FOREWORD INTRODUCTION

ISOPHYTOL

CAS N°: 505-32-8

SIDS Initial Assessment Report

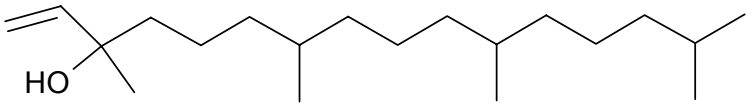
For

SIAM 16

Paris, France, 27-30 May 2003

1. Chemical Name:	Isophytol
2. CAS Number:	505-32-8
3. Sponsor Country:	Switzerland National SIDS Contact Point: Dr Georg Karlaganis Swiss Agency for the Environment, Forests and Landscape CH–3003 Berne, Switzerland
4. Shared Partnership with:	ICCA
5. Roles/Responsibilities of the Partners:	
• Name of industry sponsor /consortium	
• Process used	
6. Sponsorship History	
• How was the chemical or category brought into the OECD HPV Chemicals Programme ?	The chemical was chosen by the Sponsor Company and the Swiss authorities in the frame of the ICCA Initiative.
7. Review Process Prior to the SIAM:	
8. Quality check process:	By industry before submission to the sponsor country: Internal cross-checking by two people involved; late last literature search in public databases for confirmation. Jointly by industry and government: Independent checking by two different government agencies (health and environment), discussion with industry. no testing (×) testing ( )
9. Date of Submission:	21 February 2003
10. Date of last Update:	
11. Comments:	

SIDS INITIAL ASSESSMENT PROFILE

CAS No.	505-32-8
Chemical Name	Isophytol
Structural Formula	

SUMMARY CONCLUSIONS OF THE SIAR

Human Health

Isophytol has a low acute oral and dermal toxicity: oral mammalian LD50 above 5000 mg/kg bw, with most values greater than 8000 mg/kg bw. The acute dermal LD50 is above 5000 mg/kg bw in rabbits. One intraperitoneal LD50 in mouse is 169 mg/kg bw. Inhalative tests over 8 hours in rodents show no effect of a non-aerosol isophytol-enriched atmosphere (NOEC ≈ 0.3 mg/m^3 based on vapour pressure).

Isophytol is irritating to the skin, based on animal studies, but a 10% solution in petrolatum was not irritating to human volunteers. Isophytol is a slight eye irritator. In rabbit transient irritant reactions of the eyes were produced, which all resolved within 8 days. In two sensitisation tests the reactions were judged to be of an irritant rather than a sensitising nature, a maximisation test with 10% isophytol in human volunteers was negative.

The 28-day subchronic oral NOEL is 250 mg/kg bw/d, with only minor and reversible effects (including kidney weight changes) at the LOAEL of 1000 mg/kg bw/d. Based on histopathological data from a one-generation study with an average exposure of 64 days for females and of 98 days for males, the NOEL and NOAEL for parental systemic toxicity was below 250 mg/kg bw/d.

Isophytol was not mutagenic in two bacterial tests, whereas one bacterial test was predominantly negative with a few ambiguous results. In an *in vivo* micronucleus test no clastogenic effects were seen. Thus isophytol is considered to be not mutagenic. There are no proper carcinogenicity data.

In a one-generation reprotoxicity study, 250 mg/kg bw/d was the LOAEL for parental toxicity based on effects in kidney (dilated renal tubules; renal mineralization). 500 mg/kg bw/d was the NOAEL for maternal reprotoxic effects based on a slightly increased mean pre-coital time, a decreased fertility index and conception rate. Postnatal loss was observed at low and medium dose (2% in controls, 7% at 250, 8% at 500 mg/kg bw/d) an increase of 39% at 1000 mg/kg bw/d was observed where also clinical signs in the mothers appeared. A NOAEL of 500 mg/kg bw/d was derived for developmental toxicity of the pups based on clinical signs and decreased body weight during the lactation period.

In conclusion, the overall mammalian toxicity of isophytol is considered to be low but, based on animal data, there is a potential for irritation.

Environment

Water solubility = 5.8 mg/l (25°C); vapour pressure = 0.00003 hPa (20 °C); logP_{OW} ≈ 8.1. Isophytol preferentially partitions to soil and sediment while water and atmosphere are clearly less important compartments. Based on two out of three tests, isophytol was readily biodegradable. It is not significantly biodegradable under anaerobic conditions. No experimental bioaccumulation data have been located but in view of the high log P_{OW} a potential for bioaccumulation may reasonably be expected. In both aerobic and anaerobic sediments, however, isophytol has been shown to be a relatively short-lived intermediate in the diagenetic chemical conversion of the chlorophyll phytol side chain to kerogen, high-molecular-weight lipophilic organic matter bound in sediment and rocks.

