File No: STD/1386

October 2011

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

Esterquat

This Assessment has been compiled in accordance with the provisions of the *Industrial Chemicals (Notification and Assessment) Act 1989* (Cwlth) (the Act) and Regulations. This legislation is an Act of the Commonwealth of Australia. The National Industrial Chemicals Notification and Assessment Scheme (NICNAS) is administered by the Department of Health and Ageing, and conducts the risk assessment for public health and occupational health and safety. The assessment of environmental risk is conducted by the Department of Sustainability, Environment, Water, Population and Communities.

For the purposes of subsection 78(1) of the Act, this Public Report may be inspected at our NICNAS office by appointment only at Level 7, 260 Elizabeth Street, Surry Hills NSW 2010.

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

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Director
NICNAS

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SUMMARY

The following details will be published in the NICNAS *Chemical Gazette:*

ASSESSMENT REFERENCE	APPLICANT(S)	CHEMICAL OR TRADE NAME	HAZARDOUS SUBSTANCE	INTRODUCTION VOLUME	USE
STD/1386	Procter & Gamble Australia Pty. Limited Costco Wholesale Australia; Pty. Ltd	Esterquat	No	≤ 1,000 tonnes per annum	A component of domestic fabric softener at concentrations up to 15%.

CONCLUSIONS AND REGULATORY OBLIGATIONS

Hazard classification
Based on the data provided the notified chemical is not classified as hazardous according to the *Approved Criteria for Classifying Hazardous Substances* [NOHSC:1008(2004)].

The classification of the notified chemical using the Globally Harmonised System for the Classification and Labelling of Chemicals (GHS) (United Nations 2009) is presented below. This system is not mandated in Australia and carries no legal status but is presented for information purposes.

	Hazard category	*Hazard statement*
Environment	Acute 2	Toxic to aquatic life
Environment	Chronic 3	Harmful to aquatic life with long lasting effects

Human health risk assessment
Under the conditions of the occupational settings described, the notified chemical is not considered to pose an unacceptable risk to the health of workers.

When used in the proposed manner, the notified chemical is not considered to pose an unacceptable risk to public health.

Environmental risk assessment
On the basis of the PEC/PNEC ratio and the reported use pattern, the notified chemical is not considered to pose a risk to the environment at the proposed import quantity.

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 *Approved Criteria for Classifying Hazardous Substances* [NOHSC:1008(2004)] workplace practices and control procedures consistent with provisions of State and Territory hazardous substances legislation must be in operation.

Disposal

- The notified chemical should be disposed of to landfill.

Emergency procedures

- Spills or accidental release of the notified chemical should be handled by containment, collection and subsequent safe disposal.

Regulatory Obligations

Secondary Notification
This risk assessment is based on the information available at the time of notification. The Director may call for the reassessment of the chemical under secondary notification provisions based on changes in certain circumstances. Under Section 64 of the *Industrial Chemicals (Notification and Assessment) Act (1989)* the notifier, as well as any other importer or manufacturer of the notified chemical, have post-assessment regulatory obligations to notify NICNAS when any of these circumstances change. These obligations apply even when the notified chemical is listed on the Australian Inventory of Chemical Substances (AICS).

Therefore, the Director of NICNAS must be notified in writing within 28 days by the notifier, other importer or manufacturer:

(1) Under Section 64(1) of the Act; if
- the importation volume exceeds 1,000 tonnes per annum notified chemical;

or

(2) Under Section 64(2) of the Act; if
- the function or use of the chemical has changed from a component of domestic fabric softener at concentrations up to 15%, or is likely to change significantly;
- the amount of chemical being introduced has increased from 1,000 tonnes per year, or is likely to increase, significantly;
- the chemical has begun to be manufactured in Australia;
- additional information has become available to the person as to an adverse effect of the chemical on occupational health and safety, public health, or the environment.

The Director will then decide whether a reassessment (i.e. a secondary notification and assessment) is required.

Material Safety Data Sheet
The MSDS of the notified chemical provided by the notifier was reviewed by NICNAS. The accuracy of the information on the MSDS remains the responsibility of the applicant.

ASSESSMENT DETAILS

1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)
Procter & Gamble Australia Pty. Limited (ABN 91 008 396 245)
Level 4, 1 Innovation Road
MACQUARIE PARK, NSW 2113

Costco Wholesale Australia; Pty. Ltd. (ABN 57 104 012 893)
17-21 Parramatta Road
LIDCOMBE, NSW 2141

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

EXEMPT INFORMATION (SECTION 75 OF THE ACT)
Data items and details claimed exempt from publication: chemical name, other names CAS number, molecular and structural formulae, molecular weight, analytical data, degree of purity, impurities and use details.

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
Variation to the schedule of data requirements is claimed as follows: Bioaccumulation.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)

None

NOTIFICATION IN OTHER COUNTRIES
Europe (2010)

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
Esterquat

MOLECULAR WEIGHT
> 500 Da

ANALYTICAL DATA
Reference NMR, IR, HPLC and UV spectra were provided.

3. COMPOSITION

DEGREE OF PURITY ~98%

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20°C AND 101.3 kPa: White powder

Property	Value	Data Source/Justification
Melting Point	54°C	Measured
Boiling Point	> 150°C at 101.3 kPa.	Measured
Density	1,040 kg/m^3 at 20°C	Measured
Vapour Pressure	Not determined	Notified chemical is an ionic solid and hence the vapour pressure is expected to be low.
Water Solubility	17.6×10^{-3} g/L at 20°C	Estimated
Hydrolysis as a Function of pH	Not determined	Not required as the notified chemical is readily biodegradable.
Partition Coefficient (n-octanol/water)	log Pow = 3.8	Measured for an analogue chemical. The report author noted that the study does not need to be done as it is technically not feasible to determine the log Kow of the notified chemical due to its surface active properties.
Surface Tension	68.3 mN/m at 20°C	Measured
Adsorption/Desorption	log K_{oc} = 4.3 (soil + sludge) and 4.7 (soil)	Measured
Dissociation Constant	Not determined	The notified chemical does not contain structural elements that are capable of dissociation.
Flash Point	Not determined	Notified chemical is an ionic solid.
Flammability	Not highly flammable	Measured
Autoignition Temperature	Not auto-flammable between 20°C and 54°C (melting temperature)	Measured
Explosive Properties	Not expected to be explosive	The structural formula contains no explosophores.

DISCUSSION OF PROPERTIES
For full details of tests on physical and chemical properties, refer to Appendix A.

Reactivity
Stable under normal conditions of use.

5. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS
The notified chemical will not be manufactured within Australia.
The notified chemical will be imported in finished domestic fabric softener products at concentrations up to 15%.

MAXIMUM INTRODUCTION VOLUME OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS
Over the next five years it is estimated that a total of 1,300 tonnes will be introduced.

PORT OF ENTRY
Port Botany

TRANSPORTATION AND PACKAGING
The notified chemical will be imported in both high and low density polyethylene containers of 400 mL and 1 L volume.

USE
The notified chemical will be used as a component of domestic fabric softener at concentrations up to 15%.

OPERATION DESCRIPTION
The notified chemical will be imported in finished products and will not be manufactured or reformulated in Australia.

The finished domestic fabric softener products containing the notified chemical will be mixed with water and used for the washing of clothes. Approximately 20-40 mL of the finished product containing the notified chemical at concentrations of up to 15% will be used per wash.

6. HUMAN HEALTH IMPLICATIONS

6.1 Exposure assessment

6.1.1 Occupational exposure

EXPOSURE DETAILS
The notified chemical will not be manufactured or reformulated in Australia and hence occupational exposure will be limited to transport and warehouse workers. It is expected that transport and warehouse workers handling the imported aqueous solution containing up to 15% notified chemical will only be exposed to the notified chemical in the event of spills due to an accident or as a result of leaking container. The main route of exposure in this situation will be dermal.

6.1.2. Public exposure

The public will potentially be exposed to the notified chemical during the transfer of fabric softener containing the notified chemical to washing machines, during hand washing of clothing and during the wearing of clothing that has been washed using fabric softener. The main route of exposure will be dermal.

