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Fatty acids, $C_{14–18}$ saturated and $C_{16–18}$ unsaturated

MAK Value Documentation – Translation of the German version from 2023

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Keywords

fatty acids, C14–18 saturated and C16–18 unsaturated; irritation; respiratory tract; UVCB substance; non-ionic surfactant

Abstract

The German Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area (MAK Commission) summarized and evaluated the data for fatty acids, $C_{14–18}$ saturated and $C_{16–18}$ unsaturated [67701-06-8] to derive an occupational exposure limit value (maximum concentration at the workplace, MAK value), considering all toxicological end points. Relevant studies were identified from a literature search. It is a UVCB substance (substances of Unknown or Variable composition, Complex reaction products or Biological materials). There are no data for humans or from repeated dose studies in animals for fatty acids, $C_{14–18}$ saturated and $C_{16–18}$ unsaturated, that can be used to derive a MAK value. No data are available for the irritation potential of the substance. The systemic oral NOAEL of behenic acid (C_{22}), a structurally similar fatty acid, was found to be 1000 mg/kg body weight and day in a study carried out in rats according to OECD Test Guideline 422. The fatty acids, $C_{14–18}$ saturated and $C_{16–18}$ unsaturated, are non-ionic surfactants; therefore, effects on the pulmonary surfactant are likely to occur. A MAK value cannot be established because no data for inhalation toxicity are available. In vitro studies of the fatty acids, $C_{14–18}$ saturated and $C_{16–18}$ unsaturated, or acids of similar length and similar degree of unsaturation showed no genotoxic potential. No in vivo genotoxicity studies and no carcinogenicity studies have been carried out with the fatty acids, $C_{14–18}$ saturated and $C_{16–18}$ unsaturated. Limited studies of fatty acids of similar length and similar degree of saturation did not find effects of developmental toxicity. A sensitizing potential is not expected based on the available data. The substance does not penetrate the skin in toxicologically relevant amounts.

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MAK value	**not yet established, see Section II b of the List of MAK and BAT Values**
Peak limitation	–
Absorption through the skin	–
Sensitization	–
Carcinogenicity	–
Prenatal toxicity	–
Germ cell mutagenicity	–
BAT value	–
Synonyms	$C_{14–18}$ saturated and $C_{16–18}$ unsaturated alkylcarboxylic acids
Chemical name (IUPAC)	–
CAS number	67701-06-8
Formula	–
Molar mass	no data (variable composition)
Melting point	44–48 °C (ECHA 2018)
Vapour pressure at 25 °C	1.87×10^{-6} hPa (calculated) (ECHA 2018)
log K_{OW}	7.05–8.23 (calculated) (ECHA 2018)
Solubility at 20 °C	< 0.05 mg/l water (calculated) (ECHA 2018)
pKa value at 25 °C	4.75–5.02 (calculated) (ECHA 2018)
Hydrolytic stability	no data
Stability	no data
Production	hydrolysis of vegetable or animal oils and fats (CIREP 2019)
Purity	no data
Impurities	other fatty acids, e. g. **arachidic acid** (C_{20}:0 (20 C atoms, no double bonds); CAS No. 506-30-9), **behenic acid** (C_{22}:0; CAS No. 112-85-6), **$C_{12–14}$ fatty acids** (CAS No. 90990-10-6) (ECHA 2018)

Uses	film-forming agents (occlusive) e. g. for skin disorders (CIREP 2019); in finger paints, polishes and waxes, washing and cleaning products, air care products, biocides (e. g. disinfectants and pest control products), coating products, fertilizers, plant protection products, perfumes, fragrances, cosmetics, personal care products, in automotive care products, paints, detergents, coatings and adhesives (ECHA 2021 a)
Concentrations used	no data

This documentation is based mainly on the REACH registration data for the $C_{14–18}$ saturated and $C_{16–18}$ unsaturated fatty acids (abbreviated $C_{14–18/16–18unsatd}$) publicly available. These are UVCB substances (Chemical Substances of Unknown or Variable Composition) and have been assigned the CAS registry number 67701-06-8. The $C_{14–18/16–18unsatd}$ fatty acids may be composed of, in varying amounts: **lauric acid** (C_{12}:0; CAS No. 143-07-7), **myristic acid** (C_{14}:0; CAS No. 544-63-8), **palmitic acid** (C_{16}:0; CAS No. 57-10-3), **palmitoleic acid** (C_{16}:1; CAS No. 373-49-9), **margaric acid** (C_{17}:0; CAS No. 506-12-7), **stearic acid** (C_{18}:0; CAS No. 57-11-4), **oleic acid** (C_{18}:1; CAS No. 112-80-1), **linoleic acid** (C_{18}:2; CAS No. 60-33-3), **linolenic acid** (C_{18}:3; CAS No. 463-40-1), **trans-vaccenic acid** (C_{18}:1; CAS No. 693-72-1) and **elaidic acid** (C_{18}:1; CAS No. 112-79-8) (ECHA 2018). The source (ECHA 2018) does not include data for the amount of each individual substance in the mixture and whether these are constituents of the mixture or impurities. Fatty acids $C_{14–18/16–18unsatd}$ occur as a solid or paste. CIREP (2019) refers to the substance that has been assigned the CAS number 67701-06-8 as "isomerized linoleic acid".

Only few studies have been carried out with the mixture of $C_{14–18/16–18unsatd}$ fatty acids. This evaluation therefore includes studies that investigated individual substances in the mixture. Documentation has been published by the MAK Commission for the following substances: lauric acid (Greim 2000), myristic acid, palmitic acid and stearic acid (Greim 1999, available in German only), oleic acid (Greim 2002; Hartwig and MAK Commission 2017) and behenic acid (Greim 2008, available in German only). If specific data were unavailable, the evaluation has made use of the findings for fatty acids with similar chain lengths: **hexanoic acid** (C_6:0; CAS No. 142-62-1), **caprylic acid** (C_8:0; CAS No. 124-07-2), **azelaic acid** (nonanedioic acid; C_9:0; CAS No. 123-99-9), **capric acid** (C_{10}:0; CAS No. 334-48-5), **isostearic acid** (C_{18}:0; CAS No. 30399-84-9), **$C_{3–18}$ saturated fatty acids**, **$C_{8–18}$ fatty acids** (CAS No. 90990-08-2) and **$C_{18unsatd}$ fatty acids** (CAS No. 502962-81-4).

1 Toxic Effects and Mode of Action

The oral LD_{50} for $C_{14–18/16–18unsatd}$ fatty acids was found to be above 5000 mg/kg body weight in rats. No substance-induced findings were observed. A study carried out according to OECD Test Guideline 422 with gavage doses of behenic acid of up to 1000 mg/kg body weight and day likewise did not report substance-related effects.

Palmitic acid and stearic acid did not cause irritation of the skin in rabbits. However, $C_{12–14}$ fatty acids, palmitic acid and stearic acid induced slight, reversible effects in the eyes of rabbits during the first days after administration. Lauric acid caused severe damage to the eyes that was not reversible.

$C_{14–18/16–18unsatd}$ fatty acids have a low vapour pressure. As a result, exposure to these substances in solid or paste form is not expected to occur as a vapour, but only as an aerosol. The fatty acids have a hydrophilic end (carboxyl group) and a longer hydrophobic hydrocarbon chain; this may lead them to induce effects similar to those caused by surfactants. Studies investigating irritation or inhalation exposure that could be used as a source of data for possible effects on the respiratory tract are currently not available.

Studies in animals and humans found that the skin irritation potential of unsaturated fatty acids and their salts decreases with an increase in chain length. Therefore, medium-chain fatty acids (C_{10}) cause irritation, fatty acids with a C_{12} chain lead to minimal skin irritation and homologues with longer chains, C_{14} and higher, are not irritating.

There is no evidence that the $C_{14–18/16–18unsatd}$ fatty acids cause sensitizing effects on the skin or airways in humans or animals.

