

QUALITY GUARDS.



NAGARDO®
**THE NATURAL GUARDIAN
FOR BEVERAGE QUALITY**

Technical Leaflet

Beverage manufacturers tailor beverages to meet their customers' lifestyles – at LANXESS we tailor the corresponding beverage protection. **Nagardo®** is the natural guardian to secure and prolong the shelf life of your beverage. Safety enabled by nature.

X Nagardo®

QUALITY WORKS.

LANXESS
Energizing Chemistry

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*when used as directed



ABOUT NAGARDO® – A SAFE AND HIGHLY EFFECTIVE* PRESERVATIVE

Nagardo® is a *de novo* developed preservative first identified in the course of screening more than 100,000 different proprietary natural materials. Nagardo® comprises innovative new functional molecules based on naturally derived long-chain glycolipids from the edible Sweet Osmanthus Ear mushroom.

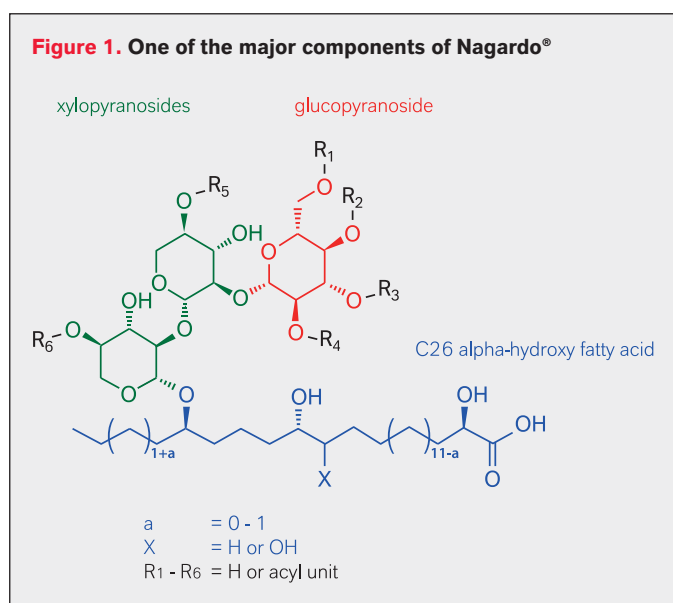
CAS Registry Number

2205009-17-0

Molecular and Structural Formula

A major component of Nagardo® is the glycolipid, as depicted in Figure 1. All other components are glycolipid congeners, sharing the saturated fatty acid and the trisaccharide moiety but differing in the acylation pattern, i.e. the number and position of acyl groups attached to the sugar units.

Nagardo® glycolipids consist of generally safe primary metabolites as building blocks well-known in various nutrition sources of mankind, such as glucose (drawn in red), xylose (green), acetate and isovalerate (black) as well as fatty acid (blue).



Production Process

Nagardo® is obtained via fermentation of glucose by the edible fungus Sweet Osmanthus Ear (wild type strain, not genetically modified). [13] This mushroom species is also known as *Dacryopinax spathularia*. Sweet Osmanthus Ear produces edible, orange-colored, spatula-shaped fruiting bodies as shown in the photo below.