The dermal exposure to the notified chemical from wearing clothing that has been washed using fabric softener containing it can be estimated using the following equation (SDA, 2005).

$$\text{Exposure} = \frac{A \times PR \times PT \times DA \times CF}{BW}$$

A = amount used: the label for the notified chemical suggests up to 40 mL per use, when this is multiplied by the concentration (15%), the average of 4 (EC TGD, 2003) washes per week the amount used per day is 3.43 mL/day. Converting this to grams using the density gives the amount of notified chemical used per day as 3.57 g/day.
PR = Product retained on the clothing at the end of the wash = 0.95% (SDA, 2005)
PT = Product transferred from the clothing to the skin = 10% (SDA, 2005)
DA = Dermal absorption = 1.4% (see section 6.2 below)

CF = Conversion factor for converting from g to μg (1,000,000)
BW = Body weight = 60 kg (SDA, 2005)

The estimated daily exposure therefore is 0.79 μg/kg bw/day. This value is also likely to be an overestimate as the amount used is calculated using a household rather than individual basis.

Based on the above calculation exposure to the notified chemical from wearing clothes that have been washed with products containing it is negligible. Dermal exposure to the notified chemical during hand washing will be limited due to the further dilution of the notified chemical in the water.

6.2. Human health effects assessment

The results from toxicological investigations conducted on the notified chemical are summarised in the table below. Details of these studies can be found in Appendix B. **Error! Reference source not found.**

Endpoint	*Result and Assessment Conclusion*
Rat, acute oral toxicity	LD50 > 10,000 mg/kg bw; low toxicity
Mouse, acute oral toxicity	LD50 > 10,000 mg/kg bw (in males); low toxicity
Rat, acute dermal toxicity	LD50 > 2,000 mg/kg bw; low toxicity
Rabbit, skin irritation (2 studies)	slightly irritating
Rabbit, eye irritation (2 studies)	slightly irritating
Guinea pig, skin sensitisation – non-adjuvant test. (2 studies)	no evidence of sensitisation up to 15%
Human, skin sensitisation – HRIPT (3 studies)	no evidence of sensitisation up to 20%
Rat, repeat dose oral toxicity – 28 days.	NOAEL > 500 mg/kg bw/day
Rat, repeat dose oral toxicity – 91 days.	NOAEL > 500 mg/kg bw/day
Mutagenicity – bacterial reverse mutation	non mutagenic
Genotoxicity – in vitro Mammalian Chromosome Aberration Test (Mouse Lymphoma L1578Y Cells)	non genotoxic
Genotoxicity – in vitro Mammalian Chromosome Aberration Test (Chinese Hamster Ovary Cells)	non genotoxic

Toxicokinetics, metabolism and distribution.
Two different toxicokinetics studies have been conducted on the notified chemical, one investigating oral absorption and the other dermal absorption (Evonik, 2010). In the oral absorption study the notified chemical radio labelled with ^{14}C was administered to 4 male Sprague-Dawley rats by gavage at a single dose of 112 mg/kg bw. At the end of the 72-hour test period, from the total radioactivity administered 48 ± 4 % was recovered in the faeces plus GI wash, 46 ± 6 % in the urine plus cage wash, 1.4 ± 0.2 % in the tissues plus carcass and 0.38 ± 0.04 % in the expired carbon dioxide. The total oral absorption of the notified chemical was therefore 48 ± 6 % over the 72-hour test period. Over 72 hours, 96 % of the absorbed radioactivity was excreted in the urine, 3 % was detected in tissues and carcass at 72 hours and < 1 % was eliminated in the expired carbon dioxide. In the dermal absorption study the notified chemical radio labelled with ^{14}C was administered to 4 male Sprague-Dawley rats at a single dose of 1.62 mg/cm^2 (62.7 mg/kg bw). About 1.03 % of the notified chemical was recovered in the urine/cage wash, ~0.16 % in expired CO2, ~0.13 % in tissue, and ~0.05 % in faeces/GI tract. A total of < 1.4 % (normalised for 100 % recovery) of the administered dose was absorbed over the 72-hour test period.

Acute toxicity.
The notified chemical is considered to be of low acute toxicity (LD50 > 10,000 mg/kg bw) via the oral route based on tests conducted in rats and mice and of low acute toxicity (LD50 > 2,000 mg/kg bw) via the dermal route based on a study conducted in rabbits.

Irritation and Sensitisation.
Based on tests conducted in rabbits the notified chemical is considered to be slightly irritating to the skin and eye.

The notified chemical did not induce sensitisation in guinea pigs at concentration up to 15% in 2 Buehler non adjuvant tests. In 3 HRIPTs (two at 20% and one at 1.5% concentration) irritation (mild erythema only) was observed. However, as the number of reactions reduced from the 48 hour to the 96 hour observation period and

no oedema was observed (which is more indicative of a sensitisation reaction) the notified chemical was considered to be slightly irritating but non-sensitising in these tests.

Repeated Dose Toxicity (sub chronic).
In two different repeated dose toxicity studies oral administration of the notified chemical to rats for a period of either 28 or 91 consecutive days at dose levels of 1, 10 and 500 mg/kg/day resulted in no adverse treatment related effects at any dose level. Therefore, the NOAELs were established as > 500 mg/kg bw/day.

Mutagenicity.
The notified chemical was found to not be mutagenic using a bacterial reverse mutation test, and is not clastogenic to Chinese hamster ovary cells or mouse lymphoma L1578Y cells *in vitro*.

Toxicity for reproduction.
In a developmental toxicity study groups of 25 mated female Wistar rats were dosed orally with the notified chemical daily from day 6 through 15 post coitum, at dose levels of 0, 50, 250 and 1000 mg/kg bw/day (Evonik, 2010). No test substance-related effects in the dosed female rats were noted as reaction to treatment. There were slightly increased incidences of post-implantation losses in the 1000 mg/kg bw/day dose group; however, the values were within the range of the historical control values recorded at the same laboratory. There were no test substance-related effects on the foetuses up to the limit dose of 1000 mg/kg bw/day.

Health hazard classification
Based on the available data the notified chemical is not classified as hazardous according to the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004).

6.3. Human health risk characterisation

6.3.1. Occupational health and safety
As worker exposure to the notified chemical will be limited to spills due to an accident or as a result of leaking container, and considering the low hazardous nature of the notified chemical, the risk to these workers is not considered to be unacceptable.

6.3.2. Public health
The general public will be exposed to the notified chemical during the transfer of fabric softener containing the notified chemical at up to 15% concentration to washing machines and during hand washing of clothing. Exposure may also occur during wearing of clothing that has been washed using the fabric softener containing the notified chemical.

The notified chemical is a slight skin and eye irritant. However, the notified chemical will be present in fabric softener at concentrations ≤ 15% and therefore the risk of irritation is not expected.

The risk of systemic effects from dermal exposure is expected to be low based on the low dermal absorption (< 1.4%) of the notified chemical. The notified chemical showed no adverse effects at doses up to 500 mg/kg bw/day in the repeat dose toxicity studies. Therefore the risk of adverse systemic effects following exposure is not considered to be unacceptable.

7. ENVIRONMENTAL IMPLICATIONS

7.1. Environmental Exposure & Fate Assessment

7.1.1 Environmental Exposure

RELEASE OF CHEMICAL AT SITE
The notified chemical will be imported into Australia as a component (≤ 15%) of finished domestic fabric softener products for direct end-use in Australia. No local reformulation or repackaging will take place and therefore no significant release is expected to occur in Australia as a result of these processes.

RELEASE OF CHEMICAL FROM USE
The notified chemical in fabric softener formulation will be used in domestic laundries that normally drain to the sewer. As such, almost all of the imported volume (anticipated maximum of 1000 tonnes per year) of the notified chemical could be released into the aquatic environment.

RELEASE OF CHEMICAL FROM DISPOSAL
Spilt material is expected to be disposed of to landfill after containment and collection. Container residues will be disposed of to landfill with the containers or washed to sewer when containers are rinsed before recycling. Residues removed during equipment cleaning are likely to be flushed to sewer.

7.1.2 Environmental fate

The notified chemical is expected to be largely degraded during sewage treatment as it is readily biodegradable. A small proportion may be discharged to receiving waters in treated effluent as the notified chemical is expected to disperse and degrade. Bioaccumulation is not expected as the notified chemical is readily biodegradable. For the details of the environmental fate studies refer to Appendix C.

7.1.3 Predicted Environmental Concentration (PEC)

The PEC can be estimated as outlined below based on the hypothetical worst case assumptions of complete discharge to receiving waters via sewage treatment works nationwide. The PEC is calculated assuming a volume of 1000 tonnes/year as a worst case, the notified chemical is readily biodegradable within 28 d and it is expected to partition to some extent to sludge (log Pow ~ 3.8).

Predicted Environmental Concentration (PEC) for the Aquatic Compartment		
Total Annual Import/Manufactured Volume	1,000,000	kg/year
Proportion expected to be released to sewer	100.000%	
Annual quantity of chemical released to sewer	1,000,000.00	kg/year
Days per year where release occurs	365	days/year
Daily chemical release:	2739.73	kg/day
Water use	200.0	L/person/day
Population of Australia (Millions)	21.161	million
Removal within STP	73%	Mitigation
Daily effluent production:	4,232	ML
Dilution Factor - River	1.0	
Dilution Factor - Ocean	10.0	
PEC - River:	64.74	µg/L
PEC - Ocean:	6.47	µg/L

STP effluent re-use for irrigation occurs throughout Australia. The agricultural irrigation application rate is assumed to be 1000 L/m^2/year (10 ML/ha/year). The notified chemical in this volume is assumed to infiltrate and accumulate in the top 10 cm of soil (density 1500 kg/m^3). Using these assumptions, irrigation with a concentration of 64.74 µg/L may potentially result in a soil concentration of approximately 0.432 mg/kg. Assuming accumulation of the notified chemical in soil for 5 and 10 years under repeated irrigation, the concentration of notified chemical in the applied soil in 5 and 10 years may be approximately 2.16 mg/kg and 4.32 mg/kg, respectively.

7.2. Environmental effects assessment

The results from ecotoxicological investigations conducted on the notified chemical and an analogue chemical are summarised in the table below. Since the acute fish toxicity study results are considered unreliable, a modelled estimate (ECOSAR (v1.00), surfactants, cationic; US EPA, 2009) for the fish toxicity of the notified chemical is also reported below. Details of these studies can be found in Appendix C.

Endpoint	*Result*	*Assessment Conclusion*
Acute Toxicity		
Fish Toxicity	96 h LC50 = 2.1 mg/L*	Toxic
Daphnia Toxicity	24 h LC50 = 14.8 mg/L	Harmful
Algal Toxicity	72 h E_rC50 = 6.3 mg/L	Toxic
Chronic Toxicity		

Fish Toxicity	35 d NOEC = 0.686 mg/L	Harmful with long lasting effects
Daphnia Toxicity	21 d NOEC = 1.0 mg/L	Harmful with long lasting effects
Algal Toxicity	72 h NOEC = 1.5 mg/L	Not classified

*calculated

Under the Globally Harmonised System of Classification and Labelling of Chemicals (United Nations, 2009) the notified chemical is considered to be acutely toxic to algae and fish, and chronically harmful to fish and daphnia.

7.2.1 Predicted No-Effect Concentration

The lower limit of the median effect concentrations from ecotoxicological studies on the notified chemical was used to calculate the PNEC. An assessment factor of 10 was used as both acute and chronic toxicity endpoints are available for the effects of the notified chemical on aquatic species from three trophic levels.

Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment		
35 day NOEC (Fish)	0.686	mg/L
Assessment Factor	10	
Mitigation Factor	1.00	
PNEC:	68.60	µg/L

7.3. Environmental risk assessment

Insert the Risk Quotient Table (PEC/PNEC)

Risk Assessment	PEC µg/L	PNEC µg/L	Q
Q - River	64.74	68.6	0.944
Q - Ocean	6.47	68.6	0.094

The Risk Quotients (Q = PEC/PNEC) for the worst case discharge scenario have been calculated to be < 1 for the river and ocean compartments. Therefore, the notified chemical is not expected to pose an unacceptable risk to the aquatic environment based on its reported use pattern at the proposed import quantity.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Melting Point/Freezing Point 54ºC

Method	OECD TG 102 Melting Point/Melting Range. EC Directive 92/69/EEC A.1 Melting/Freezing Temperature.
Remarks	Determined using DSC. No significant protocol deviations.
Test Facility	NOTOX (2009)

Boiling Point > 150ºC at 101.3 kPa

Method	OECD TG 103 Boiling Point. EC Directive 92/69/EEC A.2 Boiling Temperature.
Remarks	Decomposes at approximately 150ºC at 101.3 kPa, no sign of boiling was observed prior to decomposition.
Test Facility	NOTOX (2009)

Density 1040 kg/m^3 at 20ºC

Method	OECD TG 109 Density of Liquids and Solids. EC Directive 92/69/EEC A.3 Relative Density.
Remarks	Measured using a gas comparison stereopycnometer. No significant protocol deviations.
Test Facility	NOTOX (2009)

Water Solubility 17.6 × 10^{-3} g/L at 20ºC

Method	OECD TG 105 Water Solubility. EC Directive 92/69/EEC A.6 Water Solubility.
Remarks	A preliminary flask test indicated that the notified chemical formed an in-separable emulsion with water at concentration above 10 mg/L. Since it was not possible to remove the excess of the notified chemical from the aqueous phase by centrifugation or filtration, the flask method could not be applied for the determination of the water solubility of the notified chemical. Therefore, a definitive test was conducted by turbidity measurements according to method A2 of ASTM International E1148-02. Eight suspensions of the notified chemical in double distilled water at target concentrations of 2.08, 5.20, 10.0, 24.7, 49.0, 100, 252 and 502 mg/L were prepared in flasks. The contents of the flask were stirred for 72 h at 19.7 ± 0.5ºC. Duplicate sub-samples were taken from the test solutions and the degree of turbidity was measured for each sample using a spectrophotometer. The water solubility of the notified chemical was estimated to be 17.6 mg/L.
Test Facility	NOTOX (2009)

Surface Tension 68.3 mN/m at 20ºC

Method	OECD TG 115 Surface Tension of Aqueous Solutions. EC Directive 92/69/EEC A.5 Surface Tension.
Remarks	Concentration:15.2 mg/L The study authors note that the test result is not in line with the expected surface tension behaviour of the notified chemical. Cationic surfactants such as the notified chemical carrying two C18 alkyl chains are designed to possess surface active properties and typically exhibit surface tension values as low as 27 mN/m (depending on the area per molecule) when studied on a Langmuir film balance. Therefore due to the intrinsic properties (crystallization) of the notified chemical at temperatures below the melting point no reliable results were obtained using the ring method.
Test Facility	NOTOX (2009)

Partition Coefficient (n-octanol/water) log Pow = 3.8

Method Not provided
Remarks Measured value for an analogue chemical.
Test Facility (Evonik, 2010)

Adsorption/Desorption Koc = 20225 (log Koc = 4.3 for soil and sludge) Koc = 50882 (log Koc =4.7 for soil)

Method OECD TG 106 Adsorption - Desorption Using a Batch Equilibrium Method.
Remarks This test was conducted using two sludges and three soils. During the stability test and the adsorption/desorption kinetic experiment, it was found that the notified chemical was not fully stable under the experimental conditions based on the TLC results. A Koc =20225 was calculated as the geometric mean of the two sludges and two soils whereas a Koc = 828 was calculated as the geometric mean of the two sludges and a Koc = 494 × 103 was calculated as the geometric mean of the two soils. Only a summary of study results was provided with the application.
Test Facility Procter and Gamble (2009)

Flammability Not highly flammable

Method EC Directive 92/69/EEC A.10 Flammability (Solids).
Remarks No significant protocol deviations.
Test Facility NOTOX (2009)

Autoignition Temperature Not auto-flammable between 20ºC and 54ºC (melting temperature)

Method EC Directive 92/69/EEC A.16 Relative Self-Ignition Temperature for Solids.
Remarks No significant protocol deviations.
No autoignition was seen up to temperatures of 400ºC and the sample was black and charred at the end of the test.
Test Facility NOTOX (2009)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral

TEST SUBSTANCE	Notified chemical
METHOD	P&G protocol C 1 B (1985) OECD TG 401 Acute Oral Toxicity – Limit Test.
Species/Strain	Rat/WISW
Vehicle	Oleum arachidis
Remarks - Method	No significant protocol deviations

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
I	5 per sex	10,000	0/10

LD50	> 10,000 mg/kg bw
Signs of Toxicity	There were no deaths. No signs of systemic toxicity were noted.
Effects in Organs	No abnormalities were noted at necroscopy
Remarks - Results	Body weight gains were as expected.

CONCLUSION	The notified chemical is of low toxicity via the oral route.
TEST FACILITY	IBR (1986a)

B.2. Acute toxicity – oral

TEST SUBSTANCE	Notified chemical
METHOD	Irwin dose range study
Species/Strain	Mouse/CD-1
Vehicle	Water
Remarks - Method	Necropsy was not performed after study termination and changes in bodyweights were not recorded.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
I	4 male	0	0/4
II	4 male	316	0/4
III	4 male	1,000	0/4
IV	4 male	3,160	0/4
V	4 male	10,000	1/4

LD50	> 10,000 mg/kg bw
Signs of Toxicity	All mice dosed with 10 000 mg/kg bw showed slight apathy between 10 and 20 minutes after dosing. In addition, one animal in this group showed further indication of CNS depression when observed at 30 and 90 minutes after treatment. This animal appeared normal during the observations performed at 150 and 300 minutes; however it died overnight between day one and day two.
Effects in Organs	Necroscopy was not performed.
Remarks - Results	No signs of toxicity were recorded in animals in groups I to IV.

CONCLUSION	The notified chemical is of low toxicity via the oral route.

TEST FACILITY Huntingdon (1986a)

B.3. Acute toxicity – dermal

TEST SUBSTANCE Notified chemical

METHOD P&G protocol C 1 B (1985)
OECD TG 402 Acute Dermal Toxicity.
Species/Strain Rabbit/New Zealand White
Vehicle Oleum arachidis
Type of dressing Semi-occlusive.
Remarks - Method No significant protocol deviations

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
I	3 per sex	2,000	0/6

LD50 > 2,000 mg/kg bw
Signs of Toxicity - Local Slight to moderate erythema and moderate oedema were observed at observations up to 3 days. All signs of irritation had cleared by the 7th day.
Signs of Toxicity - Systemic There were no deaths or test-substance related clinical signs. There were no signs of systemic toxicity.
Effects in Organs No abnormalities were noted at necroscopy
Remarks - Results Body weight gains were as expected.

CONCLUSION The notified chemical is of low toxicity via the dermal route.

TEST FACILITY IBR (1986b)

B.4. Irritation – skin

TEST SUBSTANCE Notified chemical (40% in corn oil)

METHOD P&G protocol C 9-E (1986)
OECD TG 404 Acute Dermal Irritation/Corrosion.
Species/Strain Rabbit/New Zealand White
Number of Animals 3
Vehicle Corn oil
Observation Period 72 Hours
Type of Dressing Occlusive
Remarks - Method The irritation was not measured at 48 hours after exposure.
The experiment was conducted on both intact and abraded skin.

RESULTS

Table showing the results for the intact skin sites.

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3			
Erythema/Eschar	0.5	0.5	0	1	< 72 hours	0
Oedema	0	0	0	0	–	0

*Calculated on the basis of the scores at 24 and 72 hours for EACH animal.

Table showing the results for the abraded skin sites.

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3			
Erythema/Eschar	0.5	0	0.5	1	< 72 hours	0
Oedema	0	0	0	0	–	0

*Calculated on the basis of the scores at 24 and 72 hours for EACH animal.

Remarks - Results: A single 4-hour, occluded application of the test material to abraded and intact skin on the 3 rabbits produced slight erythema at the 4.5 and 24 hour observation. All treated skin sites appeared normal at the 72-hour observation.
No corrosive effects were noted.

CONCLUSION: The notified chemical is slightly irritating to the skin.

TEST FACILITY: Huntingdon (1986b)

B.5. Irritation – skin

TEST SUBSTANCE: Notified chemical

METHOD: OECD TG 404 Acute Dermal Irritation/Corrosion.
EC Directive 84/449/EEC B.4 Acute Toxicity (Skin Irritation).

Species/Strain	Rabbit/New Zealand White
Number of Animals	3 (2 female, 1 male)
Vehicle	Test substance was moistened with water before being applied.
Observation Period	72 Hours
Type of Dressing	Semi-occlusive.
Remarks - Method	No significant protocol deviations.

RESULTS

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3			
Erythema/Eschar	0.3	0	0.3	1	< 48 hours	0
Oedema	0	0	0	0	–	0

*Calculated on the basis of the scores at 24, 48, and 72 hours for EACH animal.

Remarks - Results: A single 4-hour, semi-occluded application of the test material to the intact skin of the 3 rabbits produced very slight erythema at the 24 hour observation in two rabbits. All treated skin sites appeared normal at the 48-hour observation. No corrosive effects were noted.

CONCLUSION: The notified chemical is slightly irritating to the skin.

TEST FACILITY: RCC (1993a)

B.6. Irritation – eye

TEST SUBSTANCE: Notified chemical

METHOD: OECD TG 405 Acute Eye Irritation/Corrosion.
EC Directive 92/69/EEC B.5 Acute Toxicity (Eye Irritation).

Species/Strain	Rabbit/New Zealand White
Number of Animals	3 (2 female, 1 male)
Observation Period	72 Hours

Remarks - Method 100 mg of the notified chemical was placed in each rabbit's eye.
Conjunctival discharge was not measured.

RESULTS

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3			
Conjunctiva: redness	0	0.3	0.3	1	< 48 Hours	0
Conjunctiva: chemosis	0	0	0	1	< 24 Hours	0
Corneal opacity	0	0	0	0	–	0
Iridial inflammation	0	0	0	0	–	0

*Calculated on the basis of the scores at 24, 48, and 72 hours for EACH animal.

Remarks - Results A single application of the test material to the non-irrigated eye of three rabbits produced mild conjunctival irritation. One treated eye appeared normal at the 24 hour observation with the remaining eyes appearing normal at the 48 hour observation.

CONCLUSION The notified chemical is slightly irritating to the eye.

TEST FACILITY RCC (1993b)

B.7. Irritation – eye

TEST SUBSTANCE Notified chemical

METHOD P&G protocol C2B (1985)
Species/Strain Rabbit/New Zealand White
Number of Animals 9 (2 Female, 7 male)
Observation Period 7 Days
Remarks - Method The nine rabbits were split into 2 groups. In the first group containing 6 rabbits the treated eyes were not rinsed. In the second group with three rabbits the treated eyes were rinsed with water 4 seconds after the notified chemical had been applied.
In both groups 10 mg of the notified chemical was applied to the eye.
The first observations were recorded 24 hours after the test material had been applied.

RESULTS

Results for the 3 rabbits in the treatment group where the eyes were rinsed with water.

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3			
Conjunctiva: redness	0.3	0	0.3	1	< 48 Hours	0
Conjunctiva: chemosis	0	0	0	0	–	0
Conjunctiva: discharge	0	0	0	0	–	0
Corneal opacity	0	0	0	0	–	0
Iridial inflammation	0	0	0	0	–	0

*Calculated on the basis of the scores at 24, 48, and 72 hours for EACH animal.

Remarks - Results No signs of irritation were seen in any of the 6 rabbits in the treatment group where the eyes were not rinsed.
In the group of 3 rabbits where the eyes were rinsed with water after treatment the test material produced mild conjunctival irritation in two animals at the 24 hour observation. At the 48 hour observation both eyes appeared normal.

CONCLUSION The notified chemical is slightly irritating to the eye.

TEST FACILITY Huntingdon (1986c)

B.8. Skin sensitisation

TEST SUBSTANCE Notified chemical

METHOD P&G protocol C4A (1986)
OECD TG 406 Skin Sensitisation – non-adjuvant Buehler test

Species/Strain Guinea pig/Pirbright, Dunkin Hartley Boe: DHPK (SPF-L&C.)/Boe

PRELIMINARY STUDY Maximum Non-irritating Concentration:
topical: 15% in acetone

MAIN STUDY

Number of Animals Test Group: 20 Control Group: 2 groups of 10

INDUCTION PHASE Induction Concentration:
topical: 15% in ethanol

Signs of Irritation No signs of irritation were seen during the induction phase.

CHALLENGE PHASE

1st challenge topical: 15% in acetone

2nd challenge topical: 7.5 and 15% in acetone

Remarks - Method In a dose range finding study mild irritations was seen in 3 out of 4 animals at 20% concentration, but no effects were seen at the lower concentrations.

RESULTS

Animal	*Challenge Concentration*	*Number of Animals Showing Skin Reactions after:*			
		1st challenge		*2nd challenge*	
		24 h	*48 h*	*24 h*	*48 h*
Test Group	7.5	N/A	N/A	0/20	0/20
	15	1/20	1/20	0/20	0/20
Control Group	7.5	N/A	N/A	0/10	0/10
	15	0/10	0/10	0/10	0/10

Remarks - Results 1 animal in the test group challenged at 15% concentration showed slight erythema. No skin reactions were observed after re-challenge at concentrations of 7.5 and 15%.

As there was no evidence of induction of the test group animals the test cannot be used to determine the sensitisation potential of the notified chemical.

CONCLUSION There was no evidence of reactions indicative of skin sensitisation to the notified chemical at concentrations up to 15% under the conditions of the test.

TEST FACILITY IBR (1986c)

B.9. Skin sensitisation

TEST SUBSTANCE Notified chemical

METHOD P&G protocol C 4 A-E (1986)
OECD TG 406 Skin Sensitisation – Buehler test

Species/Strain Guinea pig/Pirbright, Dunkin Hartley Boe: DHPK (SPF-L&C.)/Boe

PRELIMINARY STUDY Maximum Non-irritating Concentration:

	topical: 15% in acetone
MAIN STUDY	
Number of Animals	Test Group: 20 Control Group: 10
INDUCTION PHASE	Induction Concentration:
	topical: 15% in ethanol
Signs of Irritation	No signs of irritation were seen during the induction phase.
CHALLENGE PHASE	
1st challenge	topical: 7.5% in acetone
Remarks - Method	The notified chemical was applied three times at weekly intervals for induction.

RESULTS

Animal	*Challenge Concentration*	*Number of Animals Showing Skin Reactions after: 1st challenge*	
		24 h	*48 h*
Test Group	7.5	0/10	3/20
Control Group	7.5	0/10	0/10

Remarks - Results	At the 48 hour observation slight patcy erythema was seen in 3 animals. As there was no evidence of induction of the test group animals the test cannot be used to determine the sensitisation potential of the notified chemical.
CONCLUSION	The notified chemical may have skin sensitising ability but the test conditions employed are inadequate. Therefore, on the basis of inadequate evidence, no conclusion is made.
TEST FACILITY	IBR (1986d)

B.10. Skin sensitisation – human volunteers

TEST SUBSTANCE	Notified chemical
METHOD	P&G protocol described in IS consultancy protocol issue #1 (04/03/1985) Human Repeat Insult Patch Test Griffith, J.F., (1969), Predictive and Diagnostic Testing for Contact Sensitisation, Toxicol. Appl. Pharmacol., Suppl.3:90 Stotts, J., (1980), Planning, Conduct and Interpretation Human Predictive Sensitisation Patch Test, Current Concepts in Cutaneous Toxicity, pp 41-53.
Study Design	Induction Procedure: A three week induction period with patches applied on Mondays, Wednesdays and Fridays. Rest Period: 14 days Challenge Procedure: One 24 hour patch application at the beginning of the 5th week.
Study Group	90 of which 84 completed the study
Vehicle	Water
Remarks - Method	The study was run in two stages with 26 subjects starting the test ahead of the main group in case alteration in concentration of the test material was necessary. 0.5 mL of the test material was applied to a 2 cm^2 pad and affixed to the back of the test subjects.
RESULTS	
Remarks - Results	The notified chemical at a concentration of 1.5% produced mild skin irritation in 16/84 test subjects at challenge. Of the 16 test subjects that showed signs of skin irritation after challenge, all of them showed irritation (mild erythema only) at the 48 hour observation but the irritation was only present in 6 subjects at the 96 hour observation. Reactions that

	fade from 48 hours to 96 hours are generally due to irritation rather than sensitisation. Furthermore, oedema and papules generally more indicative of a sensitisation reaction were not observed.
CONCLUSION	A human repeat insult patch test was conducted using the notified chemical diluted with water to 1.5% under occlusive dressing. The notified chemical was slightly irritating and non-sensitising under the conditions of the test.
TEST FACILITY	I S Consultancy (1986a)

B.11. Skin sensitisation – human volunteers

TEST SUBSTANCE	Notified chemical
METHOD	P&G protocol described in IS consultancy protocol issue #3 (19/07/1991) Human Repeat Insult Patch Test Stotts, J., (1980), Planning, Conduct and Interpretation Human Predictive Sensitisation Patch Test, Current Concepts in Cutaneous Toxicity, pp 41-53.
Study Design	Induction Procedure: A three week induction period with patches applied on Mondays, Wednesdays and Fridays. Rest Period: 14 days Challenge Procedure: One 24 hour patch application at the beginning of the 6th week.
Study Group	107 of which 104 completed the study
Vehicle	Water
Remarks - Method	An irritation screening study was conducted prior to the main study.
RESULTS	
Remarks - Results	The notified chemical at a concentration of 20% produced mild skin irritation in 2/104 test subjects at challenge.
CONCLUSION	A human repeat insult patch test was conducted using the notified chemical diluted with water to 20% under occlusive dressing. The notified chemical was slightly irritating and non-sensitising under the conditions of the test.
TEST FACILITY	I S Consultancy (1992a)

B.12. Skin sensitisation – human volunteers

TEST SUBSTANCE	Notified chemical
METHOD	P&G protocol described in IS consultancy protocol issue #3 (19/07/1991) Human Repeat Insult Patch Test Stotts, J., (1980), Planning, Conduct and Interpretation Human Predictive Sensitisation Patch Test, Current Concepts in Cutaneous Toxicity, pp 41-53.
Study Design	Induction Procedure: A three week induction period with patches applied on Mondays, Wednesdays and Fridays. Rest Period: 14 days Challenge Procedure: One 24 hour patch application at the beginning of the 6th week.
Study Group	103 of which 95 completed the study
Vehicle	Water
Remarks - Method	None of the subjects that did not complete the study did so for reasons related to the study or the test substance.

RESULTS

Remarks - Results	The notified chemical at a concentration of 20% produced mild skin irritation in 14/95 test subjects and moderate skin irritation in 1/95 test subjects at challenge. Of the 15 test subjects that showed signs of skin irritation after challenge, all of them showed irritation (mild erythema only) at the 48 hour observation but the irritation was only still present in 10 subjects at the 96 hour observation. Reactions that fade from 48 hours to 96 hours are generally due to irritation rather than sensitisation. Furthermore, oedema and papules generally more indicative of a sensitisation reaction were not observed.
CONCLUSION	A human repeat insult patch test was conducted using the notified chemical diluted with water to 20% under occlusive dressing. The notified chemical was slightly irritating and non-sensitising under the conditions of the test.
TEST FACILITY	I S Consultancy (1993a)

B.13. Repeat dose toxicity

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 407 Repeated Dose 28-day Oral Toxicity Study in Rodents. EC Directive 96/54/EC B.7 Repeated Dose (28 Days) Toxicity (Oral).
Species/Strain	Rat/Charles River CD
Route of Administration	Oral – gavage
Exposure Information	Total exposure days: 28 days Dose regimen: 7 days per week Post-exposure observation period: none
Vehicle	Water
Remarks - Method	The study was originally planned as a 91 day study but was stopped at 28 days due to microbial contamination of the dosing solutions. Two different control groups were used. The first control group used water and the second group used water adjusted to pH 2.5 using hydrochloric acid. GLP compliant.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw/day*	*Mortality*
control 1	25 per sex	0	0/50
control 2 (pH 2.5)	25 per sex	0	0/50
low dose	25 per sex	1	0/50
mid dose	25 per sex	10	0/50
high dose	25 per sex	500	0/50

Mortality and Time to Death

There were no unscheduled deaths during the study.

Clinical Observations

There were no treatment related clinical signs noted during the study. There were no significant differences in the bodyweight gain between the control and treated groups. Food consumption was significantly increased in comparison to the controls during the first week for both the low and high dose test groups and also the last week for the low dose test group.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

A statistically significant decrease in the mean corpuscular haemoglobin (MCH) index was observed in the male high dose group. However, since no corresponding changes were detected in any other haematological parameter it was considered to be of no toxicological significance.

Effects in Organs
Three animals exhibited focal scarring or retinal loss. No other ophthalmoscopic abnormalities were detected and hence the findings were considered to be of no toxicological significance.

Remarks – Results
No adverse treatment related effects were seen at any dose level and hence the NOAEL can be regarded as the highest dose level tested.

CONCLUSION
The No Observed Adverse Effect Level (NOAEL) was established as > 500 mg/kg bw/day in this study, based on the absence of any adverse effects at any of the doses tested.

TEST FACILITY IRDC (1994a)

B.14. Repeat dose toxicity

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 408 Repeated Dose 90-Day Oral Toxicity Study in Rodents. EC Directive 88/302/EEC B.26 Sub-Chronic Oral Toxicity Test: 90-Day Repeated Oral Dose Study using Rodent Species.
Species/Strain	Rat/Charles River CD
Route of Administration	Oral – gavage
Exposure Information	Total exposure days: 91 days Dose regimen: 7 days per week Post-exposure observation period: none
Vehicle	Water
Remarks - Method	Two different control groups were used. The first control group used water and the second group used water adjusted to pH 2.5 using hydrochloric acid. GLP compliant.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw/day*	*Mortality*
control 1	15 per sex	0	0/30
control 2 (pH 2.5)	15 per sex	0	0/30
low dose	15 per sex	1	0/30
mid dose	15 per sex	10	0/30
high dose	15 per sex	500	0/30

Mortality and Time to Death
There were no unscheduled deaths during the study.

Clinical Observations
There were no treatment related clinical signs noted during the study. There were no significant differences in the bodyweight gain or food consumption between the control and treated groups.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis
Mean Corpuscular Volume (MCV) values were statistically significantly lower in male rats in the control group 1, the mid dose group and the high dose group when compared to animals in the pH adjusted control group. However, the MCV values were within ± 2 standard deviations of the testing laboratory´s historical control mean value for this parameter for rats of this stain, age and sex and were also not present in both sexes and since they occurred also in the control group 1, these lower values were considered not to be

toxicologically significant nor test article-related.

In male rats the blood urea nitrogen values of the 500 mg/kg bw/day group were significantly lower than those of the control group 2. These differences were within the laboratory's historical control mean values for rats of this strain, age and sex. However, since this change was not seen in both sexes and no corresponding changes were detected in any other related parameters it was considered to be of no toxicological significance.

Effects in Organs

There were no statistically significant changes in the mean relative ovary/body or ovary/brain weight ratio when compared to both control groups with the exception of a statistically significant decrease in the mean relative ovary/body weight ratio observed in the 500 mg/kg bw/day group when compared to the control group 2. The mean body weight of the control group 2 was the lowest of all female groups in contrast to that of the 500 mg/kg bw/day group, which was the highest. In addition, there were no statistically significant changes in the mean absolute ovary weights. The mean absolute ovary weight of the 500 mg/kg bw/day group falls within the range of the mean absolute ovarian weight for 13-week CD rat studies at the laboratory. Therefore, the observed decreased mean ovary/body weight ratio was considered to be of no toxicological significance.

Remarks – Results

No adverse treatment related effects were seen at any dose level and hence the NOAEL can be regarded as greater than the highest dose level tested.

CONCLUSION

The No Observed Adverse Effect Level (NOAEL) was established as > 500 mg/kg bw/day in this study, based on the absence of any adverse effects at any of the dose rates tested.

TEST FACILITY IRDC (1994b)

B.15. Genotoxicity – bacteria

TEST SUBSTANCE Notified chemical

METHOD

	OECD TG 471 Bacterial Reverse Mutation Test. EC Directive 2000/32/EC B.13/14 Mutagenicity – Reverse Mutation Test using Bacteria. Plate incorporation procedure
Species/Strain	*S. typhimurium*: TA1535, TA1537, TA98, TA100 *E. coli*: WP2uvrA (pKM101)
Metabolic Activation System	S9 fraction from Aroclor 1254-induced rat liver.
Concentration Range in Main Test	a) With metabolic activation: 1.5 – 5000 µg/plate b) Without metabolic activation: 1.5 – 5000 µg/plate
Vehicle	Ethanol
Remarks - Method	The study was conducted in two phases. The first phase was used to establish the dose-range for the confirmatory mutagenicity assay and provide a preliminary mutagenicity evaluation. The second phase of the study was used to evaluate and confirm the mutagenic potential of the test article. The primary difference between the two phases was that the second phase did not test at dose levels of 1.5 and 5.0 µg/plate. No significant protocol deviations. GLP compliant.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	≥ 1500	≥ 1500	≥ 1500	negative
Present				

Test 1	≥ 1500	≥ 1500	≥ 1500	negative

Remarks - Results

The test material was tested up to the maximum recommended dose level of 5000 µg/plate. No toxicologically significant increases in the frequency of revertant colonies were recorded for any of the bacterial strains, with any dose of the test material, either with or without metabolic activation.

All the positive control chemicals used in the test induced marked increases in the frequency of revertant colonies thus confirming the activity of the S9-mix and the sensitivity of the bacterial strains.

CONCLUSION

The notified chemical was not mutagenic to bacteria under the conditions of the test.

TEST FACILITY

BioReliance (2008a)

B.16. Genotoxicity – in vitro

TEST SUBSTANCE

Notified chemical

METHOD

Clive D. and Spector J.F.S. (1975)
Comparable to OECD TG 476 In vitro Mammalian Cell Gene Mutation Test.

Cell Type/Cell Line: Mouse Lymphoma L1578Y
Metabolic Activation System: S9 fraction from Aroclor 1254-induced rat liver.
Vehicle: Acetone
Remarks - Method: A preliminary study and three different studies were carried out with the notified chemical. This was due to the concentration used in the first studies not reducing the cultures relative growth by ≤ 20%.
No significant protocol deviations.
GLP compliant.
Methyl methanesulfonate (MMS) was used as the positive control for the cultures without metabolic activation. 7,12-Dimethyl-benz(a)anthracene was used as the positive control for the cultures with metabolic activation.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Expression Time*	*Selection Time*
Absent				
Test 1	0, 22.5, 25, 27.5, 30, 32.5, 35, 40, 50, 60, 75	4 hours	10 – 14 days	10 – 14 days
Test 2	0, 5, 10, 20, 35, 60, 75, 90, 100, 110, 120, 130	4 hours	10 – 14 days	10 – 14 days
Test 3	0, 35, 50, 60, 75, 90, 100, 110, 120, 130, 140, 150	4 hours	10 – 14 days	10 – 14 days
Present				
Test 1	0, 25, 27.5, 30, 32.5, 35, 40, 50, 60, 75	4 hours	10 – 14 days	10 – 14 days
Test 2	0, 50, 60, 75, 90, 100, 110, 120, 130, 140, 150	4 hours	10 – 14 days	10 – 14 days
Test 3	0, 110, 120, 130, 140, 150, 200, 300, 400, 500, 550	4 hours	10 – 14 days	10 – 14 days

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/mL) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	> 36	≥ 50	> 75	negative
Test 2		≥ 60	> 130	negative
Test 3		≥ 50	> 150	negative
Present				
Test 1	> 36	> 75	> 75	negative
Test 2		> 150	> 150	negative

Test 3	≥ 150*	> 550	negative

* With the exception of 200 µg/mL.

Remarks - Results	The vehicle controls had acceptable mutant frequency values that were within the normal range for the L5178Y cell line at the TK +/- locus. The positive control induced marked increases in the mutant frequency indicating the satisfactory performance of the test and of the activity of the metabolising system.
CONCLUSION	The notified chemical was not clastogenic to Mouse Lymphoma L1578Y cells treated in vitro under the conditions of the test.
TEST FACILITY	Microbiology Associates (1996a)

B.17. Genotoxicity – in vitro

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 473 In vitro Mammalian Chromosome Aberration Test.
Cell Type/Cell Line	Chinese Hamster ovary (CHO-K_1)
Metabolic Activation System	S9 fraction from Aroclor 1254-induced rat liver.
Vehicle	Ethanol
Remarks - Method	No significant protocol deviations. GLP compliant. Mitomycin C was used as the positive control for the cultures without metabolic activation. Cyclophosphamide was used as the positive control for the cultures with metabolic activation.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	0*, 25, 50*, 100*, 200*, 225, 250, 275	4 Hours	20 Hours
Test 2	0*, 25, 50*, 100*, 125*, 150, 175, 200	20 Hours	20 Hours
Present			
Test 1	0*, 25, 50, 100*, 200*, 225*, 250, 275, 300	4 Hours	20 Hours

*Cultures selected for metaphase analysis.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/mL) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	≥ 265.8	≥ 200	≥ 50	negative
Test 2	≥ 265.8	≥ 125	≥ 50	negative
Present				
Test 1	≥ 265.8	≥ 225	≥ 50	negative

Remarks - Results	The positive and vehicle controls gave satisfactory responses, confirming the validity of the test system. The test material did not induce any statistically significant increases in the frequency of cells with aberrations, or in the numbers of polyploid cells.
CONCLUSION	The notified chemical was not clastogenic to Chinese Hamster ovary (CHO-K_1) cells treated in vitro under the conditions of the test.
TEST FACILITY	BioReliance (2008b)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready biodegradability

TEST SUBSTANCE	Notified Chemical
METHOD	OECD TG 301 B Ready Biodegradability: CO_2 Evolution Test.
Inoculum	Activated sludge from a domestic wastewater treatment plant
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	TOC analyser Model 700
Remarks - Method	The test was conducted for 28 days in accordance with the above guidelines. The test substance was added to a liquid medium inoculated with sewage microorganisms and aerated with CO_2-free air at 20 to 25 ºC. CO_2 production was analysed. The two test treatment (10 and 20 mg/L) reached the pass level of >60% CO_2 production within the 10 day window.

RESULTS

Test substance (10 mg/L)		*Test substance* (20 mg/L)		*Aniline* (20 mg/L)	
Days	% Degradation	Day	% Degradation	Day	% Degradation
4	0.00	4	1.7	4	1.1
7	22.6	7	22.3	7	44.7
11	55.3	11	53.3	11	61.7
14	61.0	14	61.6	14	66.3
28	75.9	28	79.3	28	77.8

Remarks - Results	All validity criteria for the test were satisfied.
CONCLUSION	The test substance, and by inference the notified chemical, is readily biodegradable
TEST FACILITY	Procter and Gamble (1993)

C.1.2. Ready biodegradability

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 301 A Ready Biodegradability: DOC Die-Away Test. [Influent die-away experiment (Protocol Reference Number i.e P&G C14, EEC/67/548, etc)]
Inoculum	Raw sewage from sewage treatment plant
Exposure Period	48 hour
Analytical Monitoring	LSC and thin layer chromatography
Remarks - Method	This method is not considered suitable for this notified chemical due to its low water solubility of < 100 mg/L. Cold and radiolabelled notified chemical were dosed at a combined concentration of 1 mg/L in raw domestic sewage. Three vessels were used: 1 abiotic control (90 min autoclave + 1 g/L $HgCl_2$) and two test units. One of the test units was used for complete analysis while the other was used for LSC counting only (mass balance). Test conditions were: Temperature 20-23ºC, pH 8.4.

RESULTS

Test duration (hours	% of the different species			% lost by sublation	% total recovery of measured species	% lost by lyophilisation
	DAQ	MEQ	DEQ			
0	2	3	65	28	98	2
1	2	3	76	15	98	2
2	2	3	72	14	93	7
4	3	4	69	19	95	5
6	5	7	57	23	92	8
24	7	8	29	12	57	43
48	7	7	12	11	37	63

Remarks - Results

The results indicate that the percent parent remaining after 6, 24 and 48 h was 57, 29 and 12% respectively. TLC-RAD measurements showed that biodegradation products were more soluble than the notified chemical. It was found that in a abiotic test only 15% of total radiolabel was lost by lyophilisation whereas the bioactive unit had a loss of 63%. This showed that a small fraction of the notified chemical was lost through volatilization.

CONCLUSION

This study is not reliable due to methodical deficiencies. However, it can be regarded as a supportive study. The notified chemical is rapidly degraded with half-life of 7 hours into water soluble products.

TEST FACILITY

Procter and Gamble (1992)

C.1.3. Ready biodegradability

TEST SUBSTANCE

Notified chemical

METHOD

A Ready Biodegradability: Activated Sludge Die-Away Test. Protocol E93-002- Fate of FV-Base and HP-2 in Activated Sludge.

Inoculum: Activated sludge from Sewage Treatment plant

Exposure Period: 24 hour

Analytical Monitoring: LSC and thin layer chromatography

Remarks - Method: After the preliminary DieAway experiment, a definitive batch activated sludge experiment was conducted using activated sludge and radiolabelled notified chemical. One litre of the sludge was placed in each of three flasks. One flask served as an abiotic control and was amended with buffered $HgCl_2$. After insuring that the pH of the sludge was approximately 7, the flasks were placed on a shaker and dosed with BFA base to yield a final added concentration of 0.5 mg/L. At each sampling, three replicate samples were removed from each trap to determine $^{14}CO_2$.

RESULTS

Kinetic Parameters Describing the biodegradation of the notified chemical

	Primary Degradation			Mineralization to CO_2			Complete Biodegradation		
	K (Hrs)	Asymptotic Yield	$t_{1/2}$ (Hrs)	K (Hrs)	Asymptotic Yield	$t_{1/2}$ (Hrs)	K (Hrs)	Asymptotic Yield	$t_{1/2}$ (Hrs)
Range-finder	0.05 ± 0.01	NA	12.83	0.03 ± 0.01	99.9 ± 25.7	23.90	0.16 ± 0.05	74.1 ± 7.24	4.23
Replicate 1	0.10 ± 0.02	NA	6.79	0.03 ± 0.00	65.1 ± 1.12	22.35	0.07 ± 0.01	70.0 ± 4.10	9.49
Replicate 2	0.09 ± 0.02	NA	7.62	0.04 ± 0.00	62.5 ± 1.33	18.23	0.07 ± 0.01	73.4 ± 2.16	10.5
Mean Replicate	0.09 ± 0.02	NA	7.45	0.03 ± 0.00	63.0 ± 1.47	21.66	0.07 ± 0.01	70.7 ± 2.98	9.63

Remarks - Results

After 24 hours, less than 20% of the notified chemical remained and metabolites had virtually disappeared. Kinetic analysis of the data indicated that the half-life for primary biodegradation of the notified chemical was approximately 14 h and that for mineralization to CO_2 was

24 h.

CONCLUSION The half-life for disappearance of the notified chemical was 7 to 14 hours and half-life for mineralization to $^{14}CO_2$ was 18 to 24 hrs.

TEST FACILITY Procter and Gamble (1994a)

C.1.4. Bioaccumulation

TEST SUBSTANCE Notified Chemical

METHOD BCFWIN; CFCS 2009
Remarks - Method No calculated data was provided except the BCF value.
RESULTS
Bioconcentration Factor The calculated BCF value for the notified chemical is 70.8

TEST FACILITY CFCS (2009)

C.2. Ecotoxicological Investigations

C.2.1. Acute toxicity to fish

TEST SUBSTANCE Notified chemical

METHOD OECD TG 203 Fish, Acute Toxicity Test - Static

Species Zebrafish (*Danio rerio*)
Exposure Period 96 hours
Auxiliary Solvent Not provided
Water Hardness Not provided
Analytical Monitoring pH and oxygen concentrations
Remarks – Method The test was conducted at nominal concentrations of 1.0, 1.6, 2.5, 4.0, 6.3, 10.0 mg/L under static conditions for a period of 96 h according to the guidelines above. The controls were kept in dilution water. Ten fish per test solution were observed for mortality after every 24 hours. Test conditions were: 23 ± 1°C, pH 7.8 ± 1, 7.22-7.87 mg O_2/L, 12 hours dark and 12 hours light period.

RESULTS

Concentration mg/L		*Number of Fish*	*Mortality (%)*				
Nominal	*Actual*		*24 h*	*48 h*	*72 h*	*96 h*	*After 96 h*
1.0		10	0	0	0	0	0
1.6		10	0	0	0	0	0
2.5		10	0	0	0	0	0
4.0		10	10	0	0	0	10
6.3		10	50	30	0	0	80
10.0		10	80	20	-	-	100
Control		10	0	0	0	0	0

96 h LC50 5.2 mg/L.
96 h NOEC (or LOEC) 2.5 mg/L
Remarks – Results Mortality occurred in the first 48 h. After this time, seemingly moribund organisms recovered. Within the first 48 h the test solutions at concentrations of 2.5 to 10 mg/L showed a Tyndall effect. The intensity increased with increasing concentrations. Precipitates were observed on bottom of test aquarium in the exposure concentrations of 2.5 to 10 mg/L at 48 h after preparation of test solution. These observations suggest that

mortality is most likely a physical and not a toxic effect due to undissolved particles in the water phase. Five additional fish were added to the 10 mg/L solution after 48h (the time the precipitates were observed). The animals survived until test termination which supports the assumption of physical effects. Therefore, the test results/endpoints are not considered to be reliable (EPHC 2009).

CONCLUSION Results are unreliable

TEST FACILITY Procter and Gamble (1985a)

C.2.2. Chronic toxicity to fish

TEST SUBSTANCE Notified chemical

METHOD US EPA-TSCA, 40 CFR, Part 797.1600 Early Life Stage Toxicity to Fathead Minnow under Flow-Through Conditions

Species	Fathead Minnow (*Pimephales promelas*),
Exposure Period	35 days
Auxiliary Solvent	Not provided
Water Hardness	160-230 mg $CaCO_3$/L
Analytical Monitoring	LSC and thin layer chromatography radioanalysis
Remarks – Method	The test was conducted at mean measured concentrations of 0.686, 1.41, 2.68, 5.30, and 9.76 mg/L under flow-through conditions for a period of 35 days according to the above guidelines. Hatchability and pre-fry reduction survival were analysed at day 5. The interval for post-fry reduction survival began on day 5 after the reduction to 15 fry per chamber and ended on day 35 with test termination. Test conditions were: Temperature = 24.3-26.1°C, Dissolved Oxygen = 5.6-7.9 mg/L and pH = 7.7-8.3.

RESULTS

Mean Measured Concentration (mg/L)	Egg Hatchability (%)	Pre-Fry Reduction Survival (%)	Post-Fry Reduction Survival (%)
Control	88.0	80.0	80.0
0.686	88.0	88.0	91.1
1.41	90.7	90.7	33.3
2.68	89.3	89.3	53.3
5.30	87.0	84.4	0.0
9.76	75.7	64.9	22.9

LC50 (fry-mortality)	1.67 mg/L (95% confidence limits of 1.45 and 1.91 mg/L)
NOEC	0.686 mg/L
LOEC	1.41 mg/L
Remarks – Results	No statistically significant reductions in hatchability and pre-fry reduction survival were noted at any test concentration. There were statistically significant reductions in post fry-reduction survival at the mean measured concentrations of 1.41, 2.68, 5.30, and 9.76 mg/L. Based on the statistically analysed parameters of hatchability, pre-fry reduction survival, post-fry-reduction survival and growth (standard length and blotted weight), the 35 days NOEC was 0.686 mg/L.

CONCLUSION The notified chemical is classified as harmful to fish with long lasting effects.

TEST FACILITY Procter and Gamble (1996a)

C.2.3. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 202 Daphnia sp. Acute Immobilisation Test and Reproduction Test - Static
Species	*Daphnia magna*
Exposure Period	24 hours
Auxiliary Solvent	Not provided
Water Hardness	267.7 mg $CaCO_3$/L
Analytical Monitoring	pH and oxygen concentrations
Remarks - Method	The 24-hr-acute toxicity of the notified chemical to *Daphnia magna* was studied under static conditions according to OECD TG 202 (Part I). Twenty daphnia (4 replicates of 5 animals) were exposed to six nominal concentrations from 0.1 to 32 mg/L. Immobilization was observed after 24 hours. Test conditions were: 18-22°C ± 0.5°C, pH 7. 8 ± 2.0.

RESULTS

Concentration mg/L		*Number of D. magna*	*Number Immobilised (%)*
Nominal	*Actual*		*24 h*
0.10		4 × 5	0
0.32		4 × 5	0
1.00		4 × 5	0
3.20		4 × 5	10
10.00		4 × 5	40
32.00		4 × 5	70

24 h LC50	14.8 (8.4 – 26.2) mg/L
24 h NOEC (or LOEC)	1.0 mg/L
Remarks - Results	The 24 hr EC50 was 14.8 mg/L with 95% CL of 8.4 - 26.2 mg/L. The study period of 24 h was recommended before adoption of the OECD TG 202 in 2004 and therefore this study does not meet one criterion of today standard methods. This study, however, is classified as reliable with restrictions and satisfies the guideline requirements for an acute toxicity study with freshwater invertebrates.
CONCLUSION	The notified chemical is harmful to daphnia.
TEST FACILITY	(Procter and Gamble, 1985b)

C.2.4. Chronic toxicity to aquatic invertebrates

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 202 Daphnia sp. Acute Immobilisation Test and Reproduction Test; EPA OTS 797.1330 (Daphnid Chronic Toxicity Test)
Species	*Daphnia magna*
Exposure Period	21 days
Concentration Range	Nominal (mg/L) Control, 0.25, 0.50, 1.0, 2.0 and 4.0 Actual (mg/L) Control, 0.27, 0.47, 1.0, 2.0 and 3.9.
Auxiliary Solvent	Dilution water used was natural stream water also called river water
Water Hardness	144-252 mg $CaCO_3$/L
Analytical Monitoring	Liquid Scintillation counting (LSC), Thin-layer chromatography-radioanalysis
Remarks - Method	The 21 day chronic toxicity of the notified chemical to *Daphnia magna* was studied under flow through conditions according to above guidelines. Daphnids were exposed to control and test chemical at measured concentrations of 0.27 to 3.9 mg/L. Test conditions were: 20°C ± 2°C, pH

7. 8 ± 2.0, and light intensity ranged from 54 to 61 foot candles at the water surface.

Nominal loading retested, daphnid survival, mean total body length and dry weight of daphnids (*Daphnia magna*)

Nominal Conc. (mg/L)	Mean Measured Conc. (mg/L)	Mean Percent Survival	Mean Total Body Length in mm (SD)	Mean Dry Weight in mg (SD)
Control	N/A	98	4.85 (0.07)	1.15 (0.02)
0.25	0.27	98	4.79 (0.10)	1.25 (0.09)
0.50	0.47	98	4.69 (0.11)	1.19 (0.05)
1.0	1.0	95	4.65 (0.08)	1.18 (0.04)
2.0	2.0	38	3.65 (0.36)	0.29 (0.12)

Remarks - Results: Production of offsprings in the treated groups indicated that the notified chemical had an effect on the reproduction at concentrations higher than 1.0 mg/L. The 21-day EC50 based on mortality was 1.7 mg/L (95 % confidence limits of 1.5 and 1.9 mg/L). The 21-day NOEC based on survival, number of young/adult/reproduction day, and growth (length and weight) was 1.0 mg/L, the LOEC was 2.0 mg/L.

CONCLUSION: The notified chemical is harmful to daphnia with long lasting effects.

TEST FACILITY: Procter and Gamble (1996b).

C.2.5. Algal growth inhibition test

TEST SUBSTANCE: Analogue chemical

METHOD: OECD TG 201 Alga, Growth Inhibition Test (Static) # 35947

Species	*Scenedesmus subspicatus (new name Desmodesmus subspicatus)*
Exposure Period	72 hours
Concentration Range	Nominal: Control (0), 2.0, 4.0, 8.0, 16, 32, and 64 mg/L Actual: 0, 1.5, 2.8, 6.6, 29 and 64 mg/L
Auxiliary Solvent	Not provided
Water Hardness	Not provided
Analytical Monitoring	Mass spectrometry method.
Remarks - Method	After a range finding test, a definitive test at concentrations of 0, 1.5, 2.8, 6.6, 29 and 64 mg notified chemical/L (in triplicate) was conducted for a period of 72 hours according to the above guidelines. Test conditions were: 23.0 - 24.0°C ± 2.0°C, continuous illumination at 800 ± 10% footcandles, pH 6.8-8.6. Cell counts were conducted at 24, 48, and 72 hours for each replicate using a light microscope and a hemacytometer. Statistical analysis of the results was performed using one way analysis of variance and Dunnett's comparison procedure.

RESULTS

Biomass		*Growth*	
E_bC_{50} mg/L at 72 h	*NOEC mg/L*	*E_rC_{50} mg/L at 72 h*	*NOEC mg/L*
3.2 (2.7 – 3.6)	1.5	6.3 (1.5 – 64)	1.5

Remarks - Results: All validity criteria for the guideline were satisfied and no significant

	deviations from the guidelines were reported. The 72 h EC50 based on biomass is 3.2 mg/L with 95% confidence limits of 2.7 - 3.6 and the 72 h EC50 based on growth rate is 6.3 mg/L with 95% confidence limits of 1.5 - 64. The 72 h NOEC value for both biomass and growth rate was 1.5 mg/L.
CONCLUSION	The test substance and, by inference, the notified chemical is toxic to algae.
TEST FACILITY	Procter and Gamble (1995).

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