Negative results were obtained in mutagenicity studies carried out in Salmonella typhimurium with $C_{14–18/16–18unsatd}$ fatty acids, lauric acid, caprylic acid, $C_{8–18}$ fatty acids, behenic acid and hexanoic acid and in Escherichia coli WP2uvrA with behenic acid as well as chromosomal aberration tests conducted in CHL cells (a cell line derived from Chinese hamster lung) with behenic acid and a $TK^{+/-}$ mutation test performed in L5178Y mouse lymphoma cells with capric acid. Carcinogenicity studies are currently not available.

2 Mechanism of Action

The carboxyl group may be responsible for the slight irritation of the eyes induced by several of the acids in the mixture. Furthermore, the structure of the acids (comprising a long non-polar carbon chain and polar head group) may lead to effects in the respiratory tract that are similar to those caused by surfactants.

3 Toxicokinetics and Metabolism

3.1 Absorption, distribution, elimination

There are no studies available with the $C_{14–18/16–18unsatd}$ fatty acids that investigated these end points. There is evidence that azelaic acid (nonanedioic acid; C_9:0; CAS No. 123-99-9) is absorbed orally (see below; Hartwig 2013, available in German only; Täuber et al. 1992). On the basis of this finding, at least partial absorption after oral or inhalation exposure is expected to occur.

Mathematical models cannot be used to calculate the amount of $C_{14–18/16–18unsatd}$ fatty acids absorbed through the skin because the fatty acids have a high log K_{OW} of > 6.

The penetration of oleic, linoleic, lauric and capric acid into the skin of humans was studied by time-of-flight secondary ion mass spectrometry (TOF-SIMS) using Bronaugh-type flow-through diffusion cells. The results show that these fatty acids penetrate into the skin (Kezutyte et al. 2013).

The fatty acids are absorbed through the skin in amounts of < 1% (ECHA 2018).

An in vitro study with occlusive application of an aqueous solution of radioactively-labelled ^{14}C-linoleic acid and ^{14}C-oleic acid (concentration about 10.8 µg/ml) to human skin for 8 hours reported the potential absorption of 55% to 60% of the administered dose. Of this amount, 7.4% and 1.2%, respectively, were found in the receptor cells (Buist et al. 2010). A flux of about 0.01 µg/cm^2 and hour is calculated using the data for the volume of solution applied (10 µl) and the area of absorption (0.64 cm^2) (10.8 µg/ml × 0.01 ml × 0.55 (or 0.6) / 0.64 cm^2 / 8 hours). Assuming standard conditions (a surface area of 2000 cm^2 of exposed skin, 1 hour of exposure), this results in an absorbed amount of 0.02 mg.

Five grams of an acne treatment cream containing 20% azelaic acid (equivalent to 1 g) was applied once to the face (1 g), chest (2 g) and upper back (2 g) of 6 healthy male volunteers (23 ± 1 years old, weight 69 ± 7 kg, height 178.5 ± 1.6 cm) (5 mg/cm^2; duration 24 hours). One week later, the same persons were administered 1 g of azelaic acid orally as an aqueous microcrystalline suspension in the morning on an empty stomach. The amount of unchanged substance excreted with the urine was determined after both treatments. Following application of the substance to the skin, the highest concentration found in the first 24-hour urine was 7.8 ± 3.2 µg/ml (1.29% ± 0.5% of the applied dose), while amounts of 0.76% ± 0.49% and 0.12% ± 0.15% of the applied dose, respectively, were found in the second and third 24-hour urine. Overall, 2.2% of the dose was absorbed through the skin. After oral administration, 424 ± 104 µg/ml was

recovered in the first 24-hour urine, which is equivalent to 61.2% ± 8.8% of the dose. Urinary excretion was complete after 24 hours (Täuber et al. 1992). A flux of 4.6 µg/cm^2 and hour (5000 µg/cm^2 × 0.022 / 24 hours) was calculated from the study findings.

3.2 Metabolism

After dermal and oral administration, azelaic acid was excreted unchanged with the urine (Hartwig 2013; Täuber et al. 1992).

The even-numbered fatty acids are expected to be oxidized completely by β-oxidation.

4 Effects in Humans

Only few studies were carried out in humans with similar organic acids.

Local effects on skin and mucous membranes

Skin

Palmitic acid

An occlusive epicutaneous test was carried out in 1988 with 20 healthy volunteers (12 women, 8 men) to study the effects of palmitic acid on the skin. The test was performed by applying 10 µl of a 50% solution in a large Finn Chamber on Scanpor tape to the backs of the volunteers for 24 hours. Readings were taken 1, 6, 24, 48, 72 and 144 hours after removal of the chambers. Redness, oedema, dryness of the skin and fissures were not observed at any time point; the findings in all cases were 0/4 or 0/3 (fissures) (ECHA 2018). Palmitic acid did not cause irritation of the skin.

Saturated $C_{3–18}$ fatty acids and unsaturated C_{18} fatty acids

In a patch test study carried out in 1975, saturated fatty acids with chain lengths from C_3 to C_{18} and unsaturated C_{18} fatty acids were applied occlusively once a day to the skin on the backs of male volunteers (10 control persons, 10 test persons) and left on for 24 hours. This was repeated until erythema developed, but for a maximum of 10 consecutive days. The test substances were 0.5 molar and 1.0 molar solutions of saturated fatty acids or 1.0 molar solutions of oleic and *trans*-oleic acid, linoleic acid and linolenic acid; *n*-propanol was used as the solvent for all acids. The controls were administered a patch containing pure *n*-propanol. The saturated fatty acids with chain lengths of C_8 to C_{12} were found to be the most severely irritating: after 2 days, erythema developed in 4 persons treated with C_8 acids and in 2 persons treated with C_{12} acids. After 10 days, erythema was observed in 8 persons administered C_8 acids and 6 persons administered C_{12} acids. If erythema developed at all after application of the saturated C_{14}, C_{15} and C_{16} fatty acids, then only after 8 to 10 days and in 1 to 2 of 10 persons. Linoleic acid was the only unsaturated fatty acid that caused irritation, but that to a considerable degree (Stillman et al. 1975).

Caprylic acid, capric acid, lauric acid

A 4-hour patch test was developed to compare the severity of the acute irritation induced by different substances on the human skin. Similar test results were obtained after a number of different assays were carried out by the same and by different laboratories. The results diverged more substantially if the tests were not performed at the same time of the year or did not use the same study protocols. Determinations were carried out with 100% caprylic acid, capric acid or lauric acid. A 20% solution of sodium lauryl sulfate was used as the positive control. The study lasted 4 months. Capric acid was the most irritating, followed by caprylic acid and sodium lauryl sulfate; lauric acid was the least irritating (Robinson et al. 1999).

Allergenic effects

Sensitizing effects on the skin

The currently available studies did not investigate $C_{14–18/16–18unsatd}$ fatty acids themselves. However, there are data available for individual fatty acids in the mixture.

Documentation for myristic acid, palmitic acid and stearic acid was published in 1999. Studies performed with 2.2% palmitic acid and up to 13% stearic acid did not find any evidence of sensitizing effects. Photosensitization did not occur in humans after application of 2.2% palmitic acid and up to 13% stearic acid (Greim 1999).

No studies are available that investigated the fatty acids in occupational exposure settings. Fatty acids found in cosmetic products were studied in individual cases, but the test concentrations were very low and the test substances were always mixtures of different substances.

Sodium stearate

In a 21-day epicutaneous test carried out with 100 test persons, no evidence suggesting a sensitizing potential was found following application of an aqueous bath soap containing 0.3% to 0.75% sodium stearate. A stick deodorant containing 7% sodium stearate had low sensitizing potency (no other details) (CIREP 2019). However, the findings cannot be included in the evaluation because of the low test concentration, the exposure to a mixture of substances and because tests were not performed with the individual substances.