Isophytol was not acutely toxic to fish and algae at loadings (nominal concentrations) far higher than the water

solubility in older studies which were not optimised as regards investigations of chemicals with low solubility. Some older daphnid EC50 values vary widely, from 0.11 to 20.3 mg/l, due to nominal concentrations reported and different ways of preparing test solutions. A recent semi-static OECD 202 test under GLP with analytical monitoring resulted in an acute EC50 of 0.130 mg/l (based on average measured concentrations, because the concentration of the substance significantly diminished during each of the two days of the semi-static test). Entrapment of daphnids to the surface of the test media was noted at all test concentrations, but not in the controls. Isophytol had low toxicity to microorganisms, from activated sludge to various species of bacteria and yeasts, with all reported NOECs at least 100 mg/l (nominal concentration). Based on the lowest acute EC50 with measured concentrations, an aquatic PNEC of 0.13 µg/l is proposed for freshwater using an assessment factor of 1000. Nonstandard tests with marine crustaceans resulted in not otherwise specified "weak" effects at 500 mg/l (nominal concentration) in one case, respectively in a minimal inhibitory concentration for the attachment of larvae to surfaces of ≤ 1 µg/cm^2 in the other.

In a chronic and reproductive test with the ubiquitous soil and sediment nematode *Caenorhabditis elegans* the NOEC was high (15,000 mg/kg sediment dry weight). No proper data for effects on terrestrial plants have been located, but isophytol was not toxic in nonstandard *in vitro* tests with maize leaves and safflower cell cultures. Isophytol may work as a semiochemical for certain rice moths, but there are no reports on proper insect toxicity. No avian data have been located.

In conclusion, isophytol showed no acute toxicity towards fish and algae, but seemed to have effects at low concentrations to daphnia after short time exposure. Interpretation of the effects on the daphnids are however complicated because of the disappearance of the substance during the test, and because the effects may have been caused by physical entrapment of the daphnids and not because of toxicity of isophytol. Isophytol is barely toxic for microorganisms and for a common soil and sediment dweller. There is no indication for toxicity against terrestrial plants and insects.

Exposure

Worldwide, approximately 35,000–40,000 tonnes isophytol *per annum* are estimated by industry to be produced. In addition, there is some natural biosynthesis by plants as evidenced by analytical determinations, however, the ubiquitous formation of isophytol from chlorophyll, as postulated in the Merck Index, is not supported by the original literature consulted. More than 99% of synthetic isophytol is used as an intermediate in the synthesis of vitamins E and K_1 and of further terpenoid compounds, while clearly less than 1% is used in fragrance mixtures and less than 0.1% is estimated to be added to food and beverages for flavouring. The initial formulators of isophytol produced at the Swiss plant have comparable emission controls and waste treatment facilities as the manufacturer, hence only minor losses to the environment are expected. Some isophytol is released to the atmosphere, where it is expected to be rapidly degraded abiotically with an estimated half-life below 30 minutes. In the aquatic compartment, isophytol is rapidly biodegraded under aerobic conditions, while anaerobic biodegradation is negligible. In sediment there is evidence for the formation of isophytol as a relatively short-lived intermediate in the abiotic transformation of chlorophyll-derived phytol to high-molecular-weight organic compounds locked in sediment respectively rock. No measured environmental concentrations have been located.

Chemical production workers are rarely exposed to isophytol, due to closed synthesis; where direct contact is possible, standard occupational hygiene measures limit exposure. There may be some limited exposure on filling transport containers. The public is exposed to isophytol as an ingredient of perfumes and cosmetics, however, concentrations in the final products are clearly < 0.2%. Isophytol is listed as a food ingredient in the European Union, but not in the United States; while no quantitative data have been located the actual use in food must be minimal.

RECOMMENDATION

The chemical is currently of low priority for further work.

RATIONALE FOR THE RECOMMENDATION AND NATURE OF FURTHER WORK RECOMMENDED

Human health: The only hazard identified is irritation to skin and slight irritation to eyes. Given the main use as a chemical intermediate and the low content of the substance in consumer products in the Sponsor country, the substance is considered to be of low priority for further work. Countries may desire to investigate any exposure scenarios that were not presented by the Sponsor country.

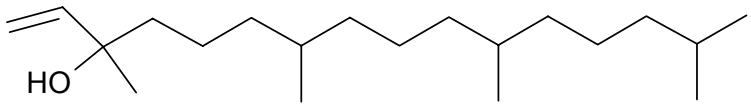
Environment: The chemical possesses properties indicating a hazard for the environment. These hazards do not warrant further work (but they should nevertheless be noted by chemical safety professionals and users). Based on data presented by the Sponsor country, exposure to the environment is anticipated to be low.

