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NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

FULL PUBLIC REPORT

Glycerides, castor-oil mono-, hydrogenated, acetates

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 the Environment, Water, Heritage and the Arts.

For the purposes of subsection 78(1) of the Act, this Full Public Report may be inspected at our NICNAS office by appointment only at 334-336 Illawarra Road, Marrickville NSW 2204.

This Full 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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This notification has been carried out under the approved foreign scheme provisions (Canada) of Section 44 of the Act. The health and environment hazard assessment of the Canadian report was provided to NICNAS and where appropriate used in this assessment report. The other elements of the risk assessment and recommendations on safe use of the notified polymer were carried out by NICNAS.

Glycerides, castor-oil mono-, hydrogenated, acetates

1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)
Danisco Australia Pty Ltd (ABN 60 096 139 392)
45–47 Green Street
BOTANY NSW 2019

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:
Import volume

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
No variation to the schedule of data requirements is claimed.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
EU, USA, Canada

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
GRINDSTED SOFT-N-SAFE, TS-ED 532

CAS NUMBER
736150-63-3

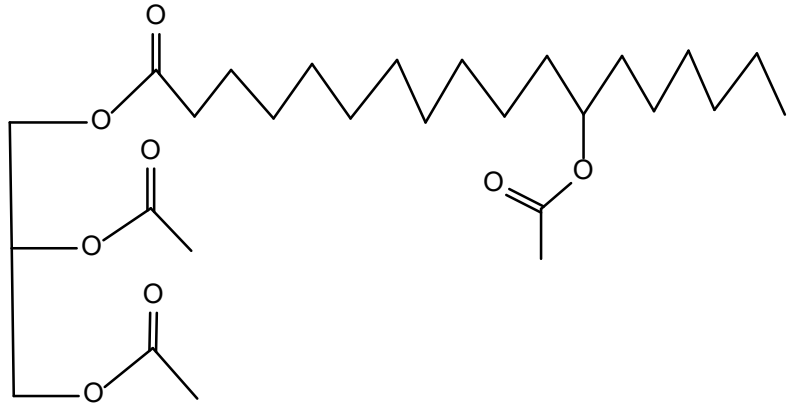
CHEMICAL NAME
Glycerides, castor-oil mono-, hydrogenated, acetates

OTHER NAME(S)
AMG-HCO, ACETEM CAO 90-00

MOLECULAR FORMULA
$C_{27}H_{48}O_8$ (for the major component of the notified chemical)

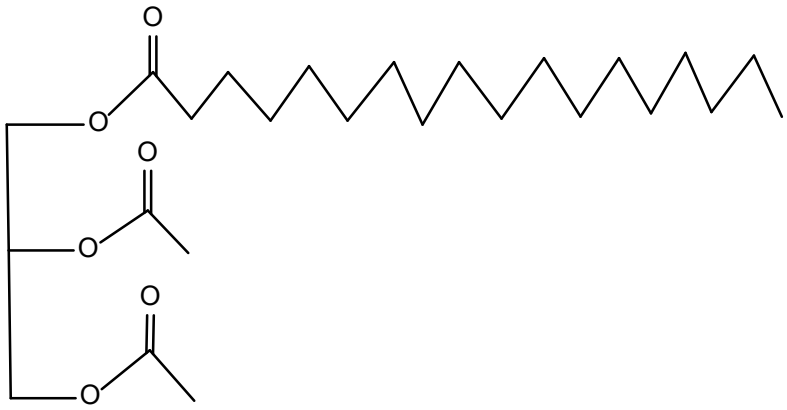
COMPOSITION AND STRUCTURAL FORMULA

The main components of the notified chemical are acetylated monoglycerides of 12-hydroxy octadecanoic acid, octadecanoic acid, and hexadecanoic acid. Examples of these main components are included below:



Octadecanoic acid, 12-(acetyloxy)-, 2,3-bis(acetyloxy)propyl ester $C_{27}H_{48}O_8$

+



Octadecanoic acid, 2,3-bis(acetyloxy)propyl ester $C_{25}H_{46}O_6$

MOLECULAR WEIGHT

500.7 (for the major component of the notified chemical)

ANALYTICAL DATA

Reference NMR, IR, MS, GC, UV spectra were provided.

3. COMPOSITION

DEGREE OF PURITY 95% (90-100%) (based on content of fully acetylated monoglycerides of 12-hydroxystearic acid, stearic acid and palmitic acid)

HAZARDOUS IMPURITIES/RESIDUAL MONOMERS None

NON HAZARDOUS IMPURITIES/RESIDUAL MONOMERS (>1% by weight)

Chemical Name	Octadecanoic acid, 12-(acetyloxy)-, 2-hydroxy-, 3-acetyloxypropyl ester		
CAS No.	Not assigned	*Weight %*	2% (0-5%)

Chemical Name	Octadecanoic acid, 12-oxy, 2,3-bis(acetyloxy) propyl ester		
CAS No.	Not assigned	*Weight %*	1.5% (0-5%)
Chemical Name	Octadecanoic acid, 12-(acetyloxy)-, 2-(acetyloxy)-1,3-propanediyl ester		
CAS No.	Not assigned	*Weight %*	1.1% (0-4%)
Chemical Name	Octadecanoic acid, 3-(acetyloxy)-2-hydroxypropyl ester		
CAS No.	820-17-7	*Weight %*	1% (0-2%)

Note: Additional impurities present at low levels have also been identified.

ADDITIVES/ADJUVANTS None

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20°C AND 101.3 kPa: Transparent liquid.

Property	Value	Data Source/Justification
Melting Point/Freezing Point	-21.5 to 3.5°C	Measured
Boiling Point	>300°C at 101.3 kPa	Boiling point could not be determined as decomposition starts at a temperature (300°C) below the true boiling point
Density	1003.0 kg/m^3 at 20°C	Measured
Vapour Pressure	4.8 x 10^{-5} kPa at 20°C	Measured
Water Solubility	< 0.33 mg/L at 20°C 0.06-0.09 mg/L	Measured
Hydrolysis as a Function of pH	Not determined because of low water solubility	The rate of hydrolysis will be limited by the low water solubility
Partition Coefficient (n-octanol/water)	log Pow = 3.5 – 6.4 at 25°C	Measured (HPLC method)
Adsorption/Desorption	log K_{oc} = 5.4 at 25°C	Measured (HPLC method)
Dissociation Constant	Not determined because of low water solubility	The notified chemical is not expected to dissociate in the environmental pH range, based on the structure.
Particle Size	Not determined	The notified chemical exists in liquid form.
Flash Point	244°C at 101.3 kPa	Measured
Flammability	Not determined	Unlikely to be flammable as the notified chemical does not contain pyrophoric groups and no flammable gas is evolved either in contact with water or humid air
Autoignition Temperature	370°C	Measured
Explosive Properties	Not determined	Unlikely to be explosive, as the notified chemical does not contain plosophoric groups.

DISCUSSION OF PROPERTIES

An upper limit for water solubility of 0.33 mg/L was reported in an earlier study. This has now been refined to 0.06-0.09 mg/L using a more sensitive analytical method for the main component of the notified chemical. For full details of this water solubility determination, please refer to Appendix A.

Reactivity

The notified chemical is expected to be stable under normal conditions of use.

Migration of the notified chemical from PVC film into Sunflower Oil and Aqueous Simulants (see also Appendix A for additional studies)
The migration of the notified chemical from a 12 µm PVC food wrap film containing 6.8% notified chemical into sunflower oil has been determined by means of GC-MS. The migration test was conducted at 40ºC for 10 days. The study reveals that a large fraction will migrate to the sunflower oil. A total of three migrations were conducted and the average migration of the notified chemical was 10.3 mg/dm^2 film sample.

In another study, the migration of the notified chemical from a 12 µm PVC food wrap film containing 6.8% notified chemical into aqueous food simulants, 3% w/v aqueous acetic acid and 15% v/v aqueous ethanol has been determined. The migration test was conducted at 40ºC for 10 days. The study reveals that the migration of the notified chemical was 0.010 and 0.011 mg/dm^2 film sample in 3% w/v aqueous acetic acid and 15% v/v aqueous ethanol, respectively.

5. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS
The notified chemical will not be manufactured in Australia and will be imported into Australia.

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

Year	*1*	*2*	*3*	*4*	*5*
Tonnes	100-3000	3000-10000	3000-10000	3000-10000	3000-10000

PORT OF ENTRY
Victoria, NSW, Perth and Brisbane

IDENTITY OF RECIPIENTS
The notifier will be the recipient of the imported notified chemical and the imported notified chemical is expected to be used in plastics and other products by customers around Australia.

TRANSPORTATION AND PACKAGING
The notified chemical will be imported into Australia in 215 L epoxy coated steel drums or 1000 L polyethylene (HDPE) lined pallet containers and transported either by airfreight or trucks.

USE
The notified chemical will be used as a plasticiser/softener in PVC and other plastic articles or films. It will be used in food contact materials (packaging film, storage containers, microwave oven trays), flooring, toys, and also as a colorant carrier in textile dyes and toys. The use as a plasticiser/softener and as a colorant carrier will be at concentrations ranging from 2-34% and 0.1-0.5% of the notified chemical in the final product, respectively.

The notified chemical will also be used as a plasticiser/softener in medical devices at concentrations ranging from 2-34%.

OPERATION DESCRIPTION
The notified chemical will not be manufactured in Australia and will be imported in neat form and reformulated in Australia into products for use as plasticizer/softener in PVC and other plastic/films and as a colorant carrier.

For use as plasticizer/softener in PVC and other plastic/films, the notified chemical will be blended with plastics and other additives used for plasticized material or compound. The formulated product containing the notified chemical will be either used on site or sent to other sites, for extrusion or injection moulding into the final products. The end use application of the plasticized material containing the notified chemical could be in food contact materials such as packaging film, storage containers, microwave oven trays, and in flooring, toys and medical devices.

For use as a colorant carrier, the notified chemical will be blended with pigments, dyes and other ingredients into colorants. The end use application of the colorant material containing the notified chemical could be in textile dyes and in toys. The colorants containing the notified chemical will be distributed for compounding with plastics and other ingredients, for ultimate extrusion or injection moulding or textile dyeing.

6. HUMAN HEALTH IMPLICATIONS

6.1 Exposure assessment

6.1.1 Occupational exposure

NUMBER AND CATEGORY OF WORKERS

Category of Worker	*Number*	*Exposure Duration (hours/day)*	*Exposure Frequency (days/year)*
Unloading of container	1-5	5-8	50
Cleaners of mixing vessels	1-25	1	240
Samples and laboratory workers	1-5	<1	240
End use	1000s	1-8	240

EXPOSURE DETAILS

Worker exposure to the notified chemical in neat form during the importation, transport and storage is not expected, except in the unlikely event of an accident where the packaging may be breached.

Inhalation exposure is not expected to occur during the occupational use scenarios. However, there is a potential for dermal and ocular exposure to the notified chemical during blending with plastics, pigments, dyes and other additives/ingredients. Although detailed information is not available on different formulation procedures, it is most likely that the notified chemical will be stored in tanks and transferred by pipelines into automated weighing systems and to the mixing vessels. Before the unloading of the notified chemical into storage tanks, a transfer tube is manually inserted into the container and the tube is also manually removed once the unloading has finished. During all these processes, workers are expected to use suitable personal protective equipment such as masks, gloves and overalls. Therefore, exposure during these processes will be low.

The cleaning of the mixing vessels will be performed manually by sweeping the vessels with dry cloths and dry cleaning it with brushes. During the cleaning process, workers are instructed to wear suitable protective clothing and dust masks and therefore, exposure is expected to be low.

Furthermore, during all stages of the formulation process, fume extraction systems are normally installed to prevent exposure of operators/workers.

6.1.2. Public exposure

Direct exposure of the general public may occur through the sale and use of consumer products containing the notified chemical as a plasticizer/softener, such as packaging film, storage containers, PVC liners/gaskets for metal caps, microwavable trays, vinyl flooring, and toys. Uses will include food-contact applications. The anticipated concentration of notified chemical in end-use products in the form of finished plastic products is 2 to 34%.

The notified chemical may also be blended with pigments, dyes and other ingredients into colorants for use as a textile dye or to be distributed for compounding with plastics for extrusion or injection moulding. Use level ranges from 0.1 to 0.5% of the final plastic product when used as a colorant. In this case, exposure to the general public is expected to be low, based on the lower concentration of the notified chemical used.

Several migration studies investigated the potential of the notified chemical to leach from PVC or polypropylene into fatty or aqueous media, focussing on the potential to leach into food in contact with the plastic. The highest migration of 10.3 mg/dm^2 occurred from PVC film to sunflower oil. Significantly lesser proportions were leached from aqueous based media, and from plastic plaques, which have less surface area than film. Therefore, it is expected that the general public will be exposed to the notified chemical through ingestion of food that has been contact in with articles containing the notified chemical.

A plausible worst case scenario for non-food public exposure would be the use of the notified chemical in plastic toys and childcare articles. Dermal exposure to children may occur during normal handling, and oral exposure may occur through intentional or inadvertent chewing, sucking and biting of these articles containing the notified chemical.

However, migration out of consumer products that are not intended to come into contact with foodstuffs is expected to be lower because the temperatures and length of exposure are expected to be lower than the food contact scenarios. Based on the use patterns and migration of the notified chemical, exposure of humans to the notified chemical is expected to be low in consumer products that do not come in contact with food.

6.2. Human health effects assessment

6.2.1. Toxicology studies on the notified chemical

The results from toxicological investigations conducted on the notified chemical are summarised in the table below. The test substance used in the toxicity studies was identified as TS-ED 532 and contained >85% of the notified chemical. Details of the studies can be found in section 6.2.3 and in Appendix B.

Endpoint	*Result and Assessment Conclusion*
Rat, acute dermal toxicity	LD50 >2000 mg/kg bw low toxicity
Rabbit, skin irritation	slightly irritating
Rabbit, eye irritation	slightly irritating
Mouse, skin sensitisation – Local lymph node assay	no evidence of sensitisation
Rat, repeat dose oral toxicity – 90 days.	NOAEL > 5000 mg/kg bw/day
Mutagenicity – bacterial reverse mutation	non mutagenic
Genotoxicity–in vitro chromosomal aberration test in human lymphocytes	non genotoxic
Genotoxicity–in vitro chromosomal aberration test with mouse lymphoma L5278Y cells	non genotoxic
Genotoxicity – in vitro Mammalian Cell Gene Mutation Test	non genotoxic
Genotoxicity–in vivo mouse bone marrow micronucleus test	non genotoxic
Developmental studies: Preliminary (rat and rabbit) Main (rat)	No developmental effect
Reproductive two generation study (preliminary)	No reproductive effect

6.2.2 Summary of Human Health Effects

Toxicokinetics, metabolism and distribution:
Uptake of radioactivity into the systemic circulation was rapid with a peak concentration (representing <2% of the dose) in blood occurring within 6 hours post-dosing. Elimination of radioactivity from the blood was slow at both doses. The mean plasma elimination half-life was between 51.9-55.6 hours. Radioactivity was eliminated from the body as $^{14}CO_2$ with 62% accounted for within 12 hours of dosing, 70.8% within 24 hours and 77% after 72 hours. The remaining radioactivity was excreted in urine (6.5%) and feces (24.6%). Metabolism is expected to be rapid.

Acute toxicity:
The notified chemical was of low acute dermal toxicity in rats. Acute oral and inhalation toxicity studies were not conducted.

Irritation and Sensitisation.
The notified chemical was slightly irritating to the skin and eyes of rabbits. The notified chemical was not a skin sensitiser in a local lymph node assay

Repeated Dose Toxicity (subchronic):
In a subchronic toxicity study, the notified chemical was administered to rats in the diet for targeted dose levels of 0, 500, 1600 or 5000 mg/kg bw/day for 92 or 93 days. The No Observed Adverse Effect Level (NOAEL) was established as > 5000 mg/kg bw/day in both sexes, based on no adverse treatment-related effects at the highest dose tested.