Isostearic acid

Studies with the substance mixture isostearic acid are cited secondarily in a publication: In a Human Repeated Insult Patch Test (HRIPT) carried out with a total of 333 test persons, formulations containing 2.5% to 2.85% isostearic acid did not yield any evidence of contact sensitization. In another HRIPT, allergic reactions were not observed in 103 and 168 persons, respectively, after application of 10% and 35% isostearic acid in mineral oil. The potential of isostearic acid to induce phototoxic or photosensitive reactions was then investigated in 28 of the 168 persons who had earlier participated in the HRIPT. In the test, a formulation composed of 35% isostearic acid in mineral oil was applied to the forearm. The application sites of 19 of the test persons were treated with UVA radiation (320–400 nm, 4.4 $\mu W/cm^2$ determined at the skin surface at a wavelength of 360 nm) for 15 minutes, while the application sites of 9 of the test persons were first treated with UVB radiation (at 2 × the mean erythema dose from a 150-watt Xenon Arc Solar Simulator at 280–320 nm) and then exposed for 5 minutes to UVA radiation. Only reversible reactions were observed and the authors concluded that isostearic acid does not cause photosensitization (no other details; CIREP 1983).

Oleic acid

Documentation and a supplement have been published for oleic acid (Greim 2002; Hartwig and MAK Commission 2017). From April 2000 to July 2002, 233 patients with exposure to metal-working fluids underwent epicutaneous testing with a large range of possible constituents of metal-working fluids at 5 clinics of the Information Network of Departments of Dermatology (IVDK). Positive reactions were not observed in any of the 229 test persons tested with oleic acid (5% in petrolatum); 1 test person produced a questionable reaction (Geier et al. 2003).

Various fatty acids

Formulations for cosmetic products containing 1% to 13% oleic acid, palmitic acid or stearic acid did not induce photosensitization (CIREP 2019).

Only one finding relating to occupational exposure is available; however, this involves exposure to a mixture of substances. A 37-year-old mechanic who was exposed to water-based metal-working fluids every day at work developed itchy eczema on his hands and forearms. On day 3 of epicutaneous testing, the patient produced a 2+ reaction to a

1% condensation product of boric acid in water, monoethanolamine and "fatty acids"; the latter made up 21% of the condensate. The symptoms subsided after the mechanic switched occupations. Another 26 patients were tested with the condensation product in a concentration that was 5 times as high (5%); 3 of these produced a questionable positive reaction and 2 developed irritation (Devantier Jensen and Andersen 2003). This case has not been included in the evaluation because the test substance was a condensation product and the reaction cannot be attributed to the fatty acids.

In spite of the extensive use of these fatty acids in cosmetic products, there are no other findings of sensitizing effects in humans.

Sensitizing effects on the airways

There are no data for sensitizing effects on the airways.

5 Animal Experiments and in vitro Studies

5.1 Acute toxicity

5.1.1 Inhalation

There are no studies available for the $C_{14–18/16–18unsatd}$ fatty acids or any of the individual substances these contain.

5.1.1.1 Caprylic acid

The 4-hour LC_{50} for caprylic acid vapour was above 162 mg/m^3 in rats (ECHA 2018).

5.1.1.2 C_{10} acid isomers

Mortality was not observed among rats that were exposed to the saturated vapour of a mixture of different C_{10} isomers for 8 hours (no other details; HERA 2002).

5.1.2 Oral administration

In a study carried out in 1989 according to OECD Test Guideline 401 with gavage administration to groups of 5 male and 5 female Wistar rats, the LD_{50} for $C_{14–18/16–18unsatd}$ fatty acids dissolved in propylene glycol was above 2000 mg/kg body weight (ECHA 2018; HERA 2002).

In another study carried out according to OECD Test Guideline 401 with groups of 5 male and 5 female rats (no other details), the oral LD_{50} for the sodium salt of $C_{14–18/16–18unsatd}$ fatty acids, tested as a 25% suspension in water, was above 5000 mg/kg body weight. There were no signs of clinical toxicity, no mortality and no findings at necropsy (HERA 2002; OECD 2014).

5.1.3 Dermal application

There are no studies available for the $C_{14–18/16–18unsatd}$ fatty acids.

5.1.3.1 Stearic acid

A study published in 1979 with occlusive application of a test substance to the skin of 3 male and 3 female New Zealand White rabbits reported a dermal LD_{50} value of above 2000 mg/kg body weight for the stearic acid contained in the mixture. The treated skin of all animals was reddened upon removal of the substance. In 1 female, the redness had become very severe by day 7 after treatment. Several animals had oedema and some had scabs. The breathing of 1 male was laboured on day 6 after treatment and the animal was found dead the following day (ECHA 2018; Greim 1999).

5.2 Subacute, subchronic and chronic toxicity

There are no studies available for the $C_{14–18/16–18unsatd}$ fatty acids.

5.2.1 Inhalation

There are no studies available.

5.2.2 Oral administration

5.2.2.1 Behenic acid

A study was carried out according to OECD Test Guideline 422 with the administration of the long-chain analogue behenic acid in gavage doses of 0, 100, 300 or 1000 mg/kg body weight and day in corn oil to 13 Sprague Dawley rats per sex and dose group. The test substance had a purity of 85.9% and contained 10.9% C_{14} to C_{20} fatty acids and 2.3% C_{24} fatty acids as impurities. In the males, the mean body weights ($p < 0.01$) and the relative liver weights ($p < 0.05$) were increased between days 8 and 29 in the group given a dose of 100 mg/kg body weight and day in comparison with the values determined in the control animals. In the females, feed consumption was reduced ($p < 0.01$) during lactation in the group given a dose of 100 mg/kg body weight and day and the absolute kidney weights were reduced ($p < 0.05$) at 300 mg/kg body weight and day. As these findings were not dependent on the dose and there was no histopathological correlate, they are not considered relevant for the evaluation. In the blood, alkaline phosphatase was reduced in all dose groups ($p < 0.05$) and the glucose levels were decreased in the high dose group ($p < 0.05$). At 300 mg/kg body weight and day, the blood chloride levels were increased ($p < 0.05$) and the calcium ($p < 0.01$) and total protein ($p < 0.05$) levels were decreased. These findings were not dose-dependent and are therefore considered coincidental. The NOAEL (no observed adverse effect level) for behenic acid was 1000 mg/kg body weight and day (ECHA 2018; Greim 2008).

5.2.2.2 Lauric acid

In a study carried out in 1960, groups of 5 male Osborne Mendel rats were given 0 or 10% lauric acid with the feed for 18 weeks. This is equivalent to about 5000 mg/kg body weight and day assuming that each rat consumed 5 g of feed per 100 g body weight. There were no substance-induced findings (ECHA 2018; Fitzhugh et al. 1960; Greim 2000, available in German only).

5.2.3 Dermal application

There are no studies available.

5.3 Local effects on skin and mucous membranes

There are no studies available for the $C_{14–18/16–18unsatd}$ fatty acids.

5.3.1 Skin

5.3.1.1 Palmitic acid

In 1988, a study was carried out according to the version of OECD Test Guideline 404 valid at that time with occlusive application of 0.5 g of palmitic acid in powder form to the shaved dorsal skin of 4 Himalayan rabbits. No redness or oedema was observed (both effects: grade 0 of 4) and the substance was not regarded to be an irritant (ECHA 2018).

5.3.1.2 Stearic acid

A study was carried out in 1974 to investigate the irritation potential of stearic acid by occlusively applying 0.5 cm^3 of undiluted substance to the shaved intact or abraded dorsal skin of 6 New Zealand White rabbits. The substance was left on for 24 hours. The primary dermal irritation index calculated directly after removing the patch was 0 of 8. No redness or oedema was observed (both effects: grade 0 of 4). The substance did not cause irritation of the skin in rabbits (ECHA 2018).

5.3.2 Eyes

5.3.2.1 $C_{12–14}$ Fatty acids

In 2010, a study was carried out in compliance with OECD Test Guideline 405 with the application of 100 mg of $C_{12–14}$ fatty acids (pH 5–6) in solid form to 3 New Zealand White rabbits. Neither corneal opacity (mean score 0 of 4) nor effects on the iris (mean score 0 of 2) were observed. After 24, 48 and 72 hours, 2 animals had individual mean scores for conjunctival redness of 0.67 on a scale with a maximum of 3 and 1 animal had a score of 1.0. The effects were completely reversible within 4 days. All 3 animals had individual mean scores for conjunctival swelling of 0.67 on a scale up to 4 after 24, 48 and 72 hours; this effect was completely reversible within 72 hours. Effects on the cornea were not detected in any of the animals by fluorescein test 72 hours after substance administration. No signs of systemic toxicity or mortality were observed up to 21 days after substance administration. The $C_{12–14}$ fatty acids were not found to cause irritation of the eyes according to the evaluation scheme of the OECD test guideline (ECHA 2018). The slight findings observed during the first 3 days after administration, however, were evidence of a mild irritant effect.