SIDS Initial Assessment Report

1 IDENTITY

1.1 Identification of the Substance

CAS Number: 505–32–8
Chemical Name: 3,7,11,15-Tetramethyl-1-hexadecen-3-ol (Isophytol)
Molecular Formula: C_{20} H_{40} O
Structural Formula:



Molecular Weight: 296.52 g/mol
Synonyms: 2,6,10,14-Tetramethylhexadec-15-en-14-ol
2,6,10-Trimethyl-14-vinyl-pentadecan-14-ol

1.2 Purity/Impurities/Additives

Purity: ≥ 95% v/v (synthetic isophytol, minimum specification)

1.3 Physico-Chemical properties

Table 1 Summary of physico-chemical properties

Property	Value
Physical state	
Melting point	≤ –20 °C
Boiling point	313 °C
Density	0.846 g/cm^3 (20 °C)
Vapour pressure	2.98 × 10^{-5} hPa (20 °C)
Water solubility	5.8 mg/l (25 °C)
Partition coefficient n-octanol/water (log value)	> 6 (experimental, GLP) ≈ 8.1 (median of 9 estimates, used for modelling)
Henry's law constant	≤ 6.92 × 10^{-4} atm×m^3/mol
Surface Tension	28.47 mN/m (20 °C; pure substance)
Flash Point	135 °C (only data with reliability 4 available)

Isophytol is a poorly water-soluble organic compound, a clear oily liquid at room temperature. It is a terpenoid alcohol that is biosynthesised by some plants. Isophytol has been produced for many years in high volumes through total chemical synthesis. It is for the best part (>99%) used in the synthesis of vitamins E and K_1, while the remainder (<< 1%) is used as a fragrance and cosmetics ingredient and to a very much minor extent as a flavour compound.

1.4 Listings and Inventories

Isophytol is listed in the OECD HPVC List, the Australian AICS, the Canadian DSL, the Chinese Inventory of Existing Chemical Substances, the European Community EINECS, the European Community HPVC List, the Japanese ENCS, the Korean ECL, the Philippines PICCS, the Swiss List of Toxic Substances and the United States of America TSCA Inventory [SciFinder, 2002].

2 GENERAL INFORMATION ON EXPOSURE

2.1 General Discussion

Chemical synthesis.

Total chemical synthesis of isophytol may start from the addition of acetylene (CAS 74–86–2) to acetone (67–64–1) resulting in 3-methyl-1-butyn-3-ol (115–19–5), which is hydrated in the presence of a palladium catalyst to 3-methyl-1-buten-3-ol (115–18–4), which is reacted with either diketene or acetic acid ester to the acetoacetate and the latter thermally reacted to 2-methyl-2-hepten-6-one (110–93–0). Alternatively, 3-methyl-1-buten-3-ol is reacted with isopropenyl methyl ether (116–11–0) to 2-methyl-2-hepten-6-one. In a third synthetic pathway, isoprene hydrochloride is reacted with acetone in the presence of an alkaline condensating agent or in the presence of organic bases as catalysts to 2-methyl-2-hepten-6-one.

2-Methyl-2-hepten-6-one is then reacted with acetylene to dehydrolinalool (29171–20–8), to which isopropenyl methyl ether is added to make pseudoionone (141–10–6). The three double bonds are hydrated to form 6,10-dimethyl-2-undecanone (1604–34–8), which is reacted with acetylene to 3,7,11-trimethyl-1-dodecyn-3-ol (1604–35–9). Isopropenyl methyl ether is added to form 6,10-14-trimethyl-4,5-pentadecadiene-2-one (16647–10–2), which is hydrated to hexahydrofarnesyl acetone (502–69–2). This is again reacted with acetylene to 3,7,11,15-tetramethyl-1-hexadecyn-3-ol (dehydroisophytol, 29171–23–1), which is finally hydrated to isophytol. The repeated addition of acetylene and hydration was first described by Fischer and Löwenberg [1929].

Natural origins.

Isophytol has been reported from several (at least 15) species of flowering plants and from two red algae [various authors, see IUCLID p. 11/133 “Additional Remarks”]. This broad systematic distribution suggests that isophytol is a common compound in plant biochemistry that may have a long evolutionary history. However, no high concentrations have been reported nor is detection truly wide-spread, in contrast to other terpenoid alcohols.

The Merck Index [1999] states that isophytol is a "decomposition product of chlorophyll", which would make it a very common substance. However, no literature has been located that would support this statement in the broadly general form used. There is not one single clear identification of isophytol as either a precursor or direct metabolite of chlorophyll; in contrast, the isomer phytol, having the hydroxy group in terminal position, has been shown to be both a precursor and direct metabolite of chlorophyll (and other compounds). On the other hand, for both anaerobic and aerobic, freshwater and marine sediments there is good experimental evidence for abiotic isomerisation of phytol to isophytol [Brooks and Maxwell, 1974; de Leeuw *et al.,* 1977; Didyk *et al.,* 1978; Rontani *et al.,* 1999]. Brooks and Maxwