File No: NA/944

November 2001

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME

FULL PUBLIC REPORT

1,6-dichloro-1,6-dideoxy-β-D-fructofuranosyl-4-chloro-4-deoxy-α-D-galactose

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Director
Chemicals Notification and Assessment

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SPLENDA/SUCRALOSE
1,6-dichloro-1,6-dideoxy-β-D-fructofuranosyl-4-chloro-4-deoxy-α-D-galactose

1. APPLICANT

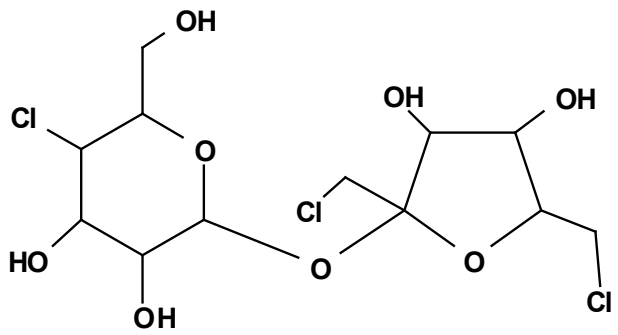
Johnson & Johnson Pacific Pty Ltd (ACN 001 121 446) of Stephen Road BOTANY NSW 2019 has submitted a limited notification statement in support of their application for an assessment certificate for 1,6-dichloro-1,6-dideoxy-β-D-fructofuranosyl-4-chloro-4-deoxy-α-D-galactose.

2. IDENTITY OF THE CHEMICAL

No claims for exempt information were made by the applicant.

Chemical Name:	1,6-dichloro-1,6-dideoxy-β-D-fructofuranosyl-4-chloro-4-deoxy-α-D-galactose.
Chemical Abstracts Service (CAS) Registry No.:	56038-13-2
Other Names:	Trichlorogalactosucrose; TGS; 4,1',6'-trichlorogalactosucrose.
Marketing Name:	SPLENDA Brand Sweetener; Sucralose
Molecular Formula:	$C_{12}H_{19}Cl_3O_8$

Structural Formula:



Molecular Weight: 397.64

Method of Detection and Determination:	Infrared spectrum

3. PHYSICAL AND CHEMICAL PROPERTIES

Appearance at 20°C & 101.3 kPa:	Free-flowing, white crystalline powder.
Melting Point:	125.5°C
Specific Gravity:	Not determined
Vapour Pressure:	Not determined – see comments below
Water Solubility:	283 g/L at 20°C
Partition Co-efficient (n-octanol/water):	Log P_{OW} = -0.51±0.05
Hydrolysis as a Function of pH:	See comments
Adsorption/Desorption:	Not determined
Dissociation Constant:	Not determined
Particle size distribution:	Not supplied
Flash Point:	Not flammable
Surface Tension:	Negligible
Flammability Limits:	Not flammable (MSDS)
Autoignition Temperature:	Not supplied
Explosive Properties:	Not explosive unless in dust form (MSDS)
Reactivity/Stability:	Stable under normal conditions

3.1 Comments on Physico-Chemical Properties

The vapour pressure of the notified chemical was not determined for this notification. However, the notifier indicates that its vapour pressure is expected to be negligible.

The notifier did not determine the melting point, water solubility, partition coefficient and surface tension, but provided an article by Jenner and Smithson (1989) which dealt with these parameters. The water solubility was determined using a thermostatically controlled Wheaton jacketted glass vessel. The sucralose solutions were stirred for 22 hours then left to stand for 1 hour, after which samples were analysed by HPLC. The water solubility was determined to

be 283 g/L at 20ºC.

The partition coefficient was determined by the shake flask method. Aliquots of saturated sucralose solution were mixed with water/octanol solution in test tubes, stopped and shaken 100 times in 5 mins. The tubes were then centrifuged for 10 mins at 20ºC. Samples from the resultant layers were analysed by HPLC. The log P was determined to be –0.51±0.05.

A Kruss model K8600 tensiometer, ring method, was used to determine the surface tension. Initially the surface tension of double distilled water was determined followed by dilute sucralose solutions prepared with the double distilled water. It was found that the surface tension of the double distilled water was only negligibly lowered by the sucralose. Thus, sucralose has negligible surface tension, ie is not surface active.

The degradation of the notified chemical was investigated at 62, 50, 40 and 30°C at pH 1, 1.5, 2 and 3 (Tate and Lyle Group Research and Development, 1983) to determine the stability of sucralose in beverages. A quantity of the notified chemical was added to the each of the buffered solutions and the resulting solutions were stored at the above temperatures. The storage time and sampling times varied according to pH and temperature with sampling starting on day 1 and the last sample taken on day 336 for pH 3 at 30 and 40ºC. All samples were analysed by HPLC. It was found that the notified chemical broke-down by simple hydrolysis to 1,6-dichloro-1,6-dideoxy-D-fructosfuranose and 4-chloro-4-deoxy-D-galactopyranose, the constituent monomers. The rate of hydrolysis increased with temperature and decreasing pH, eg at pH 1 and 62ºC 98.8% of sucralose is hydrolysed after 120 hours, at pH 2 and 62ºC only 30.4% is hydrolysed after 120 hours. While this study dealt with low pHs, in the environmental pH range of 4 to 9, significant hydrolysis is unlikely to occur.

No adsorption/desorption tests were conducted for this notification. The notified chemical's high water solubility, low partition coefficient and lack of surface tension indicate that it is a hydrophilic compound likely to partition mainly into the aqueous phase.

Sucralose contains no acidic or basic groups.

4. PURITY OF THE CHEMICAL

Degree of Purity: 98.0-102.0 % Calculated on the anhydrous basis

Hazardous Impurities:

Chemical name:	Arsenic (as As)
CAS No.:	7440-38-2
Weight percentage:	Less than 3 mg/kg
Toxic properties:	Toxic (T) by inhalation (R23) and if swallowed (R25)
Chemical name:	Heavy metals (as Pb)
Weight percentage:	10 mg/kg or less

Toxic properties:	Toxic (T), may cause harm to the unborn child (R61), possible risk of impaired fertility (R62), harmful by inhalation (R20) and if swallowed (R22), danger of cumulative effects (R33).
Chemical name:	Methanol
Synonyms:	Methyl alcohol
CAS No.:	67-56-1
Weight percentage:	0.1 % or less
Toxic properties:	Toxic (T) by inhalation (R23) and if swallowed (R25)

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

Chemical name:	4-chloro-4-deoxygalactose
Synonyms:	4-CG
Weight percentage:	Very low
CAS No.:	
Chemical name:	1,6-dichloro-1,6-dideoxyfructose
Synonyms:	1,6-DCF
Weight percentage:	Very low

Additives/Adjuvants: None

5. USE, VOLUME AND FORMULATION

The notified chemical is in use worldwide as a non-nutritive sweetener in food and beverages and as an excipient in pharmaceutical products. In Australia these uses have been approved by the Australia New Zealand Food Authority (ANZFA) and the Therapeutic Goods Administration (TGA).

In this notification the notified use is as a sweetener at 0.06% in mouth wash products. The usual concentration of sucralose in foods is 0.025-0.15 %.

The notified chemical will be imported as a 25% component of the product Splenda in sealed 4 and 20 kg plastic (HDPE) containers. Formulation of the mouth wash products will be performed by FH Faulding at 1538 Main North Rd, Salisbury South, South Australia. During formulation of the mouth wash products, Splenda will be added directly to a 6000 L stainless steel manufacturing vessel and mixed with other materials. The formulated mouthwash will be pumped directly from the mixing vessel via an in built connecting hose to into 250 and 500 mL PET bottles with polypropylene screw top caps for sale to consumers.

For this use the notified chemical will be imported at 175 kg per year.

6. OCCUPATIONAL EXPOSURE

Transport and Storage
Waterside, warehouse (4 workers, 20 minutes/day, 24 days/year) and transport (8 workers, 15 minutes/day, 24 days/year) workers are unlikely to be exposed to the notified polymer unless the packaging is breached.

Formulation
Approximately 2 factory workers will have the potential for exposure to the notified chemical during the formulation of the mouth wash products (maximum duration of exposure of 10 minutes/day, 12 days/year). Possible dermal and ocular exposure to spills and splashes containing the notified chemical may occur during the addition of Splenda to the vessel, during the mixing of the mouth wash products, and when connecting lines for filling into PET bottles. Inhalation exposure to aerosols during mixing may also occur. The mixing process occurs in a semi automated closed system in an area with an exhaust ventilation system. Workers will wear overalls, dust masks, protective gloves and eye protection.

One QC sampling worker (duration of exposure of 5 minutes/day, 12 days/year) will take a 50 mL sample from the bulk liquid mouth wash formulations, which will be forwarded to a QC laboratory for analysis by QC testing workers (2 workers, 20-60 minutes/day, 12 days/year). QC sampling and testing worker may receive dermal and ocular exposure to drips and spills containing the notified chemical during sampling and testing of mouth wash formulations.

Retail outlets
Retail outlet workers (approximately 500 workers, 1-10 minutes/day, 26-365 days/year) are unlikely to be exposed to the notified polymer unless the packaging is breached.

7. PUBLIC EXPOSURE

The public may be exposed to the notified chemical through transport accidents and environmental contamination, although such events are unlikely. The notified chemical is an ingredient of a mouth wash products intended for consumer use. Public exposure to the notified chemical via the oral cavity can be expected to be widespread. If any contact with it occurs by these means it is most likely to be dermal. Very small volumes of the mouth wash may also be ingested. The notified chemical has negligible volatility and is unlikely to be inhaled. The potential for public exposure to the notified chemical is therefore high.

8. ENVIRONMENTAL EXPOSURE

8.1 Release

The notifier estimates that during formulation of the mouthwash up to 0.1% per annum of

notified chemical will be released into the environment as a result of spills, equipment cleaning and import container residue. This equates up to 0.175 kg per annum. It is possible that the washwater generated by container or process equipment cleaning may be recycled back into the process. Otherwise, it will be released to sewer.

It is expected that the plastic import drums containing residual polymer solution will be either incinerated or cleaned and the plastic recycled. The mouthwash containers (250 or 500 mL PET bottles), which will be sold to consumers, will be disposed of in domestic landfill. It is estimated that less than 0.1% of the volume of the bottle will remain once it has been 'emptied', which equates to less than 175 g per annum of the notified chemical being disposed of to landfill.

The notifier has estimated that approximately 10 mL of mouthwash will be used each time someone rinses their mouth, with only 1 mL will remain in their mouth and 9 mL being spit out down the drain. Thus, the majority (approximately 99%) of the notified chemical may ultimately be released to the environment, on the understanding that any ingested will not be absorbed but pass straight through the gut.

8.2 Fate

Wastes (0.1%) resulting from the cleaning process equipment and containers and spills may be released into the sewer. The majority of the notified chemical will be released into the sewer following mouth rinsing.

In landfill, the notified chemical contained in the disposed consumer PET bottles may leach out but at very low levels and in a very diffuse manner.

The notifier has provided the results of a ready biodegradation test in an aerobic aqueous media following a modified OECD TG 301E (1981) (Aquatox Ltd, 1984a). The biodegradation was determined by the removal of dissolved organic carbon produced from a mineral salt medium after it was inoculated with a mixed population of micro-organisms (activated sludge) and stored in the dark at 22ºC for 28 days. Sodium benzoate was used as the reference substance. The results indicated that 5% of the notified chemical had degraded over this time, while approximately 92% of the standard degraded in 28 days. The results indicate that notified substance is not readily biodegradable.

The biodegradability of the notified chemical in a sediment/water system and water system was investigated by Imperial Chemicals Industries PLC (1987). The biodegradation was determined by the measurement of $^{14}CO_2$ generated from the medium after it was inoculated with either soil or micro-organisms population (activated sludge) and shaken at 20ºC for 130 days. Sodium benzoate was used as the reference substance. For two of the soil inoculum, the results indicated that approximately 56 days was required for microbial adaptation before degradation began, thus resulting in 63 and 45.2% degradation by day 130. With the third soil inoculum the adaptation period appeared to be 100 days, with 14.2% degradation by day 130. No degradation was observed in the activated sludge inoculum. These results indicate that the notified chemical is inherently biodegradable but not readily degradable.

Due to its high water solubility and low log P_{ow}, the notified chemical should not bioaccumulate (Connell, 1990).

9. EVALUATION OF TOXICOLOGICAL DATA

Because of its intended uses in food, beverages and pharmaceuticals, the notified chemical has undergone much toxicological investigation including human volunteer studies. The toxicological data have been extensively reviewed by government agencies and the chemical approved for use in products for human ingestion by many national authorities.

9.1. Pharmacokinetics and Metabolism

When administered orally, between 11-27% of the sucralose is absorbed from the gastrointestinal tract in male humans. The remaining sucralose is excreted unchanged in faeces (USFDA Department of Health and Human Services 1998).

Following gastrointestinal absorption, between 20-30% of the sucralose is broken down to two metabolites in human. The remaining sucralose is excreted unchanged in urine (USFDA Department of Health and Human Services 1998).

Studies in rats indicate that repeated dosing with sucralose does not induce microsomal enzymes. Furthermore chronic dosing did not produce evidence of metabolic adaptation (USFDA Department of Health and Human Services, 1998).

9.2 Acute toxicity

The acute oral toxicity of sucralose and its hydrolysis products, 4-chloro-4-deoxygalactose (4-CG) and 1,6-dichloro-1,6-dideoxyfructose (1,6-DCF), have been assessed in mice and rats. The results of these studies are summarised in table 9.2.1.

Table 9.2.1. Acute oral toxicity of sucralose and its hydrolysis products.

Species	Sex	Route	LD_{50} (mg/kg bw)
Sucralose			
Mouse	Not specified	Oral	>16 000
Rat	Male	Oral	>10 000
Sucralose hydrolysis products			
Mouse	Male & female	Oral	3499
Rat	Male & female	Oral	1629

(TGA, unknown date)

9.3 Genotoxicity

The genotoxicity of sucralose and the two sucralose hydrolysis products, 4-CG and 1,6-DCF have been assessed. The results of these studies are summarised in tables 9.3.1-4.

Table 9.3.1. Sucralose mutagenicity studies.

Test system	Test object	Sucralose concentration	Result

S. typhimurium (+ & - S9)	TA98, TA100, TA1535, TA1537, TA1538.	16-10 000 µg/plate	Negative
S. typhimurium (+ & - S9)	TA98, TA100.	16-10 000 µg/plate	Negative
E. coli (+ & - S9)	W3110, P3478	0.5 -1 000 µg/plate	Negative
Mouse lymphoma (+ & - S9)	TK +/-	1 335 – 10 000 µg/mL	Positive at 7 500 & 10 000 µg/mL. Cell viability was less than 50%
Mammalian cytogenetics (human)	Peripheral blood lymphocytes (in vitro)	8 - 200 µg/mL	Negative
Mammalian cytogenetics (rat)	Bone marrow (in vivo; oral route)	5 x 200 mg/kg bw	Negative
Mouse micronucleus test	In vivo; oral route	1 000 – 5 000 mg/kg bw, 24, 48 and 72 h	Negative

(TGA, unknown date)

Table 9.3.2. 4-CG mutagenicity studies.

Test system	Test object	4-CG concentration	Result
S. typhimurium (+ & - S9)	TA98, TA100, TA1535, TA1537, TA1538.	16-10 000 µg/plate	Negative
Mouse lymphoma	TK +/-	1.3 – 10 000 µg/mL	Negative
Mammalian cytogenetics (human)	Peripheral blood lymphocytes (in vitro)	40 – 1 000 µg/mL	Negative
Mammalian cytogenetics (rat)	Bone marrow (in vivo; oral route)	5 x 50, 150 & 150 mg/kg bw	Negative

(TGA, unknown date)

Table 9.3.3. 1,6-DCF genetic toxicity studies.

Test system	Test object	1,6-DCF concentration	Result
S. typhimurium (+ & - S9)	TA98, TA100, TA1537, TA1538.	16 -10 000 µg/plate	Negative
S. typhimurium	TA1535.	16-10 000	Negative

		µg/plate	
S. typhimurium	TA1535.	250 - 5 000 µg/plate	Positive (2-3 fold increase in revertant colonies).
S. typhimurium (+ & - S9)	TA98, TA100, TA1535, TA1537, TA1538	16 -10 000 µg/plate	Negative
S. typhimurium	TA1535.	2 000 - 5 000 µg/plate	Positive (2-3 fold increase in revertant colonies).
S. typhimurium (+ & - S9)	TA98, TA100, TA1537, TA1538.	16 -10 000 µg/plate	Negative
S. typhimurium	TA1535.	60 - 6 000 µg/plate	Negative
S. typhimurium (+ S9)	TA1535.	60 - 3 000 µg/plate	Negative
S. typhimurium	TA1535.	6 000 µg/plate	Positive (2-3 fold increase in revertant colonies).
Mouse lymphoma	TK +/-	13 - 42 µg/mL	Negative
Mouse lymphoma	TK +/-	56 - 133 µg/mL	Positive (increased mutation frequency was associated with decrease viability).
Mouse lymphoma	TK +/-	13 - 169 µg/mL	Negative
Mouse lymphoma	TK +/-	10 - 40 µg/mL	Negative
Mouse lymphoma (+ S9)	TK +/-	53 - 127 µg/mL	Positive (increased mutation frequency was associated with decrease viability).
Mammalian cytogenetics (human) (+ & - S9)	Peripheral blood lymphocytes (in vitro)	1.5 - 40 µg/mL	Negative
Mammalian cytogenetics (rat)	Bone marrow (in vivo; oral route)	1 000 mg/kg bw	Negative
Mammalian cytogenetics (rat)	Bone marrow (in vivo; oral route)	5 x 50, 150 & 500 mg/kg bw; 24 h interval	Negative
Sex-linked	*Drosophila*	0.2 – 2 mg/mL;	Negative

recessive lethal	*melanogaster* (in vivo)	3 days.	
Mouse micronucleus test	Bone marrow (in vivo; oral route)	415 – 1 660 mg/kg bw/day	Negative
Mouse micronucleus test	Bone marrow (in vivo; oral route)	1 000 – 2 500 mg/kg bw	Negative
Mouse sister chromatid exchange	Bone marrow (in vivo; oral route)	200 – 2 000 mg/kg bw	Negative
Covalent DNA binding (rat; oral; in vivo)	Liver, kidney, small intestine, colon, stomach & bone marrow	21 mg/kg bw	Negative

(TGA, unknown date)

Table 9.3.4. Sucralose hydrolysis products (sucralose-HP) mutagenicity studies.

Test system	Test object	Sucralose-HP concentration	Result
Dominant lethal assay (mouse)	In vivo; oral route	30 - 270 mg/kg bw/day	Negative

(TGA, unknown date)

9.4 Repeated dose toxicity

9.4.1 Bodyweight gain/food consumption

A number of studies have been conducted examining the acceptability and palatability of sucralose when administered to rats in drinking water or diet. It was determined that sucralsoe levels up to 3200 ppm were acceptable in drinking water and that levels above 800 ppm resulted in reduced food consumption (USFDA Department of Health and Human Services 1998).

A pair feeding study was conducted to determine if any reduced weight gain associated with sucralose intake was due to an effect of the test substance and not a reduction in food consumption. Five groups of female Sprague-Dawley CD rats were used in the study. Group 1 was allowed unrestricted access to a diet containing 3% sucralose. Rats in group 2 were fed a daily amount of basal diet equivalent to the mean group intake consumed on the previous day by rats in group 1. Rats in group 3 were allowed unrestricted access to basal diet. Group 4 was administered by gavage an amount equivalent to group 1. Group 5 served as the control for group 4 and received distilled water by gavage. A significant decrease in bodyweight gain and food consumption was detected in groups 1 and 2 relative to the group 3 control (USFDA Department of Health and Human Services 1998).

In a separate study, groups of Sprague-Dawley rats (10/sex/group) were dosed by gavage with 2000 mg/kg bw/day for 13 weeks, 3000 mg/kg bw/day for 9 weeks or 4000 mg/kg bw/day for 4 weeks. No treatment related changes were detected. Food consumption and

bodyweight gain were slightly increased (103-109%) when compared to controls (USFDA Department of Health and Human Services 1998).

A study has been conducted examining if decrease food consumption and bodyweight gain associated with dietary sucralose intake is due to increased spillage of the sucralose containing diet. In this study 3 groups of Sprague-Dawley rats (15/sex/group) were fed either a basal diet or a basal diet containing 3 or 5% sucralose. A 5-8% decrease in food consumption, associated with a 10-15% reduction in bodyweight gain, was observed in treated rats. During the first two weeks rats fed a diet containing sucralose showed significantly higher spillage when compared to controls (USFDA Department of Health and Human Services 1998).

A diet restriction study was also conducted to examine decreased food consumption and bodyweight gain associated with dietary sucralose intake. Results from this study indicate that 3% dietary sucralose resulted in a significant decrease in weight gain in Sprague-Dawley CD rats that was attributable to the test substance. 1% dietary sucralose (equivalent to 500 mg/kg bw/day) had no effect on bodyweight gain and was considered the NOEL for this toxic endpoint (USFDA Department of Health and Human Services 1998).

Sucralose related bodyweight gain effects were also investigated in groups of Sprague-Dawley rats (20/sex/group) dosed by gavage with 0-3000 mg/kg bw/day for 26 weeks. Food intake for males dosed with 3000 mg/kg bw/day was 3.9% greater than controls. Interestingly, the adjusted mean body weight gain of males dosed with 3000 mg/kg bw/day was significantly decreased (4.6%; $p = 0.035$) compared to controls. The NOEL for the bodyweight gain effect observed in this study was determined to be 1500 mg/kg bw/day (USFDA Department of Health and Human Services 1998).

9.4.2 Chronic toxicity/carcinogenicity studies

The toxicity of sucralose has been examined in a combined chronic toxicity/carcinogenicity study consisting of a breeding phase, a carcinogenicity phase and a chronic toxicity phase. In the breeding phase 140 (70 /sex) Sprague-Dawley CD rats were fed diets containing 0, 0.3, 1 or 3% sucralose for a 4 week period prior to mating and during gestation. Two weaning pups (1/sex) from each of 50 litters were allocated to the carcinogenicity phase of the study while 60 additional rats (30/sex) were selected for the chronic toxicity phase. Rats were sacrificed after 52, 78 and 104 weeks of sucralose treatment. The reproductive performance and fertility of parental rats during the breeding phase were normal. The survival of rats in the chronic and carcinogenicity phases of the study were unaffected by sucralose. There was no evidence of treatment related neoplasm in any rat during the carcinogenicity phase. A minimal increase in the incidence of renal pelvic mineralisation and epithelial hyperplasia lesions were detected in rats, primarily females treated with 3% sucralose, in the chronic and carcinogenicity phases. During the chronic and carcinogenicity phases of the study all sucralose treated rats showed decreased bodyweight gain. At the end of the chronic toxicity phase the reduction in bodyweight gain was 12-25% while food intake was reduced by 5-10% compared to controls. A NOEL could not be determined from this study (USFDA Department of Health and Human Services 1998).

The carcinogenicity of sucralose has also been tested in a study where groups of Charles River CD-1 mice (52/sex/group) were fed 0, 0.3, 1 and 3% sucralose in the diet for 104 weeks. During the treatment period the mean body weight gain of mice dosed with 3%

sucralose was significantly reduced compared to controls, even though food consumption was normal. A significant decrease in erythrocyte count was also detected in females dosed with 3% sucralose. No evidence of treatment related neoplasia was detected. The dietary NOEL was determined to be 1% sucralose (equivalent to 1500 mg/kg bw/day) (USFDA Department of Health and Human Services 1998).

The chronic toxicity of sucralose has been tested in a study where groups of beagle dogs (4/sex/group) were dose with 0, 0.3, 1 and 3% sucralose in the diet for 52 weeks. An increase in body weight gain, accompanied by a general increase in food consumption, was observed at all dose levels. The dietary NOEL was determined to be 3% sucralose (equivalent to 750 mg/kg bw/day) (USFDA Department of Health and Human Services 1998).

The chronic toxicity of sucralose hydrolysis products has been tested in a study where groups of Sprague-Dawley CD rats (50/sex/group) were dose with an equimolar mixture of 4-CG and 1,6-DCF at 0, 200, 600 and 2000 ppm in the diet for 104 weeks. No evidence of treatment related neoplasia was detected. A small increase in the incidence of hepatocellular clear cell foci was observed in treated rats. The mean bodyweight gain of females treated with 2000 ppm was reduced by 24%, which was accompanied by a 14% reduction in food intake. The dietary NOEL was determined to be 600 ppm of sucralose hydrolysis products (equivalent to 30 mg/kg bw/day) (USFDA Department of Health and Human Services 1998).

9.4.3 Immunotoxicity

The immunotoxicity of sucralose was assessed in groups of Sprague-Dawley rats (13/sex/group) dosed by gavage with 0-3000 mg/kg bw/day for 28 days. A significant decrease in mean thymus weight was noted in males dosed with 3000 mg/kg bw/day. Due to difficulties in interpreting the large variation in data at observed at 1500 mg/kg bw/day, the NOEL for immunological endpoints was determined to be 750 mg/kg bw/day (USFDA Department of Health and Human Services 1998).

9.4.4 Neurotoxicity

Mice and monkeys that received sucralose or an equimolar mixture of sucralose hydrolysis products at doses up to 1500 mg/kg bw/day did not exhibit any clinical signs of neurotoxicity or morphological changes in central nervous system tissues (USFDA Department of Health and Human Services 1998).

9.5 Reproductive/Developmental Toxicity

In a two generation reproductive toxicity study, groups of 60 Sprague-Dawley CD rats (30/sex) were dosed with 0, 0.3, 1 and 3% sucralose in the diet for 10 weeks prior to breeding and throughout two successive generations. Reproductive endpoints (estrous cycles, mating performance, fertility index, gestation length and gestation index), litter size and offspring viability were considered normal in either generation. A decrease in body weight gain was observed during the premating periods of the first (11-25%) and second (2-12%) generations. A slight decrease in food intake was also observed during the premating periods of the first (5-9%) and second (3-5%) generations. A significant decrease in thymic weight was detected in both generations at the 3% dose level. The findings of this study indicate that sucralose does not causes reproductive effects in rats up to 3% in the diet (USFDA Department of Health and Human Services 1998).

The ability of sucralose to cause reproductive/developmental toxicity was examined in a teratology study. Sucralose was dose orally (gavage) at 0, 500, 1000 and 2000 mg/kg bw/day to groups of 20 Sprague-Dawley CD rats from day 5 through day 15 of gestation. Bodyweight gain, food consumption, number of live young, and foetal and placental weight were unaffected by the treatment. The number of implantation sites, pre-implantation loses, and post-implantation losses were normal. The findings of this study indicate that sucralose does not causes maternal toxicity, embryo toxicity, foetal toxicity of induce teratology in rats at oral doses up to 2000 mg/kg bw/day (USFDA Department of Health and Human Services 1998).

The ability of sucralose to induce teratology was examined in groups of 16-18 pregnant rabbits dosed orally (gavage) with 0, 175, 350, and 700 mg/kg bw/day during days 6-19 of gestation. Eleven treatment unrelated deaths were recorded during the study. 2/18 treatment related deaths were noted in the 700 mg/kg bw/day group. Prior to death, both rabbits showed weight loss and reduced food intake. 3/18 rabbits in the 700 mg/kg bw/day group failed to become pregnant. Of the 9 pregnant rabbits in the 700 mg/kg bw/day, only 5 carried to term and produced viable young. At 700 mg/kg bw/day a decrease in the mean number of viable young per litter and an increase in post-implantation losses were observed. Gastrointestinal tract disturbances were also noted in the high dose group. Although maternal and foetal toxicity was observed at 700 mg/kg bw/day, no evidence of teratology was detected (USFDA Department of Health and Human Services 1998).

The ability of sucralose hydrolysis products to cause reproductive/developmental toxicity was examined in a two generation reproductive toxicity study. Groups of 60 Sprague-Dawley CD rats (30/sex) were dosed with an equimolar mixture of 4-CG and 1,6-DCF at 0, 200, 600 and 2000 ppm in the diet for 10 weeks prior to breeding and through two successive generations. In both generations estrus cycles, mating performance, fertility, gestation length, litter size and offspring viability were normal. Body weight gain of females at all doses and males at 2000 ppm was significantly reduced in the premating period for both generations. A reduction in weight gain was observed in females during pregnancy and in offspring from birth to weaning in both generations. 4-CG and 1,6-DCF at levels up to 2000 ppm in the diet caused no alterations in the reproductive performance of rats over two generations (USFDA Department of Health and Human Services 1998).

The ability of sucralose hydrolysis products to induce teratology was examined in groups of 20 pregnant Sprague-Dawley CD rats dosed orally (gavage) with an equimolar mixture of 4-CG and 1,6-DCF at 0, 30, 90 and 270 mg/kg bw/day during day 6-15 of gestation. No dose related increase in teratology was detected. Placental weight and bodyweight gain of dams in the 270 mg/kg bw/day group were significantly reduced (USFDA Department of Health and Human Services 1998).

9.6 Studies in humans

9.6.1 Diabetic studies

In a single dose crossover study the level of plasma glucose and serum c-peptide in the serum of insulin-dependant (type I diabetics) and non insulin-dependant (type II diabetics) diabetic patients was unaffected by a single dose of sucralose (1000 mg) (USFDA Department of Health and Human Services 1998).

In a separate study sucralose was administered orally at 667 mg/day for 6 months to patients with type II diabetes. A small yet statistically significant increase in haemoglobin glycosylation was observed from 1-6 months in the treatment group. This increase was determined not to be a direct effect of sucralose (USFDA Department of Health and Human Services 1998).

In a follow up study, human red blood cell preparations from diabetic and non-diabetic patients were treated with 100 mg/L sucralose. Sucralose was not found to increase haemoglobin glycosylation in this study (USFDA Department of Health and Human Services 1998).

In a second study where sucralose was administered orally at 667 mg/day no effect on haemoglobin glycosylation was observed in the treatment group. It was concluded that 667 mg/day sucralose had no effect on long term glucose homeostasis (as measured by haemoglobin glycosylation) in type II diabetics (USFDA Department of Health and Human Services 1998).

9.6.2 Clinical trials

The effect of sucralose on healthy humans was assessed in 8 subjects (4 per sex). Sucralose was administered orally at 0, 1, 2.5, 5 and 10 mg/kg bw at 24 hour intervals. This was followed by administration of sucralose at 2 mg/kg bw/day for 3 days then 5 mg/kg bw/day for 4 days. No adverse reactions or complaints were noted throughout the study. All haematological and biochemical markers examined were normal, as were ECG parameters, urine volume and blood insulin levels (TGA, unknown date).

In a separate study the effect of sucralose in healthy human volunteers was compared to that of fructose. In this study sucralose was administered to 79 human volunteers at 125 mg/day for weeks 1-3, 250 mg/day on weeks 4-7 and 500 mg/day on weeks 8-13. Fructose was administered to 31 human volunteers at 100 g/day. All ECG parameters and haematological, urinalysis and biochemical markers examined were normal (TGA, unknown date).

In a double blind cross over study eight healthy human volunteers were administered sucralose alone (10 mg/kg bw), sucrose alone (100 g) and a mixture of sucralose (10 mg/kg bw) and sucrose (100 g) at 24 hour intervals in random order. The serum concentration of glucose and fructose was similar in patients that received sucrose and sucralose when compared to those that received sucrose alone. Sucralose had no effect on insulin levels when administered alone and did not alter insulin responses to sucrose (TGA, unknown date).

9.7 Overall Assessment of Toxicological Data

Sucralose was poorly absorbed after oral administration in humans.

The notified chemical was of very low acute oral toxicity in rats (LD50 > 16 000 mg/kg bw) and mice (LD50 > 10 000 mg/kg bw). The sucralose hydrolysis products, 4-CG and 1,6-DCF, when tested as an equimolar mixture were of low and very low acute oral toxicity in rats (LD50 = 1629 mg/kg bw) and mice (LD50 = 3499 mg/kg bw) respectively.

Sucralose was non mutagenic in three Ames tests and non clastogenic in human lymphocytes and rat bone marrow cells. Sucralose was weakly mutagenic in a mouse lymphoma mutation assay. 4-CG was non mutagenic in an Ames test and a mouse lymphoma assay. 4-CG was non clastogenic as determined by a human lymphocyte assay and a rat bone marrow test. Although 1,6-DCF was found to be weakly mutagenic in 3/9 Ames tests and 2/5 mouse lymphoma assays, it was non clastogenic as determined by two rat bone marrow chromosomal aberration assay and a human lymphocyte test. 1,6-DCF did not induce sister chromatid exchanges or micronuclei in mouse bone marrow cells. A sex linked recessive lethal assay in *Drosophilia melanogaster* and a covalent DNA binding potential study in rats were negative. The sucralose hydrolysis products 4-CG and 1,6-DCF were not genotoxic as determined by a dominant lethal test in the mouse when tested as an equimolar mixture.

There was no evidence of treatment related neoplasm in rats fed a diet containing up to 3% sucralose (equivalent to 3000 mg/kg bw/day) during the carcinogenicity phase of a combined chronic toxicity/carcinogenicity study and during a 104 week carcinogenicity study. No evidence of treatment related neoplasia was detected in rats dosed with an equimolar mixture of the sucralose hydrolysis products, 4-CG and 1,6-DCF, at up to 2000 ppm in the diet for 104 weeks.

Decreased bodyweight gain was observed in rats and mice fed diets containing 3% sucralose for 104 weeks. This effect was not observed in beagle dogs dosed with 3% sucralose (equivalent to 750 mg/kg bw/day) in the diet for 52 weeks. A minimal increase in the incidence of renal pelvic mineralisation and epithelial hyperplasia lesions were detected in rats, primarily females treated with 3% sucralose. A significant decrease in erythrocyte count was detected in female mice dosed with 3% sucralose. Decreased bodyweight gain and a small increase in the incidence of hepatocellular clear cell foci was observed in female rats treated dose with an equimolar mixture of 4-CG and 1,6-DCF at 2000 ppm in the diet for 104 weeks. A number of studies have been conducted examining the acceptability and palatability of sucralose as a cause of reduced bodyweight gain when administered in drinking water or diet. It was determined that reduced bodyweight resulted from reduced palatability of diets containing sucralose. The dietary NOEL for mice and rats was determined to be 30 000 ppm (equivalent to 1500 mg/kg bw/day). The dietary NOEL for the sucralose hydrolysis products was determined to be 600 ppm (equivalent to 30 mg/kg bw/day).

The notified chemical was not teratogenic in rats and rabbits, was not neurotoxic in mice and monkeys, and had no effect on male and female reproduction in rats, or insulin secretion and carbohydrate metabolism in normal and diabetic human volunteers. Sucralose was found to induce a reduction in thymus weight in rats dosed orally with 3000 mg/kg bw/day. The NOEL for immunological endpoints was 750 mg/kg bw/day.

The sucralose hydrolysis products, 4-CG and 1,6-DCF, when test as an equimolar mixture was not teratogenic, not neurotoxic, and had no effect on male and female reproduction.

The notified chemical is not determined to be a hazardous substance according to the NOHSC *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 1999).

10. ASSESSMENT OF ENVIRONMENTAL EFFECTS

The following ecotoxicity studies have been supplied by the notifier and were carried out

according to OECD Principles of Good Laboratory Practice and Test Methods, or accepted equivalent.

Test	*Species*	*Results*
96 h Acute toxicity US EPA	Bluegill sunfish (*Lepomis macrochirus*)	LC_{50} >3200 mg/L
96 h Acute toxicity OECD TG 203	Rainbow trout	LC_{50} =1800 mg/L LC_{50} >2400 mg/L
48 h Acute toxicity OECD TG 202	Daphnia magna	EC_{50} >1800 mg/L
21 d Chronic toxicity OECD guidelines	Daphnia magna	NOEC =1800 mg/L
96h Acute toxicity OECD TG 201	Green Algae (*Selenastrum capricornutum*)	NOEC =1800 mg/L E_bC_{50} >1800 mg/L E_rC_{50} >1800 mg/L
3 h respiratory toxicity OECD TG 209-respirometric technique	Activated sludge micro-organisms	NOEC = 100 mg/L

* NOEC - no observable effect concentration

Imperial Chemicals Industries PLC (1985a) studied the toxicity of the notified chemical on Bluegill sunfish using the US EPA Office of Toxic Substances Guidelines for Testing Chemicals (EG-9). The fish were exposed to the notified chemical at the nominal concentrations of 0, 320, 560, 1000, 1800 and 3200 mg/L under static conditions at 22ºC for 96 hours. Mortalities were observed in 1800 and 3200 mg/L were attributed to stress due to decreased dissolved oxygen. These were the only mortalities observed during the 96 hours except for 1 in the control. Therefore, the LC_{50} is greater than 3200 mg/L.

Aquatox Ltd (1984b) investigated the acute fish toxicity of the notified chemical using Rainbow trout and following OECD TG 203. Two definitive tests were conducted under static conditions at a temperature of 14ºC. In the first the nominal concentrations used were 0, 560, 1000 and 1800 mg/L. In this study, 50% mortality was observed at 1800 mg/L. The estimated LC_{50} of 1800 mg/L was calculated by the moving averages method. In the second study the nominal concentrations used were 0, 560, 1000, 1800 and 2400 mg/L. Only 20% mortality observed in the highest concentration (2400 mg/L) with none in any other concentrations. Therefore, the LC_{50} is greater than 2400 mg/L with the NOEC being 1800 mg/L.

The acute toxicity of the notified chemical was studied by Aquatox Ltd (1984c) following the OECD TG 202. Daphnia were observed for 48 hours under static conditions at the nominal concentrations of 0, 180, 560, 1000 and 1800 mg/L. At 1800 mg/L no immobilisation was observed, while at 180 and 1000 mg/L 5% immobilisation was observed. Therefore from the study results it can only be said that the EC_{50} is greater than the maximum concentration studied (ie >1800 mg/L).

Imperial Chemicals Industries PLC (1986a) studied the chronic toxicity of the notified chemical to Daphnia magna following the OECD guidelines. Daphnia, older than 24 hours of age, were exposed to the notified chemical under semi-static conditions at 20ºC for 21 days. The nominal concentrations used were 0, 180, 320, 560, 1000 and 1800 mg/L. During the study mortality and number of offspring were observed. No effects were observed over the full 21 days, therefore, the LC_{50} is greater than 1800 mg/L and the NOEC is 1800 mg/L.

Imperial Chemicals Industries PLC (1986b) investigated the toxicity of the notified chemical on green algae following the OECD TG 201, with an extended test duration of 96 hours. The nominal concentrations studied were 0, 180, 320, 560, 1000 and 1800 mg/L. For the 96 hours the test vessels were maintained at 24ºC. Based on area under the growth curve, and logarithm growth rate, the E_bC_{50} and E_rC_{50} were found to be greater than 1800 mg/L, while the NOEC was 1800 mg/L.

These studies indicate that the notified chemical is practically non-toxic to aquatic organisms (fish, Daphnia and algae).

The toxicity of the notified chemical to aerobic micro-organisms was studied by Imperial Chemicals Industries PLC (1985b) following the OECD TG 209 (Respirometric technique). The nominal concentrations of the notified chemical were 0, 10, 32, 100, 180 and 320 mg/L. 3,5-Dichlorophenol was used as the control substance. Each test beaker included 200 mL of activated sludge. The beakers were kept at 22ºC and aerated continuously for 3 hours. After 3 hours a respirometric cell was used to determine the oxygen uptake rate. The results indicated that at 180 and 320 mg/L of notified chemical there had been a slight inhibition of respiration, with no impact at other concentrations. The NOEC therefore, is 100 mg/L and the LC_{50} is greater than 320 mg/L. These results indicate that the notified chemical is very slightly toxic to sewage sludge micro-organisms (Mensink, 1995).

11. ASSESSMENT OF ENVIRONMENTAL HAZARD

The intended use pattern of the notified chemical is expected to result in the majority of the chemical being eventually released to the environment. However, this will be in a dilute manner, as the notified chemical contained within a mouthwash will be released from domestic use at low concentrations. The ecotoxicity data indicates the notified substance is practically non-toxic to fish, daphnia and algae and very slightly toxic to sewage micro-organism based on measured concentrations.

In a worst case based on maximum annual imports of 175 kg per annum, all of which is released to sewer and assuming no removal during sewage treatment processes, a national population of 19,000,000 and an average personal contribution of 150 L/day to overall sewage flows, the predicted concentration in sewage effluent on a nationwide basis is estimated as 0.17 μg/L.

Amount of sucralose entering sewer annually	175 kg
Population of Australia	19 million
Amount of water used per person per day	150 L
Number of days in a year	365
Estimated PEC	0.17 μg/L (0.17 ppb)

When released to receiving waters the concentration is reduced by a further factor of at least 10, so the Predicted Environmental Concentration (PEC) is around 0.017 μg/L.

The nationwide PEC estimate indicates that after discharge to receiving waters the environmental concentration of the notified chemical will be at least 6 orders of magnitude less than the demonstrated toxicity to micro-organisms (LC_{50} >320 mg/L).

Wastes containing the notified chemical including residues from imported drums and from repackaging will also be disposed of in landfill where it may leachout at very low concentrations.

Therefore, the environmental exposure and overall environmental hazard from the notified chemical is expected to be acceptable.

12. ASSESSMENT OF PUBLIC AND OCCUPATIONAL HEALTH AND SAFETY EFFECTS

Hazard Assessment

Based on the toxicological data provided, the notified chemical would not be acutely toxic via the oral routes. It is not likely to be genotoxic or clastogenic.

Decreased bodyweight gain, a minimal increase in the incidence of renal pelvic mineralisation and epithelial hyperplasia lesions and a decrease in erythrocyte count have been observed upon repeated exposure to 3% sucralose. The dietary NOEL for mice and rats was determined to be 30 000 ppm (equivalent to 1500 mg/kg bw/day).

The notified chemical was not teratogenic or neurotoxic and had no effect on reproduction, insulin secretion and carbohydrate metabolism. Sucralose was found to induce a reduction in thymus weight in rats dosed orally with 3000 mg/kg bw/day. The NOEL for immunological endpoints was 750 mg/kg bw/day.

The notified chemical would not be classified as a hazardous substance according to NOHSC *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 1999) in terms of the toxicological data provided.

Occupational Health and Safety

Factory workers will have the potential for exposure to the notified chemical during the formulation of the mouth wash products containing 0.06 % notified chemical. Possible dermal and ocular exposure to spills and splashes containing the notified chemical may occur during the addition of Splenda (25 % notified chemical) to the mixing vessel, during the mixing of the mouth wash products, and when connecting lines for filling into PET bottles. Dermal and ocular exposure will be controlled by the use of overalls, protective gloves and eye protection. Inhalation exposure to any aerosols generated during the mixing of the mouth wash is expected to be negligible, as the mixing process occurs in a semi automated closed system in an area with an exhaust ventilation system.

QC sampling and testing workers may receive dermal and ocular exposure to drips and spills containing the notified chemical during sampling and testing of mouth wash formulations. Although not indicated by the notifier, QC sampling and testing workers should wear laboratory coats, protective gloves and eye protection when handling solutions containing the notified chemical to control exposure.

Retail outlet workers are unlikely to be exposed to the notified polymer unless the packaging is breached. Waterside, warehouse and transport workers are unlikely to be exposed to the notified polymer unless the packaging is breached.

Given the non-hazardous nature of the notified chemical and the low potential for exposure, the health risk to workers handling the notified chemical is negligible.

Public Health

The notified chemical is approved for use in foods and therapeutic goods for oral ingestion. The transient nature of the contact with the oral cavity, the very low concentration of the notified chemical in the mouth wash, the low enteric absorption rate and the low toxicity of the notified chemical and its metabolites, suggest that the notified chemical will not pose a significant hazard to public health when used as proposed.

13. RECOMMENDATIONS

Control Measures

Occupational Health and Safety

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

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

Secondary notification

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

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

The Director will then decide whether secondary notification is required.

No additional secondary notification conditions are stipulated.

14. MATERIAL SAFETY DATA SHEET

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

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

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Attachment 1

The Draize Scale (Draize, 1959) for evaluation of skin reactions is as follows:

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

The Draize scale (Draize *et al*., 1944) for evaluation of eye reactions is as follows:

CORNEA

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

CONJUNCTIVAE

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

IRIS

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

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