5.3.2.2 Lauric acid

In 1989, a study was carried out using a protocol similar to that of the version of OECD Test Guideline 405 valid at that time in which 50 mg lauric acid in powder form was instilled into the eyes of 3 New Zealand White rabbits. The changes induced in the cornea and conjunctiva were not reversible over a period of 21 days. After 24, 48 and 72 hours, 2 animals had individual mean scores for corneal opacity of 1.0 on a scale with a maximum of 4 and 1 animal had a score of 0.7. A score of 0.7 of a maximum of 2 was determined for effects on the iris in 2 animals and a score of 1.0 in 1 animal. The effects were reversible within 7 days. After 24, 48 and 72 hours, 2 animals had a score for conjunctival redness of 3.0 of a maximum of 3 and 1 animal had a score of 2.7. The scores obtained for conjunctival swelling were 1.7 of a maximum of 4 for 2 animals and 1.3 for 1 animal; these effects were reversible within 21 days. All animals had watery eyes. Damage to the corneal epithelium was determined by fluorescein test in all animals and at all-time points of assessment. New blood vessels began to form on examination day 7 (ECHA 2018). Therefore, lauric acid causes severe eye damage.

5.3.2.3 Palmitic acid

In another study carried out in 1988 using a protocol similar to that of the version of OECD Test Guideline 405 valid at that time, 0.1 g of palmitic acid in powder form was introduced into 1 eye of each of 4 Himalayan rabbits. Effects were not found in the cornea or iris at any time point. Slight redness was detected during the first 24 hours (mean value 1 of a maximum of 3; 4 animals with a score of 1), conjunctival swelling was evident after 60 minutes with a mean value of 0.75 of a maximum of 4 (3 animals with a score of 1) and exudation was observed in 1 animal (mean value 0.25 of a maximum of 3). The irritation score for redness in the conjunctiva was 0.3 of a maximum of 3 (score for all 4 animals after 24, 48 and 72 hours). All effects had subsided after 48 hours and the substance was not considered to be irritating (ECHA 2018). However, the slight findings observed during the first days after administration are regarded as evidence of mild irritation.

5.3.2.4 Stearic acid

A similar study carried out in 1976 with the instillation of stearic acid into 1 eye of each of 6 rabbits (no other details) found mild conjunctivitis in 2 animals; this subsided completely within 72 hours. The mean irritation score for erythema after 24, 48 and 72 hours was 0.2 on a scale with a maximum of 3. Stearic acid did not cause any effects on the cornea or iris. The substance was not irritating (ECHA 2018).

5.4 Allergenic effects

5.4.1 Sensitizing effects on the skin

There are no studies available that were carried out with $C_{14–18/16–18unsatd}$ fatty acids. However, data are available for individual fatty acids in the mixture.

Maximization tests and local lymph node assays (LLNA) were carried out with oleic acid, linoleic acid and linolenic acid. These are described in the following and the data are listed in Table 1. The studies of Kreiling et al. (2008) and Yamashita et al. (2015) investigated all 3 fatty acids.

5.4.1.1 C_{18} unsaturated fatty acids

An LLNA carried out in compliance with OECD Test Guideline 429 with the lithium salts of C_{18} unsaturated fatty acids yielded negative results. Using groups of 4 mice (CBA/CaOlaHsd) per concentration, stimulation indices of 0.86, 1.48 and 1.68, respectively, were determined for 2.5%, 5% and 10% formulations in ethanol/distilled water (7:3) (ECHA 2021 b).

5.4.1.2 Stearic acid

Formulations for skin lotions containing 2.8% stearic acid were not photosensitizing to the skin of Hartley guinea pigs (CIREP 2019).

5.4.1.3 Oleic acid

A maximization test (5% oleic acid for intradermal induction, 50% oleic acid for topical induction and 25% oleic acid for the challenge) that was already described in the supplement for oleic acid (Hartwig and MAK Commission 2017) yielded questionable or borderline positive results. On the basis of these findings, the substance does not require labeling. In the first challenge test, a positive reaction was obtained in 1 and 4 of 10 guinea pigs after 48 and 72 hours, respectively. These results were reproducible in only 2 animals. A second challenge test led to positive reactions in 3 of 10 animals after both 48 and 72 hours. Only 2 animals produced reactions in both challenge tests (Kreiling et al. 2008). This maximization test cannot be evaluated as unequivocally positive because of the unclear reaction.

A maximization test with oleic acid (99%) that was carried out according to OECD Test Guideline 406 yielded negative results. Induction was performed by intradermal injection, probably with a 5% solution of the test substance (the published data are contradictory), followed by epicutaneous application of the test substance (100%). None of the 10 animals developed a reaction after 2 provocations with a 1% solution of the test substance in polyethylene glycol 300 (PEG300). In the discussion section, the authors noted that the concentration used for the challenge was quite low at 1% in comparison with the concentration of 100% used for epicutaneous induction. Overall, however, they considered the test method valid (Basketter et al. 2009). As the challenge concentration was very low, the result of this maximization test is not regarded as unequivocally negative.

Oleate yielded a positive result in a maximization test that was carried out with groups of 10 female guinea pigs (Hsd Poc: DH) with 5% ammonium oleate (CAS No. 544-60-5) (99%) in physiological saline solution for intradermal induction, 50% ammonium oleate in petrolatum for topical induction and 25% for the challenge treatments. None of the animals produced a positive reaction 24 hours after the first challenge; however, 1 animal displayed a reaction after 48 hours and 4 of 10 animals after 72 hours. After the second challenge, 2 animals reacted after 24 hours, 3 animals

after 48 hours and 3 animals after 72 hours. All animals including the controls displayed a grade 1 skin reaction after both challenge treatments and at all readings (weak erythema and/or oedema). As a result, only skin reactions higher than grade 1 were evaluated as positive results (ECHA 2017).

In an LLNA that was already described in the supplement for oleic acid (Hartwig and MAK Commission 2017), the lymphocytes were stimulated by several unsaturated fatty acids such as linoleic acid, linolenic acid and undecylenic acid, as well as with oleic acid (99%), although without any clear concentration dependency in the case of oleic acid. At concentrations of 10%, 25% and 50% (in acetone/olive oil, 4:1, v/v), stimulation indices of 2.6, 14.9 and 6.9, respectively, were calculated for oleic acid. The authors evaluated this formally positive result in the discussion section. At the time of publication, they suspected that oleic acid does not act as a hapten (Kreiling et al. 2008). Other mechanisms that are currently being discussed are described in the summary below.

An LLNA in BALB/c mice investigated the sensitizing potential of metal-working fluids and their constituents. Only the result obtained with the 10% formulation can be evaluated because the stimulation index of 1.6 at an increase in ear thickness of only 11% constituted a negative result. Stimulation indices of 2.4 and 5.6, respectively, were calculated for oleic acid concentrations of 25% and 50% (no other details) in acetone/olive oil (4:1, v/v). However, in accordance with the OECD test guideline, the results obtained with oleic acid concentrations of 25% and 50% are not considered reliable because the ear thickness was increased by 29% and 52% (Anderson et al. 2009). The test result with 10% oleic acid was negative.

In another LLNA that examined oleic acid (no other details), formulations of 25%, 50% and 100% (4:1 acetone/olive oil, v/v) yielded formally positive results with stimulation indices of 3.4, 5.7 and 6.5, respectively. However, no data are given for the determination of irritation. The authors concluded that the results were false positives and without human relevance because the development of irritant or allergic contact dermatitis has not been reported although oleic acid is frequently used for the treatment of chronic atopic dermatitis (Basketter et al. 2009).

Additionally, findings are available from an LLNA modified according to Daicel (LLNA-DA) that was carried out in compliance with OECD Test Guideline 442A and included an investigation of the elicitation phase (LLNA-DAE). In an LLNA-DAE, the test substance is applied to the reverse side of the right ear of the test animals on days 1, 2 and 3 and to the reverse side of both ears on day 10. On day 10, the test substance is applied to the reverse side of the left ear of the animals in the control group. The increase in the weight of the excised lymph nodes of the left ear is then determined on day 12. If the mass of the lymph nodes of the treated animals has changed to a statistically significant degree in comparison with that of the control animals, this is evaluated as a positive finding and as evidence of elicitation. The DILL value (Degree of the Increase in Lymph node weight of the Left ear) is determined based on the formula: DILL value = [(mass of the left lymph nodes of the test group) – (mass of the left lymph nodes of the control group)] / (test concentration). The DILL values of substances with weak sensitizing potential lie between 0.02 and 0.10. A preliminary study tested 4 oleic acid concentrations (5%, 10%, 25% and 50%). Very severe irritation was induced by concentrations of 25% and above. In the main study, a DILL value of 0.07 was calculated for a 10% formulation of oleic acid (no other details) in acetone/olive oil (4:1, v/v). On the basis of these results, the authors evaluated the substance as having weak sensitizing potency (Yamashita et al. 2015).

5.4.1.4 Linoleic acid

A maximization test carried out according to OECD Test Guideline 406 using 5% linoleic acid (99%) for intradermal induction, 100% for topical induction and 50% in petrolatum for the challenge treatments did not produce a clear result. Two guinea pigs exhibited a response 24 hours after the first provocation, another after 48 hours. None of the animals displayed a reaction after 72 hours. However, at the same time, reactions were observed in 4 of 5 control animals after 24 hours and in 3 of 5 animals after 48 and after 72 hours. After the second provocation, a positive reaction was induced in 4, 3 and 2 of 10 animals, respectively, after 24, 48 and 72 hours. A reaction was likewise observed in 2 of 5 control animals. After both provocations, 2 of the 10 animals had a reproducible skin reaction (see Table 1). All of the observed reactions were weak (grade 1 – weak erythema and/oedema) except for a positive reaction in 1 animal of the control group after 24 hours that was characterized by erythema/oedema with clearly defined borders (grade 2)

(Kreiling et al. 2008). Although the test was preceded by a preliminary study, positive reactions were still observed among the control animals. Therefore, the result cannot be evaluated as clearly positive.

In an LLNA carried out according to OECD Test Guideline 429, stimulation indices of 1.5, 7.0 and 9.1, respectively, were determined for concentrations of 10%, 25% and 50% linoleic acid (99%) in acetone/olive oil (4:1, v/v). The ear thickness was not increased (Kreiling et al. 2008). The result of the test is therefore regarded as positive.

In an LLNA-DAE, a DILL value of 0.03 was calculated for a formulation containing 25% linoleic acid (no other details) in acetone/olive oil (4:1, v/v) (Yamashita et al. 2015). On the basis of these findings, the substance is considered as having weak sensitizing potency.

5.4.1.5 Linolenic acid

A maximization test carried out according to OECD Test Guideline 406 using 5% linolenic acid (90%, with linoleic acid as a source of impurity) for intradermal induction, 100% for topical induction and 50% in petrolatum for the challenge treatments yielded results that were assessed as negative. A 1+ reaction was observed in 2 animals after the first challenge treatment, and in 1 of 10 animals both after 24 and 48 hours and after 72 hours. One of the 5 control animals displayed a 1+ reaction after 48 hours (Kreiling et al. 2008). The test result is therefore regarded as negative.

In an LLNA carried out according to OECD Test Guideline 429, stimulation indices of 3.1, 9.3 and 10.3, respectively, were calculated for concentrations of 10%, 25% and 50% linolenic acid (90%, with linoleic acid as a source of impurity) in acetone/olive oil (4:1, v/v) (Kreiling et al. 2008). The ear thickness was not increased. For this reason, the test result is regarded as positive.

In an LLNA-DAE, a DILL value of 0.03 was calculated for a 25% formulation of linolenic acid (no other details) in acetone/olive oil (4:1, v/v) (Yamashita et al. 2015). The substance is therefore considered as having a weak sensitizing potency.

5.4.1.6 Other fatty acids

The factsheet for the "fatty acids, $C_{14–18}$ and $C_{16–18}$ unsaturated" published on ECHA's database of registered substances (ECHA 2018) includes a Buehler test that obtained negative results for capric acid and one maximization test with negative results for lauric acid and one for azelaic acid. As this documentation covers "fatty acids, $C_{14–18}$ and $C_{16–18}$ unsaturated" and data are available for members of the substance group, studies carried out with shorter-chain analogues have not been included in the evaluation.

Tab. 1 Overview of the findings of animal studies investigating oleic acid, linoleic acid and linolenic acid

Test system	Method	Oleic acid	Linoleic acid	Linolenic acid	References
GPMT	according to OECD Test Guideline 406	5% intradermal induction, 50%/25% in petrolatum for topical induction/provocation 1st provocation: number of test animals (number of controls) 24 h 0/10 (0/5) 48 h 1/10 (0/5) 72 h 4/10 (0/5) 2nd provocation: 24 h 2/10 (0/5) 48 h 3/10 (0/5) 72 h 3/10 (0/5) questionable/borderline positive[a)]	5% intradermal induction, 100%/50% in petrolatum for topical induction/provocation 1st provocation: number of test animals (number of controls) 24 h 2/10 (4[a)]/5) 48 h 1/10 (3/5) 72 h 0/10 (3/5) 2nd provocation: 24 h 4/10 (2/5) 48 h 3/10 (2/5) 72 h 2/10 (2/5) questionable (according to authors, challenge concentration too high)[a)]	5% intradermal induction, 100%/50% in petrolatum for topical induction/provocation 1st provocation: number of test animals (number of controls) 24 h 2/10 (0/5) 48 h 1/10 (1/5) 72 h 1/10 (0/5) 2nd provocation: not tested negative[a)]	Kreiling et al. 2008
	according to OECD Test Guideline 406	probably 5% intradermal induction, 100%/1% in PEG 300 for topical induction/provocation 1st and 2nd provocation: 0/10 (no other details) negative; however, very low challenge concentration	not tested	not tested	Basketter et al. 2009
LLNA	according to OECD Test Guideline 429	concentration / SI 10% / 2.6 25% / 14.9 50% / 6.9 100% / not tested positive no concentration dependency; ear thickness not increased with statistical significance (data not given)	concentration / SI 10% / 1.5 25% / 7.0 50% / 9.1 100% / not tested positive	concentration / SI 10% / 3.1 25% / 9.3 50% / 10.3 100% / not tested positive no irritation, ear thickness not increased with statistical significance (data not given)	Kreiling et al. 2008
	protocol similar to OECD Test Guideline 429	concentration / SI 10% / 1.6 25% / 2.4 50% / 5.6 100% / not tested according to OECD test guideline: excessive irritation at 25% and above; only the results at 10% can be evaluated as negative due to severe irritation (ear thickness > 25%)	not tested	not tested	Anderson et al. 2009
	according to OECD Test Guideline 429	concentration / SI 10% / not tested 25% / 3.4 50% / 5.7 100% / 6.5 no data for irritation false positive results according to authors	not tested	not tested	Basketter et al. 2009

Tab. 1 *(continued)*

Test system	Method	Oleic acid	Linoleic acid	Linolenic acid	References
LLNA-DAE	not according to an OECD test guideline	10% DILL 0.07 weak positive	25% DILL 0.03 weak positive	25% DILL 0.03 weak positive	Yamashita et al. 2015

DILL: Degree of the Increase in Lymph node weight of the Left ear; GPMT: Guinea Pig Maximization Test; LLNA: Local Lymph Node Assay; LLNA-DAE: Local Lymph Node Assay modified according to Daicel with elicitation phase; PEG300: polyethylene glycol 300; SI: stimulation index

[a] In this maximization test, all skin reactions were grade 1 with the exception of a grade 2 reaction observed after 24 hours in the control group tested with linoleic acid.

5.4.1.7 Summary

In summary, maximization tests carried out with oleic acid, linoleic acid and linolenic acid yielded negative or, at most, questionable positive results. A positive result was not obtained in an LLNA with oleic acid concentrations of up to 10%. With an increase in the number of double bonds (linoleic and linolenic acid), positive results were obtained in other LLNAs.

The discrepancies in the results of the maximization test and LLNA may have been caused by the potential of unsaturated fatty acids to undergo autoxidation by oxygen in the air and to form hydroperoxides. In particular, the open application of test substances in the LLNA, as opposed to the maximization test, means that autoxidation or photooxidation cannot be excluded. Therefore, the discrepancies in the results of the LLNA and maximization test may have been caused by radical-induced mechanisms. Oleic acid, linoleic acid and linolenic acid contain secondary allylic hydrocarbons that can be oxidized by atmospheric triplet oxygen via a radical mechanism to form allergenic hydroperoxides. In the case of the polyenoic fatty acids (linoleic acid and linolenic acid), the formation of hydroperoxides is promoted by the mesomeric stabilization of the radicals generated. However, the hydroperoxides that are produced may subsequently form α,β-unsaturated ketones by eliminating water; these in turn may react as Michael acceptors (Roberts et al. 2016).

Independently of this, lipids and oily substances can also activate T cells themselves, whereby the underlying mechanism is being increasingly researched (Nicolai et al. 2020).

5.4.2 Studies with non-animal methods

Studies using non-animal test methods are available for oleic acid, linoleic acid and linolenic acid.

5.4.2.1 Oleic acid

Oleic acid (purity 99%) was reactive (13% depletion of cysteine) in a standard Direct Peptide Reactivity Assay (DPRA) carried out according to OECD Test Guideline 442C (Kreiling et al. 2017). Precipitation was likewise observed; for this reason, the result is formally considered to be inconclusive according to the criteria. No evidence of reactivity was found in a DPRA carried out according to an independent protocol (not according to OECD Test Guideline 422C) (Yamashita et al. 2015). A negative result was likewise obtained for oleic acid (97%) using a different assay (Spectro-DPRA) (Cho et al. 2020). The results of two independent KeratinoSens assays (OECD Test Guideline 442D) (Cho et al. 2020; Kreiling et al. 2017) are regarded as inconclusive because oleic acid has a log $K_{OW} > 7$. Oleic acid yielded negative results in two h-CLAT (OECD Test Guideline 442E) (Cho et al. 2020; Kreiling et al. 2017). A positive result was obtained for oleic acid in the LCSA (Loose-fit Coculture-based Sensitization Assay according to Schreiner et al. 2007). This assay provides information on the up-regulation of CD86 on dendritic cells (generated from monocytes) co-cultured with primary keratinocytes. Oleic acid was further classified as an irritant in this model, and the result was regarded as false positive (Frohwein et al. 2016). Studies carried out to determine the structural activity relationship (TOPKAT "Toxicity Prediction by Komputer Assisted Technology" and DEREK "Deductive Estimate of Risk from Existing Knowledge")

of oleic acid did not find any evidence of a sensitizing potential (Anderson et al. 2009). However, data for the training sets were not provided. The results obtained using non-animal testing methods are compiled in Table 2.

Tab. 2 Overview of the results obtained for oleic acid (log K_{OW} = 7.64 (experimental); 7.73 (calculated)) using non-animal test methods

	Test system	Method	Results			Authors' assessment	References
in chemico	DPRA	according to OECD Test Guideline 442C	Cys: 13.68% (precipitate however)	Lys: interference		(N)	Kreiling et al. 2017
		Spectro-DPRA; not according to test guideline	Cys: 1.84%	Lys: 8.4%		N	Cho et al. 2020
		not explicitly according to OECD test guideline, concentrations similar to guideline	Cys: 0%	Lys: 8.4%	AM: 4.2%	N	Yamashita et al. 2015
in vitro	KeratinoSens	according to OECD Test Guideline 442D	I_{max} with viability > 70% (1/3 experiments) = 1.2			N	Kreiling et al. 2017
		according to Natsch et al. (2013), not explicitly according to test guideline	I_{max} = 1.29 no other data			N	Cho et al. 2020
	h-CLAT	according to draft OECD Test Guideline 442E; turbidity of the solutions, high variability at CV75 for all fatty acids, attributed to poor solubility	RFI CD86 (0/2 batches)	RFI CD54 (0/2 batches)		N	Kreiling et al. 2017
		according to Natsch et al. (2013), not explicitly according to test guideline	RFI CD86 (no data[a)]) 88 (no other data)	RFI CD86 (no data[a)]) 147 (no other data)		N	Cho et al. 2020
	LCSA	no test guideline; test concentrations: 2–25 µM	EC_{50sens} = 5 µM EC_{50vit} < 50 µM			false P	Frohwein et al. 2016
in silico	DEREK	no other data	N			N	Anderson et al. 2009
	TOPKAT	no other data	N			N	Anderson et al. 2009

AM: arithmetic mean; Cys: cysteine; DPRA: Direct Peptide Reactivity Assay; EC_{50sens}: half of the maximum increase in CD86 expression; EC_{50vit}: effective concentration for 50% viability; h-CLAT: human Cell Line Activation Test; I_{max}: maximum induction of luciferase activity; LCSA: Loose fit Coculture-based Sensitization Assay; Lys: lysine; N: negative; P: positive; RFI: relative fluorescence intensity. Results in parentheses are to be formally assessed as equivocal

[a)] no data for the number of experiments with positive results and the overall number of experiments

5.4.2.2 Linoleic acid

In the DPRA, linoleic acid (purity 99%) yielded a positive result (19% depletion of cysteine), which, however, is inconclusive because of precipitation. It was not possible to determine the depletion of the lysine peptide due to interference. A DPRA that was carried out independently (not according to OECD Test Guideline 442C) reached the positivity limit (Yamashita et al. 2015). A moderately positive result was also obtained in the Spectro-DPRA (Cho et al. 2020). The results of two independent KeratinoSens assays (OECD Test Guideline 442D) (Cho et al. 2020; Kreiling et al. 2017) are regarded as inconclusive because linoleic acid has a log K_{OW} > 7. Two h-CLAT (OECD Test Guideline 442E) yielded positive results for linoleic acid (Cho et al. 2020; Kreiling et al. 2017). The test result obtained in the LCSA was considered false positive because of irritation (Frohwein et al. 2016). The results obtained using non-animal testing methods are compiled in Table 3.

Tab. 3 Overview of the results obtained for linoleic acid (log K_{OW} = 7.05 (experimental); 7.51 (calculated)) using non-animal test methods

	Test system	Method	Results			Authors' assessment	References
in chemico	DPRA	according to OECD Test Guideline 442C	Cys: 19.05% (precipitate, however)	Lys: interference		(P)	Kreiling et al. 2017
		Spectro-DPRA; not according to test guideline	Cys: 38.6%	Lys: 7.5%		P	Cho et al. 2020
		not explicitly according to OECD test guideline, concentrations similar to guideline	Cys: 38.6%	Lys: 7.5%	AM: 23.1%	P	Yamashita et al. 2015
in vitro	KeratinoSens	according to OECD Test Guideline 442D	I_{max} with viability > 70% (1/2 experiments) = 2.4			P	Kreiling et al. 2017
		according to Natsch et al. (2013), not explicitly according to test guideline	I_{max} = 1.92 no other data			P	Cho et al. 2020
	h-CLAT	according to draft OECD Test Guideline 442E; turbidity of the solutions, high variability at CV75 for all fatty acids, attributed to poor solubility	RFI CD86 (1/2 batches): 167 (396 µg/ml)	RFI CD54 (2/2 batches): 208 (396 µg/ml) 220 (191 µg/ml)		P	Kreiling et al. 2017
		according to Natsch et al. (2013), not explicitly according to test guideline	RFI CD86 (no data[a]) 168 (no other data)	RFI CD54 (no data[a]) 480 (no other data)		P	Cho et al. 2020
	LCSA	no test guideline; test concentrations: 2–15 µM	EC_{50sens} = 3 µM EC_{50vit} < 50 µM			false P	Frohwein et al. 2016

AM: arithmetic mean; Cys: cysteine; DPRA: Direct Peptide Reactivity Assay; EC_{50sens}: half of the maximum increase in CD86 expression; EC_{50vit}: effective concentration for 50% viability; h-CLAT: human Cell Line Activation Test; I_{max}: maximum induction of luciferase activity; LCSA: Loose fit Coculture-based Sensitization Assay; Lys: lysine; P: positive; RFI: relative fluorescence intensity. Results in parentheses are to be formally assessed as equivocal

[a] no data for the number of experiments with positive results and the overall number of experiments

5.4.2.3 Linolenic acid

Linolenic acid (purity ≥ 98.5%) yielded positive results (25% depletion of cysteine) in the DPRA (OECD Test Guideline 442C). However, these results are inconclusive because of precipitation. A DPRA that was carried out independently (not according to OECD Test Guideline 442C) reached the positivity limit (Yamashita et al. 2015). Moderately positive results were likewise obtained in the Spectro-DPRA (Cho et al. 2020). Two independent KeratinoSens assays (OECD Test Guideline 442D) (Cho et al. 2020; Kreiling et al. 2017) reported positive results; however, these results are regarded as inconclusive because of the log K_{OW} of about 7. Linolenic acid yielded positive results in two h-CLAT (OECD Test Guideline 442E) (Cho et al. 2020; Kreiling et al. 2017). The result of the LCSA was likewise positive, but was regarded as false positive because of irritation (Frohwein et al. 2016). The results obtained using non-animal testing methods are compiled in Table 4.

Tab. 4 Overview of the results obtained for linolenic acid (log K_{OW} = 6.46 (experimental); 7.30 (calculated)) using non-animal test methods

	Test system	Method	Results			Authors' assessment	References
in chemico	DPRA	according to OECD Test Guideline 442C	Cys: 25.45% (precipitate, however)	Lys: interference		(P)	Kreiling et al. 2017
		Spectro-DPRA; not according to test guideline	Cys: 44.2%	Lys: 7.8%		P	Cho et al. 2020
		not explicitly according to OECD test guideline, concentrations similar to guideline	Cys: 44.21%	Lys: 6.8%	AM: 25.5%	P	Yamashita et al. 2015
in vitro	KeratinoSens	according to OECD Test Guideline 442D	I_{max} with viability > 70% (2/2 experiments) = 1.7; 1.7			P	Kreiling et al. 2017
		according to Natsch et al. (2013), not explicitly according to test guideline	I_{max} = 1.83 no other data			P	Cho et al. 2020
	h-CLAT	according to draft OECD Test Guideline 442E; turbidity of the solutions, high variability at CV75 for all fatty acids, attributed to poor solubility	RFI CD86 (0/2 batches)	RFI CD54 (2/2 batches): 274 (93 µg/ml) 220 (54 µg/ml)		P	Kreiling et al. 2017
		according to Natsch et al. (2013), not explicitly according to test guideline	RFI CD86 (no data[a]) 1680 (no other data)	RFI CD54 (no data[a]) 4800 (no other data)		P	Cho et al. 2020
	LCSA	no test guideline; test concentrations: 1–12.5 µM, only 50% viability at 10 µM and above	EC_{50sens} = 10 µM EC_{50vit} < 50 µM			false P	Frohwein et al. 2016

AM: arithmetic mean; Cys: cysteine; DPRA: Direct Peptide Reactivity Assay; EC_{50sens}: half of the maximum increase in CD86 expression; EC_{50vit}: effective concentration for 50% viability; h-CLAT: human Cell Line Activation Test; I_{max}: maximum induction of luciferase activity; LCSA: Loose fit Coculture-based Sensitization Assay; Lys: lysine; P: positive; RFI: relative fluorescence intensity. Results in parentheses are to be formally assessed as equivocal

[a] no data for the number of experiments with positive results and the overall number of experiments

5.4.2.4 Summary

As they are lipophilic, the fatty acids are only slightly soluble in aqueous media. Therefore, all of the fatty acids lie at the limits of or outside the domains of the in chemico and in vitro assays. For this reason, the results cannot be evaluated further by applying the "2 out of 3" approach or the integrated testing strategy ITSv1 (according to OECD Guideline 497).

Essentially, a similar trend was established for the fatty acids both by DPRA and by LLNA. Whereas oleic acid (1 double bond) was not found to be reactive, linoleic acid (2 double bonds) and linolenic acid (3 double bonds) were particularly reactive towards cysteine. As a result, at least a mechanism of action mediated by radicals or haptens and possibly initiated by autoxidation products in the test substances cannot be ruled out completely for polyunsaturated fatty acids.

Clear positive results were obtained in the KeratinoSens and h-CLAT assays only for linolenic acid while linoleic acid yielded borderline positive results. However, not only may fatty acids undergo autoxidation, they are also suspected of interacting in a non-specific way via mechanisms that can lead to positive results in the h-CLAT and, if applicable, in the LCSA.

In spite of the limitations explained above, the results obtained for the test substances using different in chemico and in vitro methods follow the same trend of increasing reactivity or positivity evident in the LLNA for both polyenoic fatty acids.

5.4.3 Sensitizing effects on the airways

There are no studies or data available for $C_{14–18/16–18unsatd}$ fatty acids or similar substances.

5.5 Reproductive and developmental toxicity

5.5.1 Fertility

5.5.1.1 Behenic acid

In a study carried out in 2002 according to OECD Test Guideline 422, behenic acid was given to 13 Sprague Dawley rats per sex and dose group in gavage doses of 0, 100, 300 or 1000 mg/kg body weight and day (see Section 5.2.2). No effects on the fertility of the animals were observed. The NOAEL was 1000 mg/kg body weight and day (ECHA 2018). However, this was not a generation study and the sperm were not exposed at all stages of development.

5.5.2 Developmental toxicity

5.5.2.1 Behenic acid

In a study carried out in 2002 according to OECD Test Guideline 422, behenic acid was given to 13 Sprague Dawley rats per dose group in gavage doses of 0, 100, 300 or 1000 mg/kg body weight and day (see Section 5.2.2). Developmental toxicity was not observed. The NOAEL for maternal toxicity was the highest dose tested of 1000 mg/kg body weight and day (ECHA 2018; Greim 2008). Developmental toxicity was assessed by a screening test. For this reason, a conclusive evaluation of the toxic effects on development cannot be made.

5.5.2.2 2-Propyl pentanoic acid, ethylhexanoic acid, caprylic acid

A study was carried out with 3 acids that each contain 8 carbon atoms: 2-propyl pentanoic acid, ethylhexanoic acid and caprylic acid. A single dose of the straight-chain caprylic acid of up to 18.75 mmol/kg body weight given to rats on gestation day 12 did not cause teratogenic effects. However, a single dose of the two branched-chain substances, 2-propyl pentanoic acid in a dose of 6.25 mmol/kg body weight and ethylhexanoic acid in a dose of 12.6 mmol/kg body weight, given on gestation day 12 did induce teratogenic effects. Caprylic acid was absorbed via the gastrointestinal tract in an amount that was less than half that of the other 2 acids (Scott et al. 1994).

5.6 Genotoxicity

5.6.1 In vitro

In bacterial mutagenicity tests carried out in 1999, $C_{14–18/16–18unsatd}$ fatty acid concentrations of up to 2500 µg/plate did not induce mutations in the Salmonella typhimurium strains TA98, TA100, TA1535, TA1537 and TA1538 both in the presence and in the absence of a metabolic activation system (ECHA 2018). The substances were not tested to the upper limit.

5.6.1.1 Lauric acid, caprylic acid, $C_{8–18}$ fatty acids, hexanoic acid

In bacterial mutagenicity tests carried out between 1981 and 1982, lauric acid, caprylic acid and $C_{8–18}$ fatty acid concentrations of up to 2500 µg/plate did not induce mutations in the Salmonella typhimurium strains TA98, TA100, TA1535, TA1537 and TA1538 either in the presence or in the absence of a metabolic activation system. In a test carried out in 1991, hexanoic acid concentrations of up to 5000 µg/plate yielded negative results. Hexanoic acid concentrations of 800 µg/plate and above caused cytotoxicity. The positive controls confirmed that the test system was working correctly (ECHA 2018).