Mutagenicity:
The notified chemical was found to be negative in a bacterial reverse mutation test, in two genotoxicity assays in vitro, and in a mouse bone marrow micronucleus test in vivo. Therefore, based on the available information, the notified chemical is unlikely to be a genotoxin.

Carcinogenicity:
Information is not available to assess the carcinogenic potential of the notified chemical.

Toxicity for developmental/reproduction:
Based on preliminary and main developmental studies and a preliminary reproductive study, the notified chemical showed no evidence of developmental and reproductive effects.

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

6.2.3 Summaries of toxicology studies (see also Appendix B for additional studies)

Acute dermal toxicity:
The acute dermal toxicity of the notified substance (purity 86%) to rats (HanBrl:WIST SPF) was determined in accordance with OECD Guideline for Testing of Chemicals 402. A group of ten rats (5 males and 5 females) were treated at 2000 mg/kg bw. One day prior to treatment, hair was removed from the back of each rat with electric clippers, exposing an area equivalent to approximately 10% of the total body surface area. Four mL of test substance/kg bw was applied evenly over the prepared skin using a syringe and covered with a semi-occlusive dressing wrapped around the abdomen and fixed with an elastic adhesive bandage. After 24 hours, the dressing was removed and the treated area of skin was washed with lukewarm tap water to remove any residual test substance. The treated area was blotted dry with paper towel. Absorption of the test substance was not determined. Rats were observed 1,2,3 and 5 hours then once daily for 14 days after exposure. There were no deaths and no systemic or local response to treatment observed in any animal. The body weight of animals was within the range commonly recorded for this strain and age. No macroscopic findings were observed during necropsy. The LD50 is >2000 mg/kg which can be considered low concern.

Primary dermal irritation
The primary dermal irritation of the notified substance (purity 86%) to white New Zealand rabbits was determined according to OECD Guideline for Testing of Chemicals 404. Four days before treatment, the flanks of 1 male and 2 female rabbits were clipped with electric clippers exposing an area of approximately 100 cm^2. On day of treatment 0.5 mL of undiluted notified substance was placed on a gauze patch measuring 4 cm x 4 cm that was then applied directly to intact skin of the clipped area and covered with a semi-occlusive dressing that was wrapped around the abdomen and anchored with tape. After 4 hours dressing was removed and the skin was flushed with lukewarm tap water. The rabbits were then examined for skin irritation in accordance with OECD guidelines for grading of skin reactions. Examination was repeated at 1 hour, 24 hours, 48 hours, 72 hours and 7 days after removal of the notified substance. No necropsy was performed. The body weights of all rabbits were considered to be within the normal range of variability throughout the study. Very slight to well-defined erythema was observed in all rabbits at the 1-hour reading. The severity of reddening increased in one rabbit 24 hours after treatment and very slight to well-defined erthema was still visible in all 3 rabbits at 24 hours. Very slight erythema was noted in all rabbits at the 48-hour exam and persisted in 2 rabbits up to 72 hours after treatment. No edema was observed in all rabbits at the 1-hour exam. Very slight edema was observed in 2 rabbits at the 24-hour exam. No abnormal findings were observed on the treated skin of rabbit 7 days after treatment. No staining or other alterations of the treated skin nor corrosive effects were seen during the study. The Primary Irritation Index (PII) was calculated to be 1.067 which corresponds to a classification of slight skin irritant. Based on results of this study, the notified substance is considered a slight skin irritant to rabbit skin.

Primary eye irritation

The acute eye irritation of the notified substance (purity 86%) to New Zealand White rabbits was determined according to OECD Guideline for Testing of Chemicals 405. The study included 3 rabbits with healthy eyes, including 1 male and 2 females. The notified substance was placed into the conjunctival sac of the left eye of each rabbit and the eyelids were gently held together for one second to prevent the loss of notified substance. The right eye served as the untreated control. The treated eyes were not rinsed after instillation of the test substance. The exposure duration was 72 hours and observations were made at 1, 24, 48 and 72 hours after exposure. No necropsy was performed. No clinical signs of systemic toxicity were observed in the rabbits and no mortality occurred. No staining of the treated eyes produced by the test substance was observed. No corrosion of the cornea was observed at any of the reading times. The body weights of all rabbits were considered to be within the normal range of variability. One hour after treatment, all rabbits had redness in the treated eye, one female had both chemosis and reddening of the sclera and one female had reddening of the sclera. The male did not show any effects besides redness. At 24 hours, one male and one female had redness in the treated eye. No other effects were observed at 24 hours. No effects were observed at 48 and 72 hours in any of the treated rabbits. Using the Draize scale, the worst-case group mean score is 1.33. According to the Kay and Calandra Interpretation Criteria, this corresponds to a classification of minimal irritant, with the group mean total score being 1.33 at 24 hours. The notified substance is considered minimal irritant with respect to eye irritation in the rabbit.

Dermal sensitization - Local lymph node assay

The dermal sensitization of the notified substance to mice (CBA/CaOlaHsd) was determined using the local lymph node assay in accordance with OECD Guideline for Testing of Chemicals 429. Groups of 5 female rats were dosed at: 0% (control), 10%, 25% and 50% solutions of the notified substance (purity 87%) diluted with a 4:1 mixture of acetone to olive oil. Mice were treated daily with 25 µL of the appropriate concentration of the test substance to the dorsal surface of each ear (approximately 8 mm in diameter) for three consecutive days. Five days after the 1st topical application, all mice were administered with 250 µL of 81.3 µCi/mL ^{3}HTdR (approx. 20.3 µCi ^{3}HTdR/mouse) by intravenous injection via a tail vein. Approx. 5 hours after treatment with ^{3}HTdR all mice were euthanised by intraperitoneal injection of Ketamin/Xylazin/Midazolam. Draining lymph nodes were rapidly excised and pooled per animal. The level of ^{3}HTdR incorporation was measured on a β-scintillation counter. No deaths occurred during the study period. No symptoms of local toxicity at the ears of the mice and no systemic findings were observed during the study period. The body weight of the mice throughout the study was within the range commonly recorded for this strain and age. Results for each treatment group were expressed as a stimulation index. This was obtained by comparing the proliferation in the vehicle treated control group with the values from the 3 test groups as follows: the ratio of 3HTdR incorporation into lymph node cells, expressed as dpm, relative to that recorded for control lymph nodes is derived for each test group based on the group mean dpm per node. Stimulation indexes (SIs) for the 10% dose group ranged from 1.0 to 3.9 with a mean of 2.19. For the 25% dose group, the SIs ranged from 1.1 to 2.6 with a mean of 1.99. For the 50% dose group, the SIs ranged from 1.7 to 3.3 with a mean of 2.20. The SIs for the positive control ranged from 2.9 to 6.4, with a mean of 5.42. The EC3 value could not be calculated, since none of the tested concentrations induced an SI greater than 3. None of the tested concentrations induced a statistically significant increase in DPM values when compared to the control. The positive control did cause a statistically significant increase which demonstrates the reliability and sensitivity of this assay to detect skin sensitization potential. The stimulation index is <3 which suggests the notified substance does not have the potential to cause skin sensitization.

Repeated dose oral toxicity (90-day)

In a subchronic toxicity study (OECD 408), the notified substance (purity 85.6%) was administered to Hsd: Sprague Dawley SD rats (10 animals/sex/dose) in the diet for targeted dose levels of 0, 500, 1600 or 5000 mg/kg bw/day for 92 or 93 days. Surviving animals were euthanised by exsanguination under anaesthesia and subject to pathological examination. The premature death of one female in the 1600 mg/kg/day dose group was accidental and not considered to be test article-related. There were no significant differences in body weight, body weight gain, or food consumption between the control and treatment groups with the exception of females given 1600 mg/kg/day. These females were shown to have significant increases in body weight during weeks 6 and 7 and food consumption during weeks 5,6, and 7. However, these findings were not considered adverse or test article-related. Higher alkaline phosphatase for males and females given 5000 mg/kg/day were observed at days 30 and 60 but were only statistically significant for the females and were not present at the next observation period. Results of fecal analysis from males and females indicated there was no sex-specific difference in metabolism.

The functional observations revealed no treatment-related effects in any dose group for either sex. A slight dose-dependent trend towards decreased mean response time in males was observed but was not found to be statistically significant. Based on the lack of statistical significance and the absence of this difference in females, the observation was not considered to be treatment-related. Haematology and clinical chemistry observations showed no treatment-related patterns. A statistically significant increase in alkaline phosphatase was observed at days 30 and 60 for females given 5000 mg/kg/day, however, the increase was well within the historical range in rats and was not considered toxicologically meaningful. There were no ophthalmological findings observed in either sex at any dose. No treatment-related macroscopic observations were noted. Absolute and relative liver weights were increased in the females dose with 1600 mg/kg/day. Histopathological findings were not significant and did not correlate with organ weight findings. In the absence of related findings, the changes in organ weights were considered adaptive in nature. Actual consumption of the notified substance was approximately 4479 to 1881 mg/kg/day for males and 4877 to 2280 mg/kg/day for females over the course of the test. The LOAEL is >5000 mg/kg bw/day in both sexes of rat based on the absence of adverse effects at this dose level. The NOAEL is 5000 mg/kg bw/day in both sexes of rat. Under the conditions of this test, the notified substance is considered a low oral repeat-dose toxicity hazard in rats.

Bacterial Mutation Assay (Ames test)

The mutagenicity of the notified substance was tested in the Ames test using *S. typhimurium* strains TA1535, TA1537, TA98, TA100 and TA102. The test was conducted in duplicate according to OECD Guideline for Testing of Chemicals 471. The test was conducted in duplicate, once using the direct plate incorporation method and once using the preincubation method. Generally toxic effects such as thinning of the background lawn of non-revertant cells and a reduction in revertant colony numbers, was not observed, however, small reductions in the numbers of revertant colonies were observed in strains TA100 (1 plate at 5000 µg/plate +S9) and TA1535 (1 plate at 5000, 1600 and 160 µg/plate +S9) . Test concentrations were not corrected for purity. No substantial increases in revertant colony numbers over control counts were obtained with any of the tester strains following exposure to the test substance at any concentration tested in either the presence or absence of S9 mix. All of the positive controls used in the test induced marked increases in the frequency of revertant colonies, confirming the activity of the S9 mix, and the sensitivity of the test strains. The notified substance is not considered to be mutagenic in this test system.

In vitro Genotoxicity test: Mammalian Cell Chromosomal Aberration Assay

The genotoxicity of the notified substance was tested in the Mammalian Cell Chromosomal Aberration Assay using human lymphocytes collected from the blood of 2 healthy, male donors. The test was conducted in triplicate according to OECD Guideline for Testing of Chemicals 473. In the preliminary test, cells were exposed to 313, 625, 1250, 2500 and 5000 µg/mL in DMSO both with and without a S9 mixture. In the first main test, cells were exposed to 1250, 2500 and 5000µg/mL in DMSO without the S9 mixture and 625, 1250, and 2500 µg/mL with the S9 mixture. In the second main test, cells were exposed to 40, 80, and 160 µg/mL in DMSO without the S9 mixture and 625, 1250, and 2500 µg/mL with the S9 mixture. For all tests, the solvent control included 50 µg/mL of DMSO while the positive controls consisted of 0.015 µg/mL Daunomycin(-S9) and 5 µg/mL Cyclophosphamide (+S9). In the preliminary test, the notified substance caused a reduction in mitotic index to 83% of solvent control at 625 µg/mL(-S9) and 64% at 5000 µg/mL(-S9). With S9, the test substance caused a reduction in mitotic index to 10% of solvent control at 5000 µg/mL. Therefore, due to the steep toxic response in both the absence and presence of S9, repeat tests were performed with lower doses. During the repeat tests, there were no statistically significant increases in proportion of metaphase figures with chromosomal aberrations at any dose level compared to the solvent control in cells in the presence or absence of S9. The positive controls produced statistically significant increases in the frequency of aberrant metaphases demonstrating the sensitivity of the test and the efficacy of the S-9 mix. Based on these results, the test substance is not clastogenic under the conditions of the test.

In vivo Genotoxicity test: Mammalian Chromosomal Aberration (Micronucleus) Assay

The genotoxicity of the notified substance was tested in the mammalian chromosomal aberration (micronucleus) assay in accordance with OECD 474 using mice aged 5 to 8weeks (23 to 30g). A range finding toxicity test was conducted to determine a suitable dose level for use in the micronucleus test. Groups of mice were dosed as follows: 2 male and 2 female mice were injected with a single intraperitoneal dose of 2000 mg/kg; 1 male and 1 female were dosed orally with 2000 mg/kg; 1 male and 1 female were injected with single intraperitoneal dose of 1000 mg/kg; and 2 males were injected with a single intraperitoneal dose of 2000 mg/kg. No mortalities or clinical signs of toxicity were observed throughout the duration of the test. As the dose level of 2000 mg/kg/day, the standard limit dose for this test, was tolerated in both male and females, this dose was chosen as the dose for the micronucleus test. No difference in response between males and females was observed therefore it was considered acceptable to use only males for the micronucleus test. No toxic effects were observed, therefore, the absorption of the substance could not be confirmed. For this reason intraperitoneal injection was used to ensure the substance reached the target tissues. There were no premature deaths in any of the dose groups and clinical signs of toxicity were not observed. There was no evidence of a statistically significant increase in the incidence of micronucleated polychromatic erythrocytes in mice dosed with the test material when compared to the concurrent vehicle control groups. The positive control group showed a marked increase in the incidence of micronucleated polychromatic erythrocytes which confirms the sensitivity of the test system. Based on these results the notified substance is not considered to be genotoxic under the conditions of the test.

ADME Study

The metabolism and toxicokinetics of the notified substance were investigated according to OECD Guideline 417. A summary of the study was provided by the notifier. No treatment related clinical signs were observed. Uptake of radioactivity into the systemic circulation was rapid with a peak concentration in blood occurring between 1 and 3 hours post-dosing at 500 mg/kg and at 6 hours post-dosing at 5,000 mg/kg. The peak concentration represented approximately 1.2 to 1.3% of the administered dose at 500 mg/kg and approximately 0.56% of the dose at 5,000 mg/kg. Elimination of radioactivity from the blood was slow at both doses. The mean plasma elimination half-life was 55.6 hours at 500 mg/kg and 51.9 hours at 5,000 mg/kg. Radioactivity was eliminated from the body as $^{14}CO_2$ with 62% accounted for within 12 hours of dosing, 70.8% within 24 hours and 77% after 72 hours. The remaining radioactivity was excreted in urine (6.5%) and feces (24.6%). Maximum concentrations in the tissues include liver (1.29% of the dose) at 24 hours post-dosing, kidneys (0.23% of the dose) at 6 hours post-dosing, and thymus (0.026% of the dose) at 12 hours post-dosing. Metabolism is expected to be relatively rapid and consist of extensive hydrolytic cleavage of the 12-acetyl function then catabolism of the acetate to CO_2. The recovery of a substantial proportion of the administered radioactivity as $^{14}CO_2$ suggests that deacylation may be initiated in the stomach and that there is no significant absorption of unchanged notified substance.

6.3. Human health risk characterisation

6.3.1. Occupational health and safety

Based on available studies, the notified chemical was of low acute dermal toxicity in rats. It was slightly irritating to the skin and eyes of rabbits and was not a skin sensitiser in guinea pigs. The notified chemical is not expected to be a genotoxic. In an oral 90 days toxicity study in rats, the NOAEL was established as >5000 mg/kg bw/day. The notified chemical is unlikely to have developmental and reproductive effects. The notified chemical is a glyceride which is inherently metabolisable and bioaccumulation of the notified chemical is not expected. Therefore, the main hazard of the notified chemical is slight skin and eye irritation.

The risk of skin and eye irritation would be present during importation (transport and storage), blending and the end-use of the notified chemical. During transport and storage, the risk to workers is minimal and acceptable as workers will only be exposed to the notified chemical in the case of an accident involving damage to the packaging. During blending and the end-use, the exposure of workers to the notified chemical is expected to be low due to the use of automatic/semi-automatic processes and the use of PPE, and risk is therefore considered acceptable.

Therefore, considering the exposure level, low hazards of the notified chemical, and the use of PPE, the risk of acute occupational exposure is considered acceptable. Furthermore, considering the high NOAEL (>5000 mg/kg bw/day) obtained in a 90 days repeat dose study, risk from the repeated use of the notified chemical is also considered acceptable.

6.3.2. Public health

Based on available studies, the main hazard of the notified chemical is slight skin and eye irritation. The notified chemical was of low toxicity in an oral 90 days toxicity study in rats, where the NOAEL was established as >5000 mg/kg bw/day.

Exposure to the notified chemical for the general public may occur through its use in vinyl flooring, dyes/pigments, toys and other plastic articles. Although most exposure is expected to be transient, exposure of children who may mouth items containing the notified chemical may be higher.

Migration studies from PVC and polypropylene into solvents have shown that leaching occurs at the temperatures and long time periods tested, that may be representative of food contact scenarios. However, migration out of consumer products that are not intended to come into contact with foodstuffs is expected to be low because the temperatures and length of exposure are expected to be lower. The level of exposure to the general public therefore, is expected to be low from consumer products (including dyes/pigments) that do not come in contact with food. Therefore, based on the low hazard profile of the notified chemical and limited exposure, the risk to human health from non food contact uses is considered acceptable.

Exposure to the notified chemical for the general public would also occur through its use in food contact materials such as packaging film, storage containers, microwave trays etc. As stated above, migration studies have shown that leaching occurs at the temperatures and long time periods used for testing that may be representative of food contact scenarios. Therefore, it is expected that the general public will be exposed to the notified chemical through ingestion of food that has been in contact with articles containing the notified chemical.

A quantitative risk assessment for direct exposure through the use in food contact materials has not been undertaken. However, given the low systemic toxicity, there is not expected to be a significant risk to the public. In addition, it is noted that the US Food and Drug Administration (FDA) has approved the notified chemical for use in certain food contact materials (US FDA 2007).

The FDA approved uses in food packaging include:

1. a plasticizer at levels up to 34% by weight in polyvinylchloride bottle cap sealing rings for use in contact with all foods under the following conditions: boiling water sterilized; Hot filled or pasteurized above 150^0F; hot filled or pasteurized below 150^0F; room temperature filled and stored (no thermal treatment in the container); refrigerated storage (no thermal treatment in the container); frozen storage (no thermal treatment in the container); and frozen or refrigerated storage (ready-prepared foods intended to be reheated in container at time of use, including aqueous or oil-in-water emulsion of high- or low-fat and aqueous, high- or low-free oil or fat).

2. a plasticizer at levels up to 3% by weight in polymers used to manufacture articles intended for repeated-use in contact with all foods under the following conditions: refrigerated storage (no thermal treatment in the container); frozen storage (no thermal treatment in the container); and frozen or refrigerated storage (ready-prepared foods intended to be reheated in container at time of use, including aqueous or oil-in-water emulsion of high- or low-fat and aqueous, high- or low-free oil or fat).

3. a colorant carrier or dispersant at levels up to 0.5% by weight in polymers used to manufacture articles intended for repeated-use in contact with all foods.

The notified chemical is also included in the list of food contact materials in the EU (EU Commission, 2008).

A copy of the NICNAS assessment report will be referred to Food Standards Australia New Zealand (FSANZ).

The notifier has indicated that the notified chemical will also be used as a plasticiser/softener in medical devices at concentrations ranging from 2-34%. This use is not covered in the current assessment as this use does not fall within the jurisdiction of NICNAS.

7. ENVIRONMENTAL IMPLICATIONS

7.1. Environmental Exposure & Fate Assessment

7.1.1 Environmental Exposure

RELEASE OF CHEMICAL AT SITE

No release of the notified chemical to water is anticipated during use as it will be used in contained systems within industrial facilities. The notifier estimates that daily release to sewer will not exceed 3 kg at any individual site.

RELEASE OF CHEMICAL FROM USE

The notified chemical is not expected to be released significantly during use from the plastic articles or textiles in which it is incorporated.

RELEASE OF CHEMICAL FROM DISPOSAL

Plastic articles will be recycled, disposed of to landfill or destroyed by thermal decomposition at the end of their useful lives. Textiles are likely to be disposed of to landfill. There may be some release of the notified chemical to sewer from plastics recycling activities, and slow release from discarded articles in landfill.

7.1.2 Environmental fate

The notified chemical has low solubility in water (0.06-0.09 mg/L) and a very high adsorption/desorption coefficient (log K_{oc} = 5.4) which suggests the notified substance will have low mobility in the environment due to leaching. The notified chemical also has low vapour pressure (1.1 x 10^{-7} Pa at 25°C and 4.8 x 10^{-8} Pa at 20°C) which indicates that it will not partition to air. These data suggest the notified chemical will partition to soil. Based on a Level I fugacity model, the majority of the notified chemical (97.7%) will partition to the soil. The notified chemical is expected to partition to sewage sludge during sewage treatment.

The notified chemical has a high partition coefficient which is characteristic of a bioaccumulative substance. However, as it is a glyceride and therefore inherently metabolizable, bioaccumulation is not expected. The notified chemical has been shown to be moderately concentrating in fish by testing at the yolk sac larval stage, but residues would appear to have been specifically retained in the yolk tissue. As biotransformation of xenobiotics in embryonic and larval stages has been shown to be insignificant compared to juvenile/adult stages, higher body burdens of readily biotransformed chemicals may be reached in early life stages of fish (Petersen and Kristensen, 1998). For the details of this testing, please refer to Appendix C.

Based on results of the biodegradation study provided with this notification, the notified chemical is readily biodegradable (98% in 28 days) and not expected to persist in the environment.

7.1.3 Predicted Environmental Concentration (PEC)

The PECs are estimated below based on daily release of 3 kg from a single industrial facility to a small sewage treatment plant with a daily flow of 5 ML. Note that these estimates are likely to greatly exaggerate any aquatic exposure, as they are based on the worst case assumption that no degradation or sorption to sludge occurs during sewage treatment, and provide a discharge concentration in treated effluent that exceeds the water solubility.

Predicted Environmental Concentration (PEC) for the Aquatic Compartment

Daily chemical release:	3	kg/day
Removal within STP	0%	
Daily effluent production:	5	ML
Dilution Factor - River	1.0	
Dilution Factor - Ocean	10.0	
PEC - River:	600	µg/L
PEC - Ocean:	60	µg/L

7.2. Environmental effects assessment

The results from ecotoxicological investigations conducted on the notified chemical are summarised in the table below. Details of these studies can be found in Appendix C

Endpoint	*Result*	*Assessment Conclusion*
Fish Toxicity	LC50 = 0.28-0.73 mg/L	Very toxic
Daphnia Toxicity	EC50 = 0.92 mg/L	Very toxic
Algal Toxicity	E_rC50 = 26 mg/L	Harmful

The notified chemical was tested as an emulsion because of its very low aqueous solubility. Measured concentrations were lower than the nominal test concentrations. The effects observed in fish and daphnids are considered a physical effect of the hydrophobic test substance, rather than manifestations of systemic toxicity. There were no signs of systemic toxicity at the limit of water solubility.

Summaries of ecotoxicological investigations

Acute toxicity to fish

The 96-hour acute toxicity of the notified substance to zebra fish (*Danio rerio*) was examined in accordance with OECD Guideline for Testing of Chemicals 203, however, no range-finding test was conducted. A total of 10 fish per dose were exposed to the notified substance for 96 hours under semi-static conditions. Doses of the notified substance (purity 96%) included the following nominal concentrations: 0, 0.1, 0.3, 1, 3 and 10 mg/L. Due to the hydrophobicity of the notified substance (log Kow = 6.42), the measured concentrations were much lower, including 0.28, 0.73 and 4.86 mg/L for the 1, 3, and 10 mg/L doses respectively. The measured concentration for the 0.1 and 0.3 mg/L were not reported. The notified substance has low water solubility. Therefore, based on OECD 203, the test substance was tested as an emulsion prepared by blending with Ultra Turrax high speed stirring/emulsification equipment. Details of the speed and timing used to prepare the emulsion were not provided. The single control group was maintained under identical conditions but not intentionally exposed to the notified substance. The measured concentration for the control group included 0.18 mg/L which was attributed to grease on the equipment used during the study. Mortality in the controls did not exceed 10%. The oxygen saturation was >60% throughout the test period. The EC50 value for the reference substance ($K_2Cr_2O_7$) was 182 mg/L which is in the historical range for this substance. Exposure concentrations declined rapidly during each 24-hour period between renewal of the test solutions which was attributed to partitioning of the test substance onto biomass and adhesion to the surfaces of the test aquaria. Non-lethal effects were observed at the 2 highest doses and included changes in swimming behaviour, balance, heading for the bottom, respiration, and pigmentation. The strongest effects were changes in swimming behaviour, respiration and pigmentation. Lethality was observed in the 2 highest doses only. The concentration of non-dissolved test substance is assumed to increase with increasing concentration and as lethal effects were observed only in the highest test concentrations, it is assumed that the effect reflected a physical effect of the non-dissolved test substance. Therefore, lethal effects were considered a physical effect because of the tendency of hydrophobic substances such as the test substance to sorb to the gills resulting in inhibition of respiratory function. After 96 hours, 6 of the 10 fish dosed with the measured concentration of 0.28 mg/L were dead and all of the 10 fish in the 0.73 mg/L dose group (measured concentration) were dead. Therefore, the LC50 is >0.28 to <0.73 mg/L and the NOEC is 0.28 mg/L. Based on these data, the notified substance may be considered a high acute toxicity hazard to zebrafish under the conditions of this test.

Acute toxicity to aquatic invertebrates

The 48-hour acute toxicity of the notified substance to *Daphnia magna* Stratus was examined in accordance with OECD Guideline for Testing of Chemicals 202. Two range finding tests were conducted but were considered to be invalid due to low reproducibility and inconsistency between the dose and effects was observed. For example, the test substance formed emulsions with gentle stirring, however these emulsions were unstable. Therefore, based on OECD 202, the test substance was tested as an emulsion prepared by blending with Ultra Turrax high speed stirring/emulsification equipment at 20,500 rpm for 2 minutes. The concentrations achieved exceeded the solubility of the test substance based on a water solubility estimate of <0.33 mg/L which is derived from the detection limit. A total of 20 daphnids per dose, in 4 groups of 5, were exposed to each dose of the notified substance for 48 hours under static conditions. Dissolved oxygen saturation at the end of the test was 99% in the highest dose. Less than 10% of the control daphnia were immobilized which indicates valid test conditions. The EC50 value for the reference substance ($K_2Cr_2O_7$) was 1.46 mg/L which is in the historical range for this substance. Immobility of daphnids was observed at all test concentrations. The 48 hour EC50 is 0.92 mg/L based on probit analysis using the measured concentrations. The test substance is not considered to be systemically toxic at the solubility concentration based on the idea that the effects on mobility was a physical

effect of the non-dissolved test substance rather than a systemic effect. However, based on the EC50, the notified substance can be considered of high concern to daphnids.

Algal growth inhibition test
The acute toxicity of the notified substance to green algae (*Pseudokirchneriella subcapitata*) was examined in accordance with OECD Guideline for Testing of Chemicals 201. Based on the outcome of a preliminary range-finding test with the daphnia, algae were exposed to the following doses of the notified substance for 72 hours: 0, 2.5, 5, 10, 20, 40, 80, and 160 mg/L. The notified substance has low water solubility. Therefore, based on OECD 201, the test substance was tested as an emulsion prepared by blending with Ultra Turrax high speed stirring/emulsification equipment. Details of the speed and timing to prepare the emulsion were not provided. Three replicates per dose were exposed to the notified substance under static conditions and continuous light. Test nominal concentrations are higher than the reported solubility of the test substance in water (2×10^{-4} mg/L). After 72 hours only 5 to 30% of the initial concentrations were detected. This was attributed to the hydrophobicity of the test substance resulting in the adhesion of the test substance to the test flasks. EC values for growth rate were calculated by use of the computer program TOXEDO based on measured concentrations. Biomass data was not provided with the study report. The E_rC_{50} (0-72h) is 26 mg/L. The NOEC for growth rate was 0.28 mg/L. These results can be considered a moderate acute toxicity hazard to green algae for both growth inhibition and biomass reduction.

7.2.1 Predicted No-Effect Concentration (PNEC)

The PNEC cannot be calculated as the median effect concentrations in aquatic toxicity testing were not determined. The notified chemical is not considered to be toxic to aquatic life at concentrations up to the solubility limit.

7.3. Environmental risk assessment
A Risk Quotient (PEC/PNEC) cannot be calculated as the PNEC is not known.

The notified chemical is not considered to pose a risk to the environment when it is used as proposed, as it is not expected to be released in significant quantities in aqueous waste streams, is readily biodegradable (and therefore expected to degrade rapidly during sewage treatment and in the environment) and showed no signs of toxicity in aquatic life at the limit of water solubility.

8. CONCLUSIONS AND REGULATORY OBLIGATIONS

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

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 reported use pattern, the notified chemical is not considered to pose a risk to the environment.

Recommendations

CONTROL MEASURES
Occupational Health and Safety

- Employers should implement the following safe work practices to minimise occupational exposure during handling of the notified chemical as introduced and as diluted for use in the products:
  - Avoid contact with skin and eyes

- Employers should ensure that the following personal protective equipment is used by workers to minimise occupational exposure to the notified chemical as introduced and as diluted for use in the products:
  - Gloves, protective clothing

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

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

- If products and mixtures containing the notified chemical are classified as hazardous to health in accordance with the *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(2) of the Act; if
  - the function or use of the chemical has changed from use as a plasticiser/softener and as a colorant carrier, or is likely to change significantly;
  - the amount of chemical being introduced has increased from 10,000 tonnes, 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.

No additional secondary notification conditions are stipulated.

Material Safety Data Sheet
The MSDS of the product containing 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.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Physico-chemical properties were conducted on a test substance containing approximately 86% of notified chemical.

Water Solubility 0.00006-0.00009 g/L at 20°C
(0.06-0.09 mg/L)

Method In house method.
Remarks Flask Method. The notified chemical was dispensed into water from an acetone stock solution at nominal concentrations of 58, 92, 123, 154 and 215 µg/L and analysed by a capillary liquid chromatography/ion trap mass spectrometry method. The main component of the notified chemical was quantified. The precision was poor for all but the lowest concentration, because of saturation and formation of micelles or droplets.
Test Facility Danisco (2008)

Migration of the notified chemical in polypropylene

Summary A migration study of the specific migration from polypropylene (PP) plaques containing 0.47% notified chemical to the fatty food simulant (99.9% ethanol) and the aqueous food simulant (10% aqueous ethanol) was carried out. The migration test was conducted at 40°C for 10 days and the migration was determined after 2, 24, 96 and 240 hrs, respectively, by means of GC and gravimetry.
The specific migration (mg/in^2) of the notified chemical to 99.9% ethanol was 0.0092, 0.034, 0.064, 0.10, at 2 hrs, 24 hrs, 96 hrs, and 240 hrs, respectively. as measured by GC.
Under ideal conditions, additive migration from polymers will follow Fick's law, implying migration will be a function of the square root of time. From the best-fit function of the data generated, it is determined that the migration of the notified chemical was in excellent correspondence with Fick's law.
In order to verify the specific migration determined by GC, the migration was also measured gravimetrically as the weight loss of polypropylene plaques and by weighing the amount of material migrated from the plaques. The results for specific migration of the notified chemical from polypropylene to 99.9% ethanol determined by weighing of both plaques and migrated material were seen to be in good agreement with the results obtained by GC, especially at 240 hrs interval.
The specific migration of the notified chemical to the aqueous food simulant (10% aqueous ethanol), was measured by weighing of polypropylene plaques before and after contact with the simulant at 40°C for 240 hrs. The mean value of specific migration was 0.005 mg/in^2, which was considerably lower as compared to 99.9% ethanol (0.08 mg/in^2).
Test Facility Development Laboratory Emulsifiers (2005)

Migration of the notified chemical from PVC plaques into aqueous simulants

Summary The migration of notified chemical from PVC plaques containing 33.7% notified chemical into aqueous food simulants (3% w/v aqueous acetic acid and 15% v/v aqueous ethanol) has been determined. The migration test was conducted at 40°C for 10 days and the concentration of the two main components of the notified chemical was analysed by LC-MS in samples taken after 3, 4, 7 and 10 days, respectively. After termination of the study, the food simulant was also analysed by GC.

The specific migration (mg/in^2) of the notified chemical to 15% ethanol was 0.0021, 0.0018, 0.0018, 0.0018, at 3 days, 24 hrs, 4 days, 7 days, and 10 days, respectively, as calculated per volume of simulant and area of PVC using LC-MS. The specific migration (mg/in^2) of the notified chemical to 3% acetic acid was 0.0008, 0.0004, 0.0003, 0.0004, at 3 days, 24 hrs, 4 days, 7 days, and 10 days, respectively, as calculated per volume of simulant and area of PVC using LC-MS.

	Based on the results, the main conclusion is that the main factor determining the migration of the notified chemical to aqueous food simulants is the solubility of the component in the simulant rather than the content in the PVC, mass transfer in the PVC, migration time and the surface area. Accordingly, the lack of continued migration of the notified chemical into the simulant after 4 days of the 10-day experiment suggests that the solubility limits of these components were reached.
Test Facility	Development Laboratory Emulsifiers (2006)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

Toxicological investigations were conducted on notified chemical with a purity of >85%.

B.1. Developmental toxicity -Preliminary

TEST SUBSTANCE Notified chemical

METHOD

Species/Strain	Rat/Sprague-Dawley Crl:CD
Route of Administration	Oral – gavage
Exposure Information	Exposure days: days 5 to Day 19 of gestation Post-exposure observation period: 0
Vehicle	Arachis oil BP
Remarks - Method	No significant protocol deviations. All animals were killed on Day 20.

RESULTS

Group	*Number of Animals*	*Dose mg/kg bw/day*	*Mortality*
Control	8	0	0
Low Dose	8	250	0
Intermediate Dose	8	500	0
High Dose	8	1000	0

Mortality and Time to Death

There were no deaths during the course of study.

Effects on Dams

No clinically observable signs of toxicity were detected in test or control animals throughout the study period. No adverse effects on dietary intake and body weight developments were detected. In all the treatment groups, one female in each group was observed to have implantations sites as detected by necropsy staining the uteri with 1% ammonium polysulphide solution, indicating that these females may have lost their utero at an early stage of pregnancy. Although there was no similar incidence in the control group, in the absence of any supporting evidence of effects on implantation survival for the animals with young on Day 29 of gestation, this was considered to be co-incidental and most likely unrelated to maternal treatment.

No treatment-related macroscopic abnormalities and effects on uterine parameters were detected.

Effects on Foetus

No treatment-related macroscopic abnormalities and effects in the foetal viability or in growth and development were detected at terminal kill.

CONCLUSION

The No Observed Effect Level (NOEL) for maternal and developmental toxicity was established as 1000 mg/kg bw/day in this study, based on no toxicologically significant changes in the parameters measured at the highest tested dose (1000 mg/kg bw/day). These dose levels (250, 500 & 1000 mg/kg bw/day) were considered appropriate for use in the main oral gavage prenatal development study in the rat.

TEST FACILITY SafePharm Laboratories Ltd (2009a)

B.2. Developmental toxicity -Preliminary

TEST SUBSTANCE Notified chemical

METHOD

Species/Strain	Rabbit/New Zealand White
Route of Administration	Oral – gavage
Exposure Information	Exposure days: Day 3 to Day 28 of gestation

	Post-exposure observation period: 0
Vehicle	1% aqueous sodium carboxymethylcellulose (1% CMC)
Remarks - Method	No significant protocol deviations. All animals were killed on Day 29. The study was conducted in two phases; an initial phase using non-pregnant animals to establish the probable maximum tolerated dose, followed by a second phase using time-mated animals to determine appropriate dose levels for further investigation of developmental toxicity in rabbit. The second phase of the study is reported here.

RESULTS

Group	*Number of Animals*	*Dose mg/kg bw/day*	*Mortality*
Control	6	0	0
Low Dose	6	250	0
Intermediate Dose	6	500	0
High Dose	6	1000	0

Mortality and Time to Death

There were two unscheduled deaths during the study; neither of which was considered to be related to treatment. One animal from 250 mg/kg bw/day group died due to damage to the cervical region of the neck as a result of self-inflicted trauma in the animals' home cage. Another animal from 1000 mg/kg bw/day group was killed after showing adverse clinical signs including apparent hypothermia, pallor, prostration and gasping respiration. It was not clear whether this was due to a previous underlying condition or an intubation error earlier during treatment.

Effects on Dams

No clinically observable signs of toxicity were detected in test or control animals throughout the study period. Body weight performance of treated animals did not indicate any adverse effect of treatment at dosages up to 1000 mg/kg bw/day. Food consumption showed great individual variation and, while food intake tended to be lower than control for treated groups, there was no consistent dosage relationship. Neither the type and incidence nor distribution of macroscopic findings observed at scheduled necropsy of females on terminal killings indicated any adverse effect of treatment at dosages up to 1000 mg/kg bw/day. There were no adverse effects of treatment on the number of implantations, embryo foetal survival, sex ratio or live litter size at the terminal killing. At 1000 mg/kg bw/day, mean placental weights were lower than control; however, this may reflect the higher litter size at this dosage as compared with the control.

Effects on Foetus

At 1000 mg/kg bw/day, mean foetal weights were lower than control; however, this may reflect the higher litter size at this dosage as compared with the control. There were no treatment-related adverse effects on foetal morphology at necropsy.

CONCLUSION

The NOEL for maternal and developmental toxicity was established as 1000 mg/kg bw/day in this study, based on no toxicologically significant changes in the parameters measured at the highest tested dose (1000 mg/kg bw/day). These dose levels (250, 500 & 1000 mg/kg bw/day) were also considered appropriate for use in the main oral gavage prenatal development study in the rat.

TEST FACILITY Harlan Laboratories Ltd (2009a)

B.3. Developmental toxicity -Main

TEST SUBSTANCE Notified chemical

METHOD

Species/Strain	Rat/Sprague-Dawley Crl:CD
Route of Administration	Oral – gavage
Exposure Information	Exposure days: days 5 to Day 19 of gestation Post-exposure observation period: 0

Vehicle	Arachis oil BP
Remarks - Method	No significant protocol deviations. All animals were killed on Day 20.

RESULTS

Group	*Number of Animals*	*Dose mg/kg bw/day*	*Mortality*
Control	24	0	0
Low Dose	24	100	0
Intermediate Dose	24	300	0
High Dose	24	1000	0

Mortality and Time to Death

There were no treatment related deaths during the course of study. However, one female treated with 1000 mg/kg bw/day was killed *in extremis* on Day 17. This death was considered not to be related to treatment and was considered to be due to an accident during the dosing procedure, as it has a hole in the oesophagus and a mass present in the upper right thorax. A further female from this treatment group was also terminated on Day 19 due to the early onset of littering and had an enlarged spleen and a distended stomach with red contents.