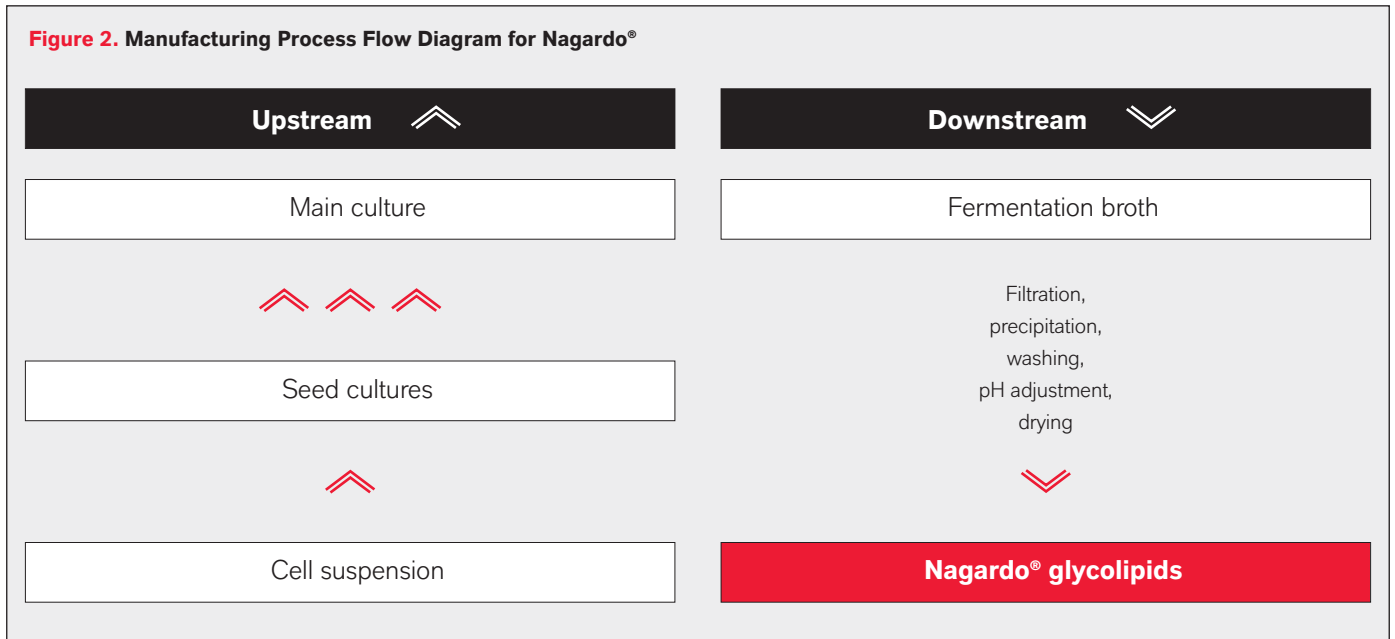
Nagardo® is recovered from the ferment by a food grade and solvent-free process using filtration and subsequent precipitation and washing steps. Nagardo® natural glycolipids are obtained without any chemical modification.

The production process follows current Good Manufacturing Practices (cGMP) as defined in 21 CFR §110 and is quality management compliant to FSSC22000, including a Hazard Analysis and Risk-based Preventive Controls (HARPC) plan as outlined by the U.S. Food and Drug Administration (FDA) Food Safety Modernization Act (FSMA). Nagardo® is certified with Kosher (OU) and Halal.



*when used as directed

Figure 2. Manufacturing Process Flow Diagram for Nagardo®



Properties of Nagardo®

- Beige to light brown powder
- Purity > 93% (total natural glycolipids; dry weight basis, sodium salt)
- Aqueous solubility > 20 g/l
- Taste neutral and colorless at use level
- Chemically stable at pH range between 2.5 to 7
- Shelf life of Nagardo® as dry powder: 36 months (ambient temperature or below; at dry conditions in close container)
- Stability of Nagardo® in aqueous solution: stable under autoclaving (121 °C for 20 min.) or pasteurization conditions (72 °C for 2 h)

Patent status

Patents granted in many countries, including: BR 112013031371 B1, CA 2838442 C, CN 103874411 B, EP 2717691 B1, JP 6124878 B2, KR 102014556 B1, MX 350043 B, US 11350628 B2

Further patent applications pending.

Regulatory status

Nagardo® natural beverage protection is approved for use in non-alcoholic beverages in a large number of countries including the United States of America (GRAS notice GRN740) and the European Union (E246). Please contact us for latest updates on product registration in your country.

Benefits that allow for new marketing and product opportunities

- Natural beverage protection
- Efficient control of spoilage organisms
- Pure sensory experience
- Broad application in various soft drink formulations
- Flexible integration into existing production processes
- Consumer friendly labeling
- Natural claims (depending on legislation)

ANTIMICROBIAL PROPERTIES

The following Table 1 depicts the minimum inhibitory concentrations (MICs; inoculation of >500 colony forming units (CFU)/mL) of Nagardo® against different types of food and beverage spoiling or pathogenic microorganisms.

The MIC data presented in Table 1 demonstrates the broad range of activity and great efficacy of Nagardo® as a preservative for beverage and food applications, in particular against various yeasts, molds and spoiling bacteria commonly found in those products.

Table 1

	ID strain collections	MIC (mg/l)
Gram-Positive Bacteria		
<i>Bacillus cereus</i>	ATCC11778	25
<i>Bacillus subtilis</i>	ATCC6633	<1.6
<i>Clostridium sporogenes</i>	ATCC3584	50
<i>Clostridium perfringens</i>	ATCC13124	60
<i>Corynebacterium variabile</i>	DSM20132	<1.6
<i>Enterococcus faecalis</i>	ATCC19433	50
<i>Lactobacillus brevis</i>	ATCC 367	<10
<i>Lactobacillus plantarum</i>	DSM12028	<3.9
<i>Listeria welshimeri</i>	DSM15452	16
<i>Listeria monocytogenes</i>	ATCC19111	200
<i>Propionibacterium acnes</i>	ATCC6919	60
<i>Staphylococcus aureus</i>	ATCC6538	<100
Gram-Negative Bacteria		
<i>Acetobacter aceti</i>	DSM 3508	50
<i>Acetobacter pasteurianus</i>	DSM 3509	6.3
<i>Asaia bogorensis</i>	own isolate	5
Filamentous Fungi		
<i>Aspergillus fumigatus</i>	ATCC 1028	20
<i>Aspergillus brasiliensis</i>	ATCC 16404	6.3
<i>Byssoschlamys fulva</i>	DSM 62097	3.1
<i>Byssoschlamys nivea</i>	DSM 1824	12.5
<i>Mucor plumbeus</i>	MUCL49355	7.9
<i>Penicillium roqueforti</i>	DSM 1079	12.5
<i>Pyricularia oryzae</i>	DSM62938	6
<i>Talaromyces luteus</i>	CBS348.51	<3.9
<i>Trichoderma virens</i>	ATCC 10045	<6.3
Yeasts		
<i>Candida albicans</i>	ATCC10231	25
<i>Candida glabrata</i>	ATCC36583	20
<i>Candida parapsilosis</i>	ATCC 22019	<6.3
<i>Dekkera (Brettanomyces) bruxellensis</i>	DSM70726	6.3
<i>Dekkera (Brettanomyces) naardenensis</i>	DSM70743	<3.9
<i>Saccharomyces cerevisiae</i>	MUCL 53497	3.1
<i>Zygosaccharomyces bailii</i>	DSM70492	3.1
<i>Zygosaccharomyces bisporus</i>	DSM70415	15.6
<i>Zygosaccharomyces florentinus</i>	DSM70506	7.8
<i>Zygosaccharomyces rouxii</i>	NCYC381	6.3
<i>Rhodotorula mucaliginosa</i>	own isolate	<10

As depicted in Table 2, Nagardo® can provide superior efficacy over a broad pH range.

Table 2: pH dependency of MIC values for Nagardo® benchmarked against potassium sorbate

Preservative	Spoiling microorganism	MIC [mg/l] in SDB medium, 28 °C, 10 ⁵ cfu/ml				
		pH 2.6	pH 3.6	pH 4.6	pH 5.6**	pH 6.6**
Potassium sorbate	<i>S. cerevisiae</i>	no growth	7.8	250	250	500
Nagardo®	<i>S. cerevisiae</i>	no growth	≤1.6*	3.1	3.1	6.3
Potassium sorbate	<i>A. brasiliensis</i>	7.8	62.5	250	500	500
Nagardo®	<i>A. brasiliensis</i>	≤1.6*	≤1.6*	3.1	6.3	12.5

* lowest concentration tested

** Please note that in beverages having a pH >4.6, a second killing step/principle is required and recommended, in agreement with applicable US FDA regulations. This is generally also recommended for non-carbonated beverages.

Application disclaimer: As with any product, use of the products mentioned in this publication in a given application must be tested (including field testing, etc.) by the user in advance to determine suitability.



APPLICATION IN BEVERAGES

Spoilage and pathogenic microorganisms such as yeasts, molds and bacteria represent a serious threat for the quality of beverages, i.e. palatability, visual appearance and safety, during production, transportation, storage and consumption. When used as directed, **Nagardo®** can provide an innovative new tool to help control growth of microorganisms during the entire span from production to consumption of beverages, fulfilling current comprehensive consumer protection and food regulatory requirements.

Nagardo® in various beverage formulations is

- Soluble in water
- Typically applied as a 1% stock solution
- Most efficacious within pH 2.5-4.6 (above pH 4.6 a second killing step is required)
- Chemically stable, also during production and heat treatment
- Taste neutral at recommended concentrations without effect on the color

The range of compatible beverages comprises:

- Carbonated & still soft drinks
- Isotonic sports drinks
- Ready to drink teas
- Flavored waters
- Energy drinks
- Alcohol-free beer
- Kombucha beverages
- ... and many more

MECHANISM OF ACTION

Tests of certain microorganisms (isolated from beverages) that have adapted to, or developed resistance to traditional preservatives such as sorbate and benzoate, showed that the mode of action (MoA) of Natural Glycolipids is most likely unique, as there is no cross-resistance/adaptation to existing preservative solutions. Due to the surfactant properties, the MoA of **Nagardo®** is assumed to be based on membrane interaction and disintegration. This hypothesis is supported by the comparison with published results for structurally similar glycolipids [11, 12].

Challenge test examples

Nagardo® was dissolved in various store-bought beverages at 3-5 ppm use level. The beverages were spoiled with a mix of yeast and mold organisms (*Saccharomyces*, *Zygosaccharomyces*, *Aspergillus*, *Byssoschlamys*, *Penicillium*). Although inoculated with prominent beverage spoiling organisms, no microbial growth was observed in the chosen beverages even after 3 months.



Nagardo® demonstrates superior antimicrobial efficacy against a large variety of spoilage organisms commonly found in beverages, essentially allowing for a replacement of chemical preservatives, while reducing dosage on average by a factor of 50.

SAFETY OF NAGARDO®

The safety evaluation of **Nagardo®** is based on various published, as well as corroborative data and information regarding the metabolism and toxicity profile of **Nagardo®** [1-5, 13]. The safety evaluation is in accordance with U.S. Food and Drug Administration (FDA) Good Laboratory Practice (GLP) regulations (21 CFR 58; FDA, 1987) and/or the Organization for Economic Co-operation and Development (OECD) Principles of GLP (ENV/MC/CHEM(98)17). [6-8].

Absorption, Distribution, Metabolism and Excretion (ADME) Profile of **Nagardo®**

The pharmacokinetics, excretion balance, and tissue distribution of [¹⁴C]-**Nagardo®** and [¹⁴C]-Long Chain Fatty Acid (LCFA) equivalents following single or repeated administration to Sprague Dawley rats were evaluated. [1]

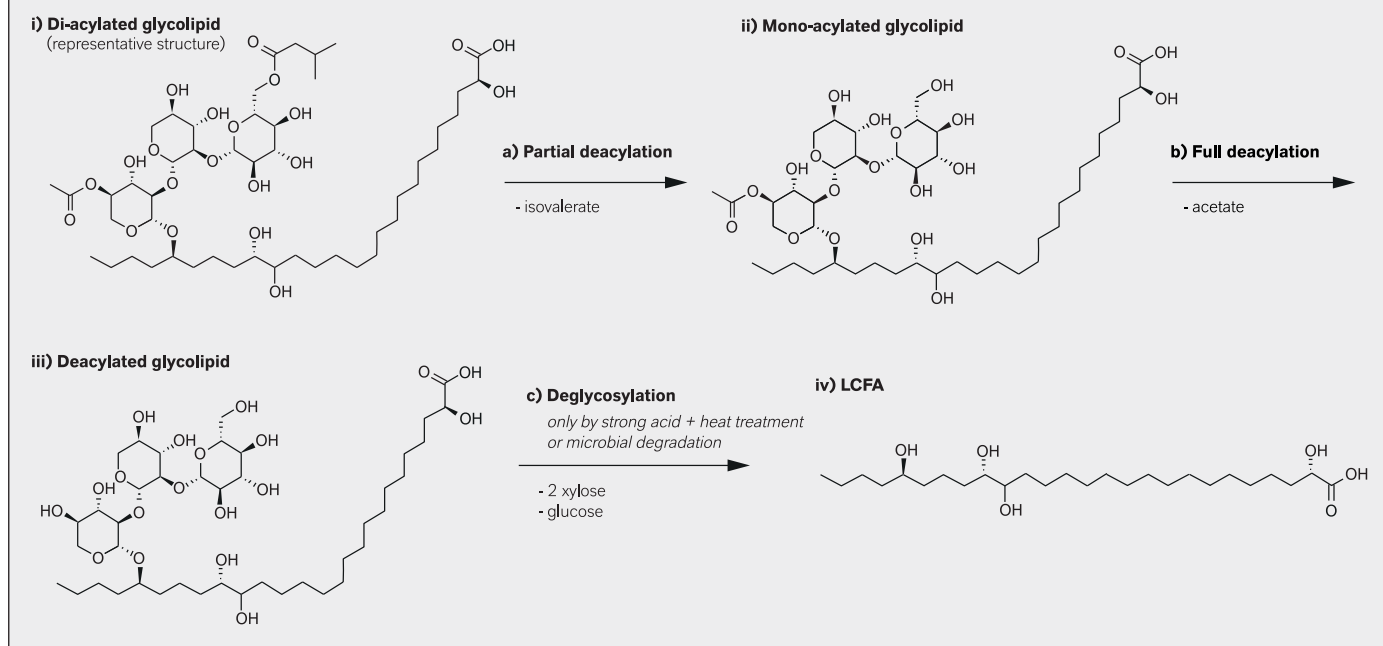
Based on the results of previous in vitro experiments in simulated gastric fluid and in vivo pharmacokinetics studies in rats with [¹³C]-**Nagardo®**, it was hypothesized that, following ingestion, **Nagardo®** passes mostly unchanged to the lower GI tract, where predominantly its ester linkages and, partially, its glycosidic linkages are prone to hydrolysis by microflora in the lower intestine to its components glucose, xylose, acetate,

isovalerate, and long chain fatty acids (LCFA) molecules (Figure 3). Since it was anticipated that its minor components – glucose, xylose, acetate and isovalerate – would be further metabolized rapidly and incorporated for normal physiological functions, the major component of **Nagardo®**, i.e. LCFA, was studied in parallel to **Nagardo®**.

This approach allowed the pharmacokinetics, excretion balance, and tissue distribution experiments to elucidate the ultimate fate of the parent compound. Based on current testing parameters, **Nagardo®** is considered to be free of common, known allergens.

Figure 3. Proposed Degradation of Nagardo® to LCFA. [1]

Initially, sequential hydrolysis of ester groups occurs. Total hydrolysis, i.e. deglycosylation towards the free fatty acids, is only observed when heated with strong acids or by microbial transformation.



Absorption, Distribution, Metabolism, and Excretion (ADME) – Summary and Conclusions

Nagardo® and its ultimate hydrolysis product LCFA are poorly absorbed by the oral route and are primarily eliminated in the feces without absorption. The apparent oral bioavailability (F) of **Nagardo®** and LCFA equivalents is approximately 11%. The pharmacokinetic, tissue distribution, and excretion balance data are consistent with an interpretation that, following ingestion, **Nagardo®** is partially hydrolyzed to its components, glucose, xylose, acetate, isovalerate, and LCFA. The small primary metabolites, glucose, xylose, acetate, and isovalerate are expected to have a fast and high bioavailability but rapid clearance, and thus to contribute marginally to the observed test article equivalents in blood and tissues after oral administration of **Nagardo®**.

These results support an interpretation that systemic exposure to **Nagardo®** or its metabolites would be very limited following oral ingestion. **Nagardo®** is marketed for use as a preservative in beverages without offering nutritional, taste or other technical benefits. Therefore, limited absorption of **Nagardo®** is a desirable property and serves to minimize exposure by the consumer to **Nagardo®** or its major hydrolysis product, LCFA. In addition to limited exposure after a single dose, there was no change to the pharmacokinetics, distribution, and elimination after repeated exposures, which is positive in regards to the application of **Nagardo®** as a food ingredient where repeated ingestion is expected. Further, because rapid hydrolysis and elimination of **Nagardo®** occurs in vivo, the antimicrobial properties of the parent material would be absent or greatly diminished in the lower intestines, so there is low concern for disruption of healthy gut microflora.

Toxicology Profile of Nagardo®

Toxicology Profile of **Nagardo®** has been demonstrated in a series of in vitro and in vivo studies conducted under GLP and compliant to current OECD guidelines as well as US FDA Redbook II recommendations. In the following table, key safety studies and their findings are listed.

Study	Species	Summary of results
Acute toxicity: Acute Toxic Class Method (OECD 423)	Rat	Single oral gavage dosage of 2000 mg/kg bw in female Wistar Crl:WI(Han) rats was associated with no signs of toxicity and no mortality. At necropsy, no treatment-related macroscopic findings were observed in any animal of any step. LC50 cut-off (rat): unclassified According to GHS and regulation (EC) 1272/2008, the test item has no labelling requirement for toxicity and is not classified.
Subchronic toxicity: 90-day oral toxicity (OECD 408)	Rat	The test item was administered via the drinking water at 1.5, 5, or 15 g/l for 90 days. Drinking water consumption for mid- and high-dose groups decreased during treatment in a dose-dependent manner, attributable to decreased palatability and high viscosity of the drinking water. As a result, food consumption and body weight gain in these groups were also reduced. No test-article related adverse effects were noted for haematological or clinical chemistry parameters when compared with actual and historical control data. Histopathology did not reveal any adverse findings. The NOAEL for systemic toxicity was 15 g/l (the highest test dose) in drinking water, correlating to approximately 1300 mg/kg bw/day.
Subchronic toxicity: 90-day non-rodent oral toxicity (OECD 409)	Dog	Oral administration of the test article in beagle dogs at doses of 150, 500, or 1000 mg/kg bw/day was done in gelatin capsules. All animals survived to scheduled necropsy without any noted effect of the test substance on body weights or food consumption. Test substance-related clinical observations were noted post-dosing and included emesis in the high dose group, which is attributed to the physical surfactant-like properties of the test item at high concentrations. Clinical chemistry, haematology and histopathology did not reveal any test-item related adverse effects. The NOAEL for systemic toxicity was 1000 mg/kg bw/day under the test conditions.

Study	Species	Summary of results
Rodent prenatal developmental toxicity study (OECD 414)	Rat	<p>The test article was administered orally by gavage to rats at dosage levels of 150, 500, or 1000 mg/kg bw/day. There were no effects on intrauterine growth and survival at any dosage level. No adverse findings were obtained by laparohysterectomy and macroscopic examination. External, internal and skeletal examinations of the fetuses did not reveal any test-item related effect.</p> <p>The NOAEL was 1000 mg/kg bw/day under the test conditions.</p>
Two-generation reproductive toxicity study (OECD 416)	Rat	<p>The test article was administered orally by gavage to rats at dosage levels of 150, 500, or 1000 mg/kg bw/day. There were no effects on F0 and F1 reproductive performance. There were no adverse effects on survival, clinical observations, body weight or food consumption parameters, macroscopic or microscopic findings, or organ weights for F0 or F1. There were no adverse effects on postnatal survival or growth during the preweaning period for F1 and F2 generations.</p> <p>The NOAEL was 1000 mg/kg bw/day for parental reproductive toxicity, parental systemic toxicity and neonatal toxicity, the highest concentration tested.</p>
Genetic toxicity: Ames test (OECD 471)	in vitro	<p>Mutagenic activity was examined in the bacterial reverse mutation test using Salmonella typhimurium and Escherichia coli strains, both in presence and absence of S9 mix.</p> <p>The test substance was found to be not mutagenic.</p>
Genetic toxicity: Micronucleus test (OECD 487)	in vitro	<p>In the in vitro human lymphocyte studies (micronucleus test), in presence and absence of a metabolic activation system (S9 mix), at all time points and at any of the concentrations analyzed, the test substance did not show a statistically significant increase in the number of binucleated cells containing micronuclei compared to controls.</p> <p>From the results, it is concluded that the test substance was not clastogenic and/or aneugenic to cultured human lymphocytes.</p>
Genetic toxicity: Mouse lymphoma assay (OECD 490)	in vitro	<p>In the mouse lymphoma thymidine kinase assay (MLA), the test item – in presence and in absence of S9-mix – did not induce an increase in the mutation frequency as compared to control.</p> <p>In conclusion, the test item was not mutagenic in the MLA assay.</p>
Metabolism/Kinetics: Adsorption, Distribution, Metabolism and Elimination (ADME) study (OECD 417)	Rat	<p>The pharmacokinetics, excretion balance, and tissue distribution of [¹⁴C]-labelled Nagardo and, separately, its hydrolysis products, were evaluated following single or repeated administration to rats at 100 mg/kg bw.</p> <p>Oral bioavailability was found to be very low at approximately 11%. The majority of the administered material was eliminated with the faeces, while maximum observed concentration in blood was below 0.1% of the orally administered dose.</p> <p>As expected, whole body autoradiography detected highest concentration of the test items in tissues of the GI tract. The remaining tissues had very low levels only. Thus, no target organs or tissues were identified. No difference between single and repeated dosing was observed.</p>
Safety during handling: Skin sensitization (Buehler test, OECD 406)	Guinea pig	<p>A 60% w/w mixture of the test substance in distilled water was topically applied to guinea pigs, once each week for a 3-week induction period. 28-days after the first dose, a challenge dose at highest non-irritation concentration (HNIC, 60%) was applied. 24 h and 48 h after each dose, the animals were scored for erythema.</p> <p>Based on the results, the test substance is not considered to be a contact sensitizer.</p>
Safety during handling: Skin sensitization and dermatological evaluation of skin-tolerance by Human Repeat Insult Patch Test (HRIPT)	Human	<p>An aqueous solution of the test item (0.5%) was applied onto human volunteers, who received the test material dispensed onto occlusive patches which were applied for 24 h on the skin three days per week for three consecutive weeks, until a series of nine 24 h exposures was done.</p> <p>No adverse reactions of any kind were noted during the study. Based on dermatological examination, the test item was found non-irritant and non-sensitizing to the skin.</p>
Safety during handling: Skin irritation (OECD 439), Skin corrosion (OECD 431), Phototoxicity (OECD 432)	in vitro	<p>The test item was applied in aqueous solutions onto the respective in vitro skin models (6.7 mg/ml for irritation and phototoxicity assay; 0.5% and 5.0% /w/w) for the skin corrosion assay).</p> <p>It was found to be non-irritant and non-corrosive and not phototoxic to skin.</p>
Safety during handling: Eye irritation (HCE)	in vitro	<p>The test item was applied in aqueous solution (6.7 mg/ml) directly onto the Human Corneal Epithelium (HCE) model.</p> <p>It was classified as non-irritant.</p>

List of abbreviations

bw	body weight
FDA	Food and Drug Administration
g	gram
GHS	Globally Harmonized Classification System
kg	kilogram
l	liter
mg	milligram
NOAEL	No Observed Adverse Effect Level
OECD	Organisation for Economic Co-operation and Development

Occupational health and safety

According to GHS classification, in accordance with the OSHA Hazard Communication Standard (29 CFR 1910.1200), **Nagardo®** is not classified as a hazardous substance or mixture. Please refer to the **Nagardo® SDS** for more details.

Summary of safety evaluation

Nagardo® and its ultimate hydrolysis product in vivo, long chain fatty acids (LCFA), are poorly absorbed by the oral route and are primarily eliminated in the feces essentially without absorption. Absorbed components appear to be almost completely metabolized to CO₂ and expired. There were no metabolites of safety concern identified for **Nagardo®** and its ultimate hydrolysis product LCFA, and no accumulation of these compounds in tissues. **Nagardo®** is considered to have low potential for systemic toxicity with an oral repeated dose (90-day) at the no-observed-adverse-effect level (NOAELs) of ≥ 1200 mg/kg bw/day in rats (oral drinking water administration) and ≥ 1000 mg/kg bw/day in dogs (oral capsule administration), the highest dose levels tested.

Nagardo® was determined to be non-genotoxic based on the results of a complete battery of in vitro genetic toxicity assays in bacteria as well as in mammalian cells including human lymphocytes. **Nagardo®** is not a reproductive or developmental toxicant as confirmed in robust 2-generation reproduction toxicity and embryofetal toxicity studies in rodents (oral gavage administration).

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Business Unit Material Protection Products

Contact:

nagardo@lanxess.com
www.nagardo.com

Europe, Middle East, Africa:

LANXESS Deutschland GmbH

Kennedyplatz 1
50569 Köln, Germany
Phone: +49 (0) 221 8885-5211

North America and Central America:

LANXESS Corporation

111 RIDC Park West Drive Pittsburgh,
PA 15275-1112, USA

Latin America:

LANXESS Indústria de Produtos Químicos e Plásticos LTDA.

Av. Maria Coelho Aguiar, 215 - Bl. B - 2º Andar
05804-902, Jardim São Luis, São Paulo-SP Brasil

Southeast Asia, Australia and New Zealand:

LANXESS Thai Co., Ltd.

208, 208 Wireless Road Tower, 5th Floor Unit 502
Wireless Road, Lumpini, Pathumwan, Bangkok 10330
Thailand

China:

LANXESS Chemical (China) Co., Ltd.

6F, 5 Corporate Avenue
150 Hu Bin Road, Huangpu District
Shanghai 200021, P.R. China

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