5.6.1.2 Behenic acid

In a test carried out in 1981 with the Salmonella typhimurium strains TA98, TA100, TA1535, TA1537 and TA1538, behenic acid concentrations of up to 1250 µg/plate did not induce mutagenic effects either in the presence or in the absence of a metabolic activation system. In studies carried out in 2002 according to OECD Test Guidelines 471 and 472 with the Salmonella typhimurium strains TA98, TA100, TA1535, TA1537 and Escherichia coli WP2uvrA, behenic acid concentrations of up to 5000 µg/plate likewise did not cause mutations either in the presence or in the absence of a metabolic activation system (ECHA 2018).

In a chromosomal aberration test carried out in 2002 according to OECD Test Guideline 473 in CHL cells, behenic acid did not induce clastogenic effects in concentrations of up to 3500 µg/ml (short-term exposure) or up to 2800 µg/ml (with exposure for up to 48 hours) either in the presence or in the absence of a metabolic activation system. The positive controls yielded the expected results (ECHA 2018).

5.6.1.3 Capric acid

In a study from 2010 that included a $TK^{+/-}$ mutation test carried out according to OECD Test Guideline 476 in L5178Y mouse lymphoma cells, capric acid did not induce mutations in concentrations of up to 1.84 mM with a metabolic activation system and in concentrations of up to 1.18 mM without activation. The highest concentrations tested were cytotoxic. The positive controls confirmed that the test system was working correctly (ECHA 2018).

5.6.2 In vivo

There are no studies available.

5.7 Carcinogenicity

There are no studies available for $C_{14–18/16–18unsatd}$ fatty acids.

Tumour promotion studies were carried out with several of the acids in the mixture. The acids, such as oleic acid and lauric acid, were applied to the dorsal skin of mice. The Commission does not consider the results relevant for classification if a substance acts only as a promoter on the dorsal skin of mice without any evidence of an initiating effect (Schwarz et al. 2015).

6 Manifesto (MAK value/classification)

$C_{14–18/16–18unsatd}$ fatty acids cause very slight systemic toxicity. A target organ has not been identified. Irritation of the skin and eyes was observed, but was dependent on the chain length.

MAK value and peak limitation. There are no data for effects induced by the $C_{14–18/16–18unsatd}$ fatty acids after repeated oral administration or exposure by inhalation. The mixture may contain (in varying amounts): lauric acid (C_{12}:0), myristic acid (C_{14}:0), palmitic acid (C_{16}:0), palmitoleic acid (C_{16}:1), margaric acid (C_{17}:0), stearic acid (C_{18}:0), oleic acid (C_{18}:1), linoleic acid (C_{18}:2), linolenic acid (C_{18}:3), *trans*-vaccenic acid (C_{18}:1) and elaidic acid (C_{18}:1) in addition to impurities such as arachidic acid (C_{20}:0) and behenic acid (C_{22}:0) (ECHA 2018).

The Commission has evaluated several of these fatty acids. A MAK value of 2 mg/m³ has been established for lauric acid (Greim 2000). Myristic acid, palmitic acid and stearic acid (Greim 1999), oleic acid (Greim 2002; Hartwig and MAK Commission 2017) and behenic acid (Greim 2008) are included in Section IIb of the List of MAK and BAT Values. The Commission has not evaluated any of the other fatty acids at present.

In a gavage study carried out according to OECD Test Guideline 422 in rats with the long-chain analogue behenic acid, the NOAEL was the highest dose tested of 1000 mg/kg body weight and day (ECHA 2018; Greim 2008). There are no inhalation studies available.

The following toxicokinetic data are taken into consideration for the extrapolation of the NOAEL for behenic acid of 1000 mg/kg body weight and day to a concentration in workplace air: the daily exposure of the animals in comparison with 5 days per week exposure at the workplace (7:5), the possible intensification of the effects over time (1:4; duration of study between subacute and subchronic), the corresponding species-specific correction value for the rat (1:4), the assumed oral absorption (100%), the body weight (70 kg), the respiratory volume (10 m^3) of the person and the assumed 100% absorption by inhalation. A concentration in air of 612.5 mg/m^3 is calculated on the basis of these data.

The $C_{12–14}$ fatty acids, palmitic acid and stearic acid induced slight local effects on the eyes of rabbits during the first days after application. Lauric acid caused severe eye damage that was not reversible within 21 days (see Section 5.3.2.2). For this reason, it is assumed that $C_{14–18/16–18unsatd}$ fatty acids likewise induce effects on the mucous membranes.

Saturated and unsaturated fatty acids occur in foods in the form of triglycerides. For this reason, they can be absorbed and metabolized by the body. In solid form, the $C_{14–18/16–18unsatd}$ fatty acids probably cause very low systemic toxicity. More critical are the effects on the lungs after possible aerosol exposure. The fatty acids may act like surfactants because they have a hydrophilic end (carboxyl group) and a longer hydrophobic hydrocarbon chain. As inhalation studies are not available, it is not possible to estimate the effects of the $C_{14–18/16–18unsatd}$ fatty acids on the respiratory tract or to derive a MAK value. The $C_{14–18/16–18unsatd}$ fatty acids are included in Section IIb of the List of MAK and BAT Values; peak limitation is not applicable.

Prenatal toxicity. A screening study for developmental toxicity carried out according to OECD Test Guideline 422 found no effects on the offspring after administration of behenic acid in gavage doses of up to 1000 mg/kg body weight and day. No valid developmental toxicity studies are available for the $C_{14–18/16–18unsatd}$ fatty acids or for similar fatty acids. As a MAK value cannot be derived, the fatty acids have not been classified in any of the pregnancy risk groups.

Carcinogenicity. Evidence of carcinogenicity was not found in studies that investigated genotoxic effects in vitro, including those carried out with analogous fatty acids, or based on their structure. For this reason, the $C_{14–18/16–18unsatd}$ fatty acids have not been classified in any of the categories for carcinogens.

Germ cell mutagenicity. In vitro studies of fatty acids with similar chain lengths did not yield clastogenic or mutagenic effects. A genotoxic potential cannot be derived from their structure. For this reason, the $C_{14–18/16–18unsatd}$ fatty acids have not been classified in any of the categories for germ cell mutagens.

Absorption through the skin. There are no studies available that investigated absorption through the skin using the mixture itself. Models cannot be applied to calculate values for absorption through the skin because the acids have a high log K_{OW}. Low dermal absorption values were determined for azelaic acid (C_9:0) in test persons. Assuming a flux of about 5 $\mu g/cm^2$ and hour, the amount absorbed under standard conditions (1 hour, 2000 cm^2) is calculated to be 10 mg. If the same amount is assumed for the $C_{14–18/16–18unsatd}$ fatty acids, the systemic toxicity of the $C_{14–18/16–18unsatd}$ fatty acids is quite low in comparison because these fatty acids (except for margaric acid) are absorbed as triglycerides with the food in gram amounts. The fluxes determined in vitro in studies with linoleic acid and oleic acid were even lower. For this reason, the $C_{14–18/16–18unsatd}$ fatty acids have not been designated with an "H" (for substances which can be absorbed through the skin in toxicologically relevant amounts).

Sensitization. No studies were carried out with the $C_{14–18/16–18unsatd}$ fatty acids. However, there are data available for individual fatty acids in the mixture. No recent findings in humans are available that suggest that myristic acid, palmitic acid and stearic acid have sensitizing potential. Data for oleic acid, linoleic acid and linolenic acid are either not available or the data do not suggest positivity. Animal studies and alternative methods that did not use animals for testing reported contradictory findings. In spite of the widespread use of these fatty acids, overall, there are no findings of potential sensitization in humans. For this reason, the $C_{14–18/16–18unsatd}$ fatty acids have not been designated with "Sh" (for substances which cause sensitization of the skin). There are no data for sensitizing effects on the respiratory tract and the fatty acids have not been designated with "Sa" (for substances which cause sensitization of the airways).

Notes

Competing interests

The established rules and measures of the Commission to avoid conflicts of interest (www.dfg.de/mak/conflicts_interest) ensure that the content and conclusions of the publication are strictly science-based.

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