Effects on Dams

No clinically observable signs of toxicity were detected in test or control animals throughout the study period. No adverse effects on food consumption and body weight developments were detected in the test or control animals. There was no adverse effect on *in-utero* offspring survival, as assessed by the mean numbers of early or late resorptions, live litter size and post-implantation losses. Sex ratio was not significantly different among groups.

Effects on Foetus

For all dose groups, there were no significant treatment-related trends in the proportions of foetuses (or litters) with evidence of visceral or skeletal anomalies. The types of visceral and skeletal anomalies were those commonly observed for this type of study. Females treated with 300 and 100 mg/kg bw/day showed a statistically significant reduction of foetuses showing ovoid eye lens. In the absence of a true dose-related response, the inter group differences were considered attributable to the increased number of control foetuses showing effect.

Females from all different groups showed an increase in foetus weight when compared with control animals. An increase in body weight is not considered of toxicological significance.

Remarks - Results

The oral administration of the notified chemical to pregnant rats during organogenesis at dose levels of 100, 300 and 1000 mg/kg bw/day did not results in any toxicological significant effects at any dose level.

CONCLUSION

The NOEL for maternal and developmental toxicity was established as 1000 mg/kg bw/day in this study, based on no toxicologically significant changes in the maternal and offspring parameters measured at the highest dose tested (1000 mg/kg bw/day).

TEST FACILITY Harlan Laboratories Ltd (2009b)

B.4. Toxicity to reproduction – two generation study - Preliminary

TEST SUBSTANCE Notified chemical

METHOD

Species/Strain	Rat/Sprague-Dawley Crl:CD
Route of Administration	Oral – diet
Remarks - Method	The study was also designed to validate, using DEHP (Bis(2-ethylhexyl) phthalate), the ability of screening tests used within the laboratory to detect potential endocrine disruptors. Briefly, F0 animals received the appropriate test diet for a minimum of

two weeks prior to mating and throughout pairing, gestation and lactation until termination. During week 3, all F0 animals were paired to mate within each dose group for a maximum of three weeks. Pregnant females were allowed to give birth and at Day 21 post partum, within treatment groups, offspring resulting from the F0 mating phase were selected to form the next generation (F1). The selected F1 males and females received their appropriate test diet and were assessed for evidence of sexual maturation.

Group Designation	Dietary Concentration (ppm)	Animal Numbers			
		F0 generation		F1 generation	
		Male	Female	Male	Female
Control	0	10	10	10	10
Positive control (DEHP)	5000	10	10	10	10
Low dose	10000	10	10	10	10
High dose	20000	10	10	10	10

Generation	Treatment Group	Aqueous Concentration (ppm)	Mean Achieved Dose Level (mg/kg bw/day)			
			Males	Females		
				Maturation	Gestation	Lactation
F0	DEHP	5000	294	356	377	687
	Low dose	10000	387	68	649	1498
	High Dose	20000	1105	1360	1467	2746
FI	DEHP	5000	593	656		
	Low dose	10000	1107	1237		
	High Dose	20000	2228	2505		

RESULTS

Mortality and Time to Death

One F0 female receiving 20,000 ppm notified chemical was killed around the time of expected parturition for animal welfare considerations. This death was considered to be coincidental and unrelated to treatment.

Effects on animals:

There were no adverse effects of the treatment with the notified chemical or DEHP on the following: Clinical signs; food intake and bodyweight or bodyweight changes for F0 animals and selected F1 animals, including females for the periods of gestation and lactation, or for F1 animals to sexual maturation.; on mating, fertility, and parturition indices for F0 animals; on corpora lutea count, implantation rate, litter size, sex ratio or offspring viability for F0 animals; F0-F1 offspring bodyweights or bodyweight changes to weaning; sexual maturation of the F1 generation.

At 5000 ppm treatment with DEHP, visible nipple counts for female offspring at Day 12 of age were lower than control; these differences were no longer apparent by Day 15 of age and male nipple counts were unaffected. Nipple counts for F0-F1 offspring received the notified chemical were unaffected by the treatment. Mean ano-genital distance for F0-F1 offspring on Day 1 of age were not adversely affected by the notified chemical or DEHP.

There were no treatment-related macroscopic abnormalities observed for F0 animals, F0-F1 offspring or for the selected F1 animals.

CONCLUSION

The dietary administration of the notified chemical at up to 20,000 ppm was not associated with any findings considered to be of toxicological significance. Therefore, this dose level is suitable for investigation of developmental neurotoxicity and toxicity over two successive generations in the rat.

TEST FACILITY SafePharm Laboratories Ltd (2009b)

B.5. Genotoxicity – in vitro

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 476 In vitro Mammalian Cell Gene Mutation Test.
Species/Strain	Mouse
Cell Type/Cell Line	Lymphoma L5178Y cells
Metabolic Activation System	S9 fraction from Aroclor 1254 induced rat liver.
Vehicle	RPMI 1640 medium
Remarks - Method	No significant protocol deviations. The treatments without metabolic activation in the second main test were repeated using a lower range of test concentrations, as the notified chemical was highly toxic to the cells at the concentration used in the test (313 to 3600 µg/mL) for the 24 hrs treatment period. At the end of the expression period, the cells were cultured with and without trifluorothymidine to determine the number of mutants and the cloning efficiency. After further incubation for 10 days, the number of wells with cell clones were counted and the mutation frequencies were calculated.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*
Absent		
Test 1	0, 625, 1250, 2500, 3600, 5000	4 hrs
Test 2	0, 313, 625, 1250, 2500, 3600	24 hrs
Test 2 (repeat)	0, 2.5, 5, 10, 20, 40, 80,160, and 320	24 hrs
Present		
Test 1	0, 625, 1250, 2500, 3600, 5000	3 hrs
Test 2	0, 156, 313, 625, 1250, 2500, 3600	3 hrs

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/mL) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	Not stated	>3600	Not stated	Negative
Test 2	Not stated	>313	Not stated	Negative
Test 2 (repeat)	---	>160	Not stated	Negative
Present				
Test 1	Not stated	>1250	Not stated	Negative
Test 2	Not stated	>2500	Not stated	Negative

Remarks - Results	The sensitivity of the test and the efficacy of the S-9 mix were demonstrated by large increases in mutation frequency in the positive control cultures.
CONCLUSION	The notified chemical was not mutagenic to lymphoma L5178Y cells treated in vitro under the conditions of the test.
TEST FACILITY	Scantox Laboratories Ltd (2002)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Bioaccumulation

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 305 Bioconcentration: Flow-through Fish Test. EC Directive 98/73/EC C.13 Bioconcentration: Flow-Through Fish Test.
Species	Zebra fish (*Danio rerio*)
Exposure Period	Exposure: 2 days Depuration: 2 days
Auxiliary Solvent	Acetone
Concentration Range	Nominal: 0.035, 0.070 mg/L Actual: 0.043, 0.075 mg/L
Analytical Monitoring	Liquid scintillation counting
Remarks - Method	The test substance was prepared using radiolabelled acetyl chloride. Testing was conducted under static renewal rather than flow-through conditions, with test media renewed 1-2 times daily. The uptake and depuration periods were reduced to 2 days from the respective guideline recommendations of 28 and 14 days, because of the life stage tested. Fish were exposed as yolk sac larvae rather than adults as specified by the guideline. The test therefore represents a worst case evaluation of bioaccumulation potential, as larval fish have a lower metabolisation capacity than adults and a higher relative lipid content. As biotransformation of xenobiotics in embryonic and larval stages has been shown to be insignificant compared to juvenile/adult stages, higher body burdens of readily biotransformed chemicals may be reached in early life stages of fish. The egg yolk can act as a temporary toxicant sink (Petersen and Kristensen, 1998).
RESULTS	
Bioconcentration Factor	1241 at lower exposure, 720 at higher exposure, at steady state.
CT50	1 month (approximately)
Remarks - Results	The moderate bioconcentration observed is likely to be an artefact of the life stage tested. This could be clarified by testing juvenile or adult fish according to the guideline.
CONCLUSION	The notified chemical is moderately concentrating in fish at the yolk sac larval stage.
TEST FACILITY	DHI (2009)

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