour incubation with S9 mix; the cells were then washed and incubated in fresh complete medium for an additional 18 hours incubation time; a similar procedure was carried out in the absence of S9; in the absence of metabolic activation, cells were also exposed continuously for 24 and 48 hours; colcemid was added two hours before harvest to

arrest cells in metaphase

Test method: OECD TG 473

Comment: no precipitation occurred for any of the test concentrations used; excessive cytotoxicity (growth inhibition), as indicated by < 25 % confluency, was observed at the highest dose used in all cases; concentrations of 13, 25 and 50 μg/mL were used for scoring except for the 48 hour exposure, where an insufficient yield of metaphase cells was found for 50 μg/mL, therefore the 6.5 μg/mL dose was used for scoring

in the initial assay, cytotoxicity (mitotic inhibition) was approximately 72 % and 38 % at the highest dose evaluated in the 24 and 48 hour continuous treatment studies: an increase in polyploid cells was seen at 50 μg/mL in the 6 hour non-activated treatment; no statistically significant increase in chromosome aberrations was observed with and without S9 in the 6 and 24 hour treatments; the percentage of cells with structural aberrations was significantly increased for the 25 and 50 μg/mL 48 hour treatments and a positive dose response trend was found

in the presence of S9, no significant increase in the percentage of cells with structural and numerical aberrations was observed

in the repeat assay, cytotoxicity (mitotic inhibition) was approximately 51 % and 80 % at the highest dose evaluated in the 24 and 48 hour continuous treatment studies: an increase in polyploid cells was seen for all doses in the 48 hour non-activated treatment; no statistically significant increase in chromosome aberrations was observed with and without S9 in any of the treatments and no dose response trend was observed

clear positive results were obtained with the positive controls in both assays indicating that the test system responded appropriately

Result: SS0001.01 was found to induce chromosome aberrations in the absence of metabolic activation under the conditions of the test; the test authors did not consider the result to be biologically significant because the increases in structural and numerical aberrations were within the historic control range, and the results for the highest 48 hour dose in the initial experiment varied widely between flasks, indicating excessive toxicity, and the results were not reproduced in a repeat experiment

	no statistically significant increase in chromosome aberrations was observed in the presence of metabolic activation

9.5.4 Rat Bone Marrow *In Vivo* Cytogenicity Study (Putman & Young, 1991)

Test Material:	SS0001.01 (C_{10-16} Glucose Amide, 45 % pure)
Species/strain:	rat/Sprague-Dawley
Number/sex of animals:	cell cycle kinetics test 3 male cytogenetic assay 10/dose; separate dose levels used for males and females
Method of administration:	gavage; single dose; dose volume 20 mL/kg test material in distilled water
Dose/Study duration:	0, 1800 mg/kg (males, cell cycle kinetics test) 0, 180, 600, 1800 mg/kg (males) 0, 210, 700, 2100 mg/kg (females) harvest times of 8 and 12 hours were used based on the results of the cell cycle kinetics test
Test method:	OECD TG
Positive controls	cyclophosphamide 20 mg/kg
Cell cycle kinetics:	average generation time for treated animals was 12.9 hours compared with 12.6 hours for control animals; one animal exhibited lethargy and diarrhoea, another exhibited breathing difficulties
Cytogenicity:	no significant change in mitotic index was observed; no significant increases in percentage of cells containing one or more aberrations or the mean aberrations per cell per animal were observed; no evidence of dose response was observed no clinical signs of toxicity were observed clear positive results were obtained with the positive control, indicating that the test system responded appropriately
Result:	SS0001.01 was negative in the acute cytogenetic assay using male and female rats

9.6 Assessment of Human Irritation and Sensitisation Data

The notifier provided summaries of a number of irritation and sensitisation studies on human subjects. The majority of the studies summarised were tests on formulations containing glucose amide components.

Test reports on three studies (two Three Application Patch Tests for determination of irritation potential and one Repeat Insult Patch Test for determination of sensitisation potential) using analogues of the notified chemical dissolved in water were provided by the notifier.

9.6.1 Three Application Patch Test, 0.25 % (w/v) $C_{12/14}$ glucose Amide (Smith, 1992)

Test Material: E4423.01 ($C_{12/14}$ GS base)

Number of panelists: 12 (11 at completion of study)

Observation period: 8 days

Method of administration: semi occluded patch, 0.5 mL 0.25 % (w/v) in distilled water applied for 24 hours on days 1, 4 and 6; a number of other chemicals were tested simultaneously

Test method: as per predetermined protocol

Panelist	***1***	***2***	***3***	***4***	***5***	***6***	***7***	***8***	***9***	***10***	***11***
Day											
4	0[a]	0	1	1	0.5	0	1	1	1	0.5	0.5
6	0.5	0.5	1	1	1	0.5	1	1	1	0.5	0.5
8	0.5	0.5	1	1	1	1	1	1	1	1.5	1

a the grading system used was

0 no apparent cutaneous involvement

1 faint but definite erythema, no eruptions or broken skin, or no erythema but definite dryness; may have epidermal fissuring

2 moderate erythema, may have a few papules or deep fissures, moderate to severe erythema in the cracks

3 severe erythema (beet redness), may have generalised papules or moderate to severe erythema with slight oedema (edges well defined by raising)

4 generalised vesicles or eschar formation or moderate to severe erythema or oedema extending beyond the area of the patch

additional half grade scores were used; a example for a score of 0.5 is given as "faint, barely perceptible erythema or slight dryness (glazed appearance)"

Result: the average irritation score was determined to be 0.76; the test substance was considered to be 'mild'

9.6.2 Three Application Patch Test, 0.01 %, 0.1 %, 1.0 % (w/v) C_{12} glucose Amide (Saylor, 1990)

Test Material:	B1516.01 (C_{12} glucose amide)
Number of panelists:	12
Observation period:	8 days
Method of administration:	semi occluded patch, 0.5 mL in distilled water applied for 24 hours on days 1, 4 and 6
Test method:	as per predetermined protocol

	Panelist	1	2	3	4	5	6	7	8	9	10	11	12
	Day												
	4	0[a]	0	0	0	0	0	0	0	0	0	0	0
0.01 %	6	0	0	0	0	0	0	0	0	0	0	0	0
	8	0	0	0	0		0	0	0	0	0	0	0
	4	0	1	0	0	2	0.5	0	0.5	0.5	0.5	0	0
0.1 %	6	0.5	1	0	0	1.5	1	0	0	1	0.5	0.5	0
	8	0.5	1	0	0	0.5	1	0.5	0.5	1	1	0.5	0.5
	4	0.5	1	0	0	2.5	0.5	0.5	0.5	0.5	0.5	0.5	1.5
1.0 %	6	0.5	1	1	0	2	1	0.5	0	1	0.5	0.5	2
	8	1	1.5	2.5	2	1	1.5	1.5	0.5	2	2.5	1	1.5

a scoring system as above

Result: the average irritation scores were determined to be 0.0 (0.01 %), 0.56 (0.1 %) and 1.07 (1.0 %); C_{12} glucose amide was found to be non-irritant at 0.01 %, mildly irritating at 0.1 % and slightly irritating at 1.0 %

9.6.3 Repeat Insult Patch Test, 0.05 % (w/v) C_{12} glucose Amide (Avila, 1990)

Test Material: B1516.01 (C_{12} glucose amide)

Number of panelists: 107

Observation period: induction, 24 days
challenge, 5 days

Method of administration: semi occluded patch, 0.5 mL 0.05 % (w/v) in distilled water, nine induction patches applied to one arm for 24 hours at 48 to 72 hour intervals over three weeks; challenge patches are applied to both the original and alternate arm for 24 hours 17 days after the end of induction; test substance dissolved in distilled water

Test method: in house test method

Induction and Challenge Outcome: during the induction phase a number of subjects exhibited responses of grade 1 and one subject a response of grade 2; during the challenge phase 5 subjects exhibited responses of grade 1; the response patterns were concluded to be indicative of primary irritation

Result:	it was concluded that the test substance did not cause contact hypersensitivity

9.6.4 Skin Effects of Formulations Containing Glucose Amides

A number of test protocols were used for testing the irritation potential of formulations containing glucose amides along with other detergent ingredients. These formulations were diluted in many cases, to concentrations generally in the range 0.05 to 1.0 %. In other cases, undiluted products containing up to 5.35 % glucose amide were tested. The results were consistent with the results of the single substance tests described above, with the maximum irritation score being 1.29, for a diluted formulation containing 0.06 % glucose amide. The undiluted products were reported to be mildly irritating to non-irritating.

A number of Repeat Insult Patch Tests in humans using formulations containing glucose amides were tabulated by the notifier. No evidence of contact sensitisation related to the glucose amides was observed.

A large number of additional human tests on formulations, designed to support claims of "mildness" for the products, were tabulated by the notifier. These were generally performed using concentrations ranging from normal use levels to ten times normal use levels. The evidence from the large number of studies is consistent with the irritation and sensitisation results reported above.

9.7 Overall Assessment of Toxicological Data

Absorption, distribution and elimination tests showed that close analogues to the notified chemical are readily absorbed from the digestive tract and distributed widely throughout tissues, but absorption through the skin is minimal. Elimination of the analogue used in the dermal study is principally through urine.

The acute oral toxicity of an analogue to the notified chemical in rats is very low (LD_{50} > 2000 mg/kg) and the acute dermal toxicity of the notified chemical in rabbits is low (LD_{50} > 2000 mg/kg). No test report for acute inhalation toxicity was provided.

The notified chemical is slightly irritating to rabbit skin, with Grade 1 erythema and oedema persisting for up to 7 days. It was found to be a severe irritant to rabbit eyes in pure form, with vascularisation of the cornea observed in one out of three animals. The irritation scores provided from this test cannot be directly assessed against the NOHSC *Approved Criteria for Classifying Hazardous Substances* (Approved Criteria) (NOHSC, 1999) as the quantity installed in the eyes was less than 10 mg, rather than the 100 mg normally used in the OECD test. The irritation scores were below the level leading to classification as an eye irritant, but installation of larger quantities may have resulted in higher scores. The irritation was found to be persistent, with effects seen in all animals at 4 days, and persisting up to 35 days in one animal. Accordingly, the notified chemical should be classified as hazardous, with the risk phrase R41 'Risk of serious damage to eyes'.

An analogue of the notified chemical was found not to be a skin sensitiser in a non adjuvant

type test.

A large number of skin irritation and sensitisation studies in humans have been performed using analogues to the notified chemical and also formulations containing the analogues. The glucose amides were found to be slight skin irritants under the conditions of the tests, but not to be skin sensitisers.

In a 28 day oral study in rats, a NOEL of 100 mg/kg/day was established. At doses of 500 and 1000 mg/kg/day, a number of histological changes to the stomach lining were observed. Reduced food consumption was also observed, along with a number of clinical chemistry changes which the study authors attributed to the poor nutritional status of these animals. Twelve of the twenty rats receiving 1000 mg/kg/day died or were sacrificed *in extremis* during the study. At the higher doses breathing difficulties were also observed.

In a 91 day oral study in rats, breathing problems were observed in animals treated with 50 mg/kg/day and above. Clinical chemistry and haematology parameters were changed in animals treated with 200 and 500 mg/kg/day. These changes were considered to be due to the poor general condition of the animals. Six out of twenty animals treated with 500 mg/kg/day died of treatment related causes during the study. No macroscopic or microscopic changes could be found during necropsy to explain the reasons for the deaths. The study authors concluded that the NOAEL was 200 mg/kg/day in this study, as all findings at this dose were slight and there were no changes in blood or urinalysis parameters indicative of toxicity, and on the basis of increased mortality and morbidity at 500 mg/kg/day. Based on clinical signs and the effects on body weight gain, a NOEL of 50 mg/kg/day was established.

In a developmental toxicity study, the notified chemical was found to not cause developmental toxicity at a dose where maternal toxicity was observed. The NOEL for developmental toxicity was determined to be 363 mg/kg/day (the highest dose tested) while the NOAEL for maternal toxicity was found to be 150 mg/kg/day based on decreased bodyweight gain at the higher dose.

An analogue of the notified chemical gave negative results in two *in vitro* mutagenicity tests (*Salmonella typhimurium* reverse mutation assay, and mouse lymphoma forward mutation assay) in the presence and absence of S9 metabolic activation. Positive results were observed in an *in vitro* study of chromosomal aberrations in CHO cells in the absence of metabolic activation, although the study authors concluded that the results were not biologically significant because of lack of reproducibility in repeat tests, and because the values were within historical control ranges. This conclusion is supported by the absence of genotoxicity in an *in vivo* study of cytogenicity in rat bone marrow cells was negative.

The notified chemical is classified as a hazardous substance according to the Approved Criteria. Severe eye effects were observed. While the scores do not meet the threshold for classification as an eye irritant, it is possible that greater irritation would have been observed in a standard test using larger quantities of the notified chemical. The persistence of the eye irritation leads to classification with the risk phrase R41. The safety phrase S25 ‘Avoid contact with eyes’ should be also be applied.

10. ASSESSMENT OF ENVIRONMENTAL EFFECTS

The notification statement included a summary table of results for a large number of ecotoxicity tests performed on the notified chemical and many close analogues. Copies of a selection of the original test reports were also supplied. These particular tests were carried out using test materials of the same composition as the notified compound – ie having a component composition similar to that indicated above in the section on Chemical Identity.

10.1 Aquatic Toxicity

Test	*Species*	**Results (nominal)*
Acute Toxicity to Fish of C12-C14 components [EC Guideline C1]	Zebra Fish *Brachydanio rerio*	LC_{50} (96 h) = 7.4 mg/L **NOEC (96 h) = 5.6 mg/L
Acute Toxicity to Fish C14 component only [US EPA Test Guideline]	Fathead Minnow *Pimephales promelas*	LC_{50} (96 h) = 2.9 mg/L NOEC (96 h) = 1.2 mg/L
Chronic Toxicity to Fish C12-C14 components [US EPA Test Guideline]	Fathead Minnow *Pimephales promelas*	***LOEC (35 day) = 10 mg/L NOEC (35 day) = 5 mg/L
Acute Immobilisation to Fresh water invertebrates C12-C14 components [EC TG C2]	*Daphnia magna*	EC_{50} (48 h) = 18 mg/L NOEC (48 h) = 10 mg/L
Acute Immobilisation to Fresh water invertebrates C14 component only [US EPA Test Guideline]	*Daphnia magna*	EC_{50} (48 h) = 5 mg/L (95 % confidence 3.3-9.2 mg/L) NOEC (48 h) = 3.3 mg/L
Chronic Toxicity to Fresh water invertebrates C12-C14 components [US EPA Test Guideline]	*Daphnia magna*	EC_{50} (21 day) = 6.8 mg/L LOEC (21 day) = 10 mg/L NOEC (21 day) = 5 mg/L
Inhibition of Algal growth C12-C14 components [OECD TG 201]	*Selenastrum capricornutum*	E_bC_{50} (72 h) = 14 mg/L E_rC_{50} (72 h) = 30 mg/L NOEC (72 h) = 5.6 mg/L
Inhibition of Algal growth C14 component only [US EPA Test Guideline]	*Selenastrum capricornutum*	E_bC_{50} (72 h) = 3.9 mg/L NOEC (72 h) = 2.9 mg/L
Bacterial respiration C12-C14 components [OECD TG 209]	Sewage bacteria.	Some inhibition of respiration – see notes below. NOEC ≈ 10 mg/L

* The results listed in this column are nominal test concentrations, but in several of the tests the solution concentrations were also measured and where appropriate, the results in terms of the measured concentrations are given in the discussion below.

** NOEC - no observable effect concentration

*** LOEC - lowest observable effect concentration

Fish

The test on Zebra fish, a fresh water species which typically grows in warm water, (Hooftman & van Drongelen-Sevenhuijsen, 1991a), was conducted using a semi static methodology (daily replacement of test media), with solutions of the notified chemical (ie a mixture containing 73.6 % of the C12 component, and 24.9 % of the C14 component) made up at nominal concentrations of 3.2, 5.6, 10, 18 and 32 mg/L together with a control containing no test substance. Each test was performed in duplicate, using 10 animals in each test vessel. Temperature was maintained at 25±1°C, and water hardness, pH and dissolved oxygen levels were around 210 mg/L (as $CaCO_3$), between 7.7 and 8.1 and always greater than 6.2 mg/L respectively.

No mortality or aberrant behaviour was observed for test concentrations below 5.6 mg/L over the full 96 hour test period. However, after 24 hours exposure to a nominal concentration of 10 mg/L, all fish (in both duplicate tests) had died, while no fish mortality was observed in either of the duplicate control vessels. The 96 hour LC_{50} value was estimated as 7.6 mg/L using a parametric analytical method proposed by Kooijman (1981). The steepness of the effect curve precluded probit analysis, but it appears that the 96 hour LC_{50} value would be between 5.6 and 10 mg/L. The results of this test indicate the test substance to be moderately toxic to this species of fish.

A second fish acute toxicity study against fathead minnow, a fresh water species, (Collins, 1992b), was also submitted. This study was conducted using a static methodology (no replacement of test media), with solutions of the test compound made up at nominal concentrations of 0 (control), 1.2, 2.0, 3.3, 5.5 and 9.2 mg/L. It should also be noted that this test was conducted with the C14 component alone, rather than the mixture of C12 and C14 components representative of the commercial product. The tests were conducted in duplicate using 5 fish in each test vessel, and the temperature was maintained between 21 and 22°C, water hardness was at 34 mg/L as $CaCO_3$, and the pH was between 6.8 and 7.3. The dissolved oxygen levels were maintained through aeration, and were typically between 6 and 8 mg/L.

No mortality or other effects were observed in the fish over the 96 hour test period for nominal concentration of 1.2 mg/L, but 10 % of the fish had died after 48 hours exposure to the 2.0 mg/L solution. All the fish exposed to the (nominally) 5.5 mg/L solution were dead after 24 hours. The data was analysed using probit analysis (Peltier & Weber, undated) to give the 96 hour LC_{50} value of 2.9 mg/L (95 % confidence interval 2.4-3.7 mg/L). Sublethal effects such as darkening of pigmentation and loss of equilibrium were also observed in fish exposed to 2 mg/L and greater which had not died. The results of this test indicate that the C14 component of the notified chemical (ie a mixture of the C12 and C14 components in the approximate proportions of 73.6 % to 24.9 %) is moderately toxic to this species of fish.

A chronic test performed using flow through methodology against embryos and larvae of Fathead minnow was also performed with the C12-C14 mixture of components (ie 74.6 %:24.9 %) (Machado, 1992). This test was performed over a 35 day period (30 days post hatch) using test solutions made up at nominal concentrations of 0 (control), 0.63, 1.3, 2.5, 5 and 10 mg/L. The solutions were analysed for test compound and the results were always within 20 % of the nominal concentrations, the mean measured concentrations being 0, 0.69, 1.5, 2.5, 4.8 and 10 mg/L respectively. Each test was conducted in its own flow through vessel suspended in a water bath maintained at 25±1°C, and the tests for each concentration (including the control) were conducted in duplicate. The rate of flow of the test media

through the vessels was such that there was 90 % replacement of media after 9 hours. During these tests the temperature was 25.5±0.5°C, water hardness was 26-42 mg/L (as $CaCO_3$), the pH between 6.8 and 7.7 and the dissolved oxygen levels between 5.8 and 8.9 mg/L.

No fish hatched after 5 days exposure to the (nominally) 10 mg/L solution, compared to better than 81 % survival for the control test. Survival at the end of the 5 day hatching period for the nominally 5 mg/L solution and the lower concentrations was between 78 and 89 %, and comparable to that of the control organisms (81 %).

Survival and general condition of the organisms after hatching was observed over a 30 day post hatch period. Those exposed to concentrations of the test substance at a nominal concentration of 5 mg/L (measured 4.8 ± 0.57 mg/L) and below had a survival rate of 93 to 95 %, not statistically different to the 98 % survival rate of the control organisms. The weight and length of the larvae after the 30 day post hatch period were also very similar at all test concentrations below the (nominal) 5 mg/L to those of the controls, and were within 95 % of the control values. The results of this test give a LOEC of 10 mg/L (measured and nominal) and a NOEC of 4.8 mg/L (nominally 5 mg/L).

Invertebrates
An acute toxicity test of the commercial C12-C14 component mix against *Daphnia magna* (Hooftman & van Drongelen-Sevenhuijsen, 1991b) was conducted in a static test over a 48 hour period using one control (no test compound) and six test solutions made up at nominal concentrations of 0 (control), 3.2, 5.6, 10, 18 and 32 mg/L at a temperature maintained at 20 ± 1°C. The test was conducted in quadruplicate using 5 daphnia in each test vessel. During the tests the water hardness was around 217 mg/L (as $CaCO_3$), the pH between 7.8 and 8.0 and the dissolved oxygen levels between 6.6 and 8.7 mg/L. It was noted that some flocculant was present in the vessel containing the highest test concentration of 32 mg/L, but that all other test media were clear. No statistically significant mortality or sublethal effects were observed over the 48 hour test period for the test concentrations of 10 mg/L and lower, but after 24 hours exposure at 18 mg/L two of the test animals were immobile, and 7 had become immobile after 48 hours exposure. All 20 animals were dead after 24 hours exposure to the 32 mg/L test solution.

The data was analysed using the parametric method of Kooijman (1981) to provide the results tabulated above. These data indicate that the notified chemical is slightly toxic to this species of daphnia.

A second acute toxicity to *Daphnia magna* was also conducted using the C14 component alone (Collins, 1992a). This test was also conducted using a static methodology using nominal test concentrations of 0 (control), 1.2, 2.0, 3.3, 5.5 and 9.2 mg/L. The 9.2 mg/L solution was slightly cloudy (probably reflecting the expected lower solubility of the C14 compound compared with the C12 component), but otherwise the test media showed no signs of containing insoluble material.

The tests at each concentration were conducted in quadruplicate using 5 daphnia in each test vessel, and throughout the 48 hour test period the temperature was between 19 and 22°C, the water hardness 160 mg/L (as $CaCO_3$), the pH between 8.0 and 8.1 and the dissolved oxygen levels between 8.4 and 9.4 mg/L. No immobilisation of the test animals was observed for the test concentration at 3.3 mg/L and lower, but after 48 hours exposure to the 5.5 mg/L solution, 65 % of the dapnia had been immobilised. All test animals were dead after 24 hours

exposure to the 9.2 mg/L solution. The results were analysed using probit analysis, and the 48 hour LC_{50} of 5.0 mg/L indicates that the C14 component of the notified chemical is moderately toxic to *Daphnia magna*.

A chronic test performed using flow through methodology against *Daphnia magna* was also performed with the C12-C14 mixture of components (ie 74.6 %:24.9 %) (Putt, 1992). This test was performed over a 21 day period using test solutions made up at nominal concentrations of 0 (control), 0.63, 1.3, 2.5, 5 and 10 mg/L. The solutions were analysed for test compound and the results were always within 25 % of the nominal concentrations (always lower), the mean measured concentrations being 0, 0.56, 0.97, 2.2, 4.3 and 8.9 mg/L respectively. Each test was conducted in its own flow through vessel suspended in a water bath maintained at 20±2°C, and the tests for each concentration (including the control) were conducted in quadruplicate using 10 daphnia in each test vessel. The rate of flow of the test media through the vessels was such that there was 90 % replacement of media after 9 hours, and the pH, dissolved oxygen and water hardness were respectively 7.9-8.3, 7.5-7.9 mg/L and 170 mg/L (as $CaCO_3$). Survival of the original daphnia and the rate of production of the offspring were monitored throughout the 21 day test period.

For those test concentrations with mean measured concentrations of 2.2 mg/L and less, there was little difference in the 21 day survival rate of adult daphnia, and that of the control – ie between 90 and 98 % compared with the 90 % of the control. However, there was only 60 % survival after 21 days for the 4.3 mg/L solution (nominally 5 mg/L), and 13 % survival for the 8.9 mg/L test solution (nominally 10 mg/L). Throughout the 21 day observation period, the adult daphnia were observed to be small, to be swimming and to exhibit other behavioural abnormalities at the two higher test concentrations.

The number of offspring per female daphnia was also counted, and while there was little difference between the control and test solutions containing test compound at a (measured) concentration of 2.2 mg/L or less, significant reduction in offspring production was observed at the two higher concentrations. After 21 days only 5 offspring per female were recorded at 8.9 mg/L , compared with an average of 212 for the control.

The results of this test were analysed using a non linear interpolation procedure to give a 21 day LC_{50} of 6.8 mg/L. The LOEC was determined as 8.9 mg/L, while the corresponding NOEC was 4.3 mg/L.

The difference between the acute 48 h NOEC and the chronic 21 day NOEC for *Daphnia magna* is around 2, indicting a low acute/chronic ratio.

Algae
Tests on algal growth inhibition (Hanstveit & Oldersma, 1991) were also performed with solutions of the C12-14 mixture of components made up in water at nominal concentrations of 0 (control), 3.2, 5.6, 10, 18, 32 and 56 mg/L, and seven replicate tests were conducted at each concentration. A blank test (no algae) was also run in order to correct for background effects. The mean temperature throughout the test was 23±1°C, and the pH of the media containing algae increased with time from 8 at the beginning of the test to 9.4 after 4 days. Both growth of algal biomass and the rate of biomass growth were monitored by counting the cell density (Coulter counter) over the 92 hour test period, and appropriate data used for the construction of growth curves. Inhibition of algal growth was apparent after about 30 hours, particularly at the higher test concentrations, and the data was analysed using parametric

models (Kooijman, 1981), and providing the results tabulated above.

These results indicate that the notified chemical is slightly toxic to this species of green algae. Microscopic examination of the algae revealed some distorted cells at the higher exposures.

A second test (Masters-Alexander, 1992) was performed using the C14 component made up in water at eight nominal concentrations between 1.43 and 184 mg/L. Three replicate tests were conducted at each concentration, and it was apparent that significant inhibition to the growth of the algae was observed at all test concentrations, with 12.5 % inhibition at exposure to 1.43 mg/L (nominal) of the test substance, increasing to 79.5 % inhibition in a 5.7 mg/L solution.

The slope of the dose-response curve was very flat, and greater than 90 % inhibition was observed at the five highest test concentrations (ie those for an 11.5 mg/L solution and higher). Accordingly, only the data for the five lower concentrations was used in the analysis, which was conducted according to Dunnett's procedure. This analysis furnished a 96 hour E_bC_{50} of 3.95 mg/L (95 % confidence interval 2.4-6.4 mg/L), and a NOEC of 2.86, indicating moderate toxicity.

Sewage Bacteria
A test for inhibition of respiration in sewage treatment bacteria (Neven, 1991a) was conducted with the mixture of C12-C14 components. Activated sludge samples (from a domestic sewage treatment plant) were incubated with 5, 10, 20, 40 and 80 mg/L of the test material, together with two controls containing no test compound. The rate of oxygen uptake in each of the samples was determined after a 3 hour incubation period. A 14 % inhibition of respiration rate over the controls was observed for the samples containing 10 mg/L, which had increased to 31 % inhibition for the 80 mg/L test. This result indicates that the notified chemical is moderately toxic to sewage treatment bacteria. It should be noted that the possibility of inhibition of bacterial activity was not addressed in the studies of aerobic biodegradation discussed above.

Summary of Aquatic Toxicology
The notified chemical, which is a mixture of the C12 and C14 linear alkyl glucose amides in the approximate proportions of 73.6 %:24.9 % is slightly to moderately toxic to those aquatic species against which it has been tested. However, certain tests conducted with the C14 component alone indicate that this compound is measurably more toxic than the new commercial mixture – and by inference, more toxic than the C12 component. Indeed, while no reports were submitted, the notifier indicated the results of certain toxicity tests performed with the C12 component alone which were –

LC_{50} (96 h) for fish (species not indicated) = 39 mg/L,
LC_{50} (48 h) for *Daphnia magna* = 44.3 mg/L,
EC_{50} (96 h) for algae = 57 mg/L (NOEC = 21.3 mg/L).

These results support the lower toxicity of the C12 component compared with that of the C14 compound.

10.2 Terrestrial Toxicity

Reports and test data were submitted on the toxic effects of the C12-C14 mixture against earthworms and three species of plants. These data are summarised in the table below.

Test	***Species***	***Results (nominal)***
Acute Toxicity to Earthworm. C12-C14 components [OECD TG 207]	*Eisenia fetida*	LC50 (14 day) > 1,000 mg/kg (dry soil) NOEC (14 day) =1,000 mg/kg (dry soil)
Growth Test on Plants [OECD TG 208]	Oats (*Avena sativa*) Mustard(*Brassica rapa*) Lettuce (*Lactuca sativa*)	NOEC (17 day) = 320 mg/L

The tests on earthworms (van der Hoeven & Henzen, 1994) were conducted in glass containers containing around 775 grams of wet (moisture content = 52.2 %) artificial soil (sphagnum peat, clay and fine sand in the proportions 1:2:7). The test material was homogeneously distributed through this media, at a level of 1,000 mg per kg of dry soil. Ten worms were placed in each of two of the containers containing the test media, and a further ten placed in two "control" containers containing only the artificial soil and no test compound. A fifth container to which no worms had been added was used as a control for monitoring pH and moisture content. The containers were placed in an environment where the temperature was maintained at 20±2°C, and the general appearance and behaviour of the worms was monitored over a 14 day period.

No mortality was observed over the test period, and the worms did not appear to have suffered adversely as a result of their exposure to the compound. These results indicate that the test compound is not toxic to the worms at levels up to 1,000 mg/kg of dry soil.

The tests on plants (Hooftman, 1997) were undertaken to ascertain the effect of the test compound on the germination of seeds, and the early growth phase of the three plant species indicated in the table. No test report accompanied the submission, but the test results were quoted in the dossier and reproduced in the table above.

The results of these tests indicate that the chemical is unlikely to exhibit toxic effects on earthworms or on plants if it becomes associated with soil, or is applied to soil associated with (for example) sewage sludge.

11. ASSESSMENT OF ENVIRONMENTAL HAZARD

The notified chemical is to be used as a surfactant in domestic dishwashing formulations, and as such almost all (around 27 tonnes per annum) is expected to be released to the sewer system. Although it does not meet the criteria for "ready biodegradability" as defined in OECD TG 301B, the chemical has been shown to be rapidly and extensively degraded under aerobic conditions, including in tests designed to simulate an activated sludge sewage treatment plant. A series of tests has demonstrated that degradation under anaerobic conditions also occurs, although at a significantly lower rate than aerobic degradation.

The chemical is slightly toxic to those aquatic species against which it has been tested, with an LC_{50} for zebra fish (the most sensitive species) estimated as 7.4 mg/L.

The notified chemical will be used throughout Australia, and consequently an estimate of the Predicted Environmental Concentration (PEC) may be made on a national basis.

The following PEC calculation assumes that the dishwashing formulations containing the surfactant are used nationwide, and that all is released to the sewer system. It is also assumed that 150 L of sewerage are generated each day by each person. Although it is likely that the compound would have some affinity for the organic component of soils, sediments or activated sludge in the sewage treatment plants, the moderate water solubility (140 mg/L), together with information obtained during biodegradability testing indicates that it may largely remain in the water column.

Import rate	27 tonne per annum
Release rate	27 tonne per annum
Population (national)	18,000,000
Volume of sewage per annum	18,000,000 × 365 ×150 = 985 × 10^9 L per annum
Mean concentration in sewage	27 μg/L

On release to receiving waters (after treatment at the sewage treatment plant), it is usually assumed that the effluent is diluted by a factor of 10. This gives a final PEC of 2.7 μg/L.

In the unlikely event of all the product being used in a single major city, (eg Sydney or Melbourne with populations of around 3,000,000), the mean concentration in the sewerage would be 175 μg/L, which would be diluted to 17.5 μg/L on release to receiving waters.

The calculation assumes that no biodegradation of the compound occurs in the sewage treatment plants prior to discharge to receiving waters. In fact it is likely that most of the chemical will have been destroyed prior to discharge, and so the calculations are a worst case scenario. However, even assuming no biodegradation, the estimated PECs are at least two orders of magnitude below levels which have been shown to be toxic to aquatic organisms. Despite the continuous release, a chronic toxicity hazard is not expected due to the low acute/chronic ratio.

During sewage treatment some of the material may become associated with sewage sludge, and this may be disposed of to landfill or used as a soil conditioning agent. However, the available data indicated that the compound is unlikely to exhibit toxic effects to either worms or plants. In any case, due to the high potential for biodegradation, the compounds are unlikely to persist in the soil compartment.

It is concluded that the notified chemical presents a low hazard to the environment when used as a component of dishwashing liquid as indicated by the notifier.

12. ASSESSMENT OF PUBLIC AND OCCUPATIONAL HEALTH AND SAFETY EFFECTS

Absorption, distribution and elimination tests showed that close analogues to the notified chemical are readily absorbed from the digestive tract and distributed widely throughout tissues, but absorption through the skin is minimal. Elimination of the analogue used in the dermal study is principally through urine.

The acute toxicity of C12-14 Linear Glucose Amide is low. It is a slight irritant to the skin of rabbits. The notified chemical is a severe and persistent irritant to rabbit eyes, and warrants health effects classification with the risk phrase R41 "Risk of serious damage to eyes" applied. The notified chemical was negative in a non-adjuvant skin sensitisation study in guinea pigs.

For longer-term systemic effects, the NOEL is 50 mg/kg/day, on clinical signs and the effects on body weight gain observed at 200 and 500 mg/kg/day in a 91 day oral rat study. In a developmental toxicity study, the notified chemical was found not to cause developmental toxicity at a dose where maternal toxicity was observed. The NOEL for developmental toxicity was determined to be 363 mg/kg/day (the highest dose tested) while the NOAEL for maternal toxicity was found to be 150 mg/kg/day based on decreased bodyweight gain at the higher dose.

The notified chemical was found not to be genotoxic in a number of *in vitro* and *in vivo* studies, although positive results were observed in an *in vitro* study of chromosomal aberrations in CHO cells in the absence of metabolic activation at a concentration where significant toxic effects occurred.

A large number of skin irritation and sensitisation studies in humans have been performed using analogues to the notified chemical and also formulations containing the analogues. The glucose amides were found to be slight skin irritants under the conditions of the tests, but not to be skin sensitisers.

Occupational Health and Safety
Occupational exposure to the notified chemical is expected to be negligible, as it will only be imported as a component of a formulated dishwashing liquid, and will not require repackaging in Australia. The health risk for transport, storage and retail workers is expected to be negligible unless the packaging is breached. Even in this case, little exposure is expected because the notified chemical will be present at a concentration of 1.43 %.

Public Health
As the notified chemical will be used in a dishwashing liquid, there will be widespread dermal exposure to the public, limited only by the commercial success of the product. Systemic exposure from dermal contact with the notified chemical in dishwashing liquid is calculated using reference values from Risk Assessment of Existing Substance: Technical Guidance Document (European Commission, 1994).

The dishwashing liquid with a concentration of 1.43 % of the notified chemical would result in a systemic exposure of 0.036 mg/kg/day, based on the following assumptions.

volume used per application = 10 g

fraction remaining on the skin = 5 %
weight fraction of notified chemical = 1.43 %
dermal absorption = 10 %
frequency of use = 3 times per day, every day
body weight = 60 kg

In comparison with the NOEL of 50 mg/kg/day established in the 13-week toxicity study in rats, the above estimated exposure would represent a safety margin of greater than 1300 for the notified chemical in this product.

The notified chemical is a slight skin irritant and a severe eye irritant in rabbits. However, the hazards associated with eye and skin irritation are likely to be offset by the low concentration (maximally 1.43 % and likely to be considerably less once diluted in water) and use pattern (predominantly dermal exposure via the hands, which could be considerably reduced by the wearing of rubber gloves) of the notified chemical. Consequently, the potential hazard from the use of the notified chemical is considered to be low. There will be minimal public exposure from transport and storage.

Based on the submitted information, it is considered that C12-14 Linear Glucose Amide will not pose a significant hazard to public health when used in the proposed manner.

13. RECOMMENDATIONS

To minimise occupational exposure to C_{12-14} Linear Glucose Amide when used in accordance with the notification, the following guidelines and precautions should be observed:

- Industrial clothing should conform to the specifications detailed in AS 2919 (Standards Australia, 1987);
- Spillage of the notified chemical should be avoided. Spillages should be cleaned up promptly with absorbents which should be put into containers for disposal;
- Good personal hygiene should be practised to minimise the potential for ingestion;
- A copy of the MSDS should be easily accessible to employees.

Further recommendations may be required if the occupational use of the notified chemical is varied from the notified use. In this case, secondary notification may be required.

14. MATERIAL SAFETY DATA SHEET

The MSDS for the notified chemical was provided in a format consistent with the *National Code of Practice for the Preparation of Material Safety Data Sheets* (National Occupational Health and Safety Commission, 1994).

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

15. REQUIREMENTS FOR SECONDARY NOTIFICATION

Secondary notification under Section 64 of the Act may be required if:

1. additional information becomes available on adverse environmental effects of the notified chemical

2. the annual import levels of the chemical exceed 100 tonnes; additional fate data such as field monitoring performed at sewage treatment plants in the Netherlands should be provided at this time

3. the concentration of notified chemical in the product is increased, or if additional products containing the notified chemical enter the public domain; secondary notification may be required to assess the hazards to public health

4. occupational and environmental exposure is varied form the exposure described in this assessment

Under the Act, secondary notification of the notified chemical may be required if any of the circumstances stipulated under subsection 64(2) of the Act arise.

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Attachment 1

The Draize Scale for evaluation of skin reactions is as follows:

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

The Draize scale for evaluation of eye reactions is as follows:

CORNEA

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

CONJUNCTIVAE

Redness	*Rating*	*Chemosis*	*Rating*	*Discharge*	*Rating*
Vessels normal	0 none	No swelling	0 none	No discharge	0 none
Vessels definitely injected above normal	1 slight	Any swelling above normal	1 slight	Any amount different from normal	1 slight
More diffuse, deeper crimson red with individual vessels not easily discernible	2 mod.	Obvious swelling with partial eversion of lids	2 mild	Discharge with moistening of lids and adjacent hairs	2 mod.
		Swelling with lids half-closed	3 mod.	Discharge with moistening of lids and hairs and considerable area around eye	3 severe
Diffuse beefy red	3 severe	Swelling with lids half-closed to completely closed	4 severe		

IRIS

Values	*Rating*
Normal	0 none
Folds above normal, congestion, swelling, circumcorneal injection, iris reacts to light	1 slight
No reaction to light, haemorrhage, gross destruction	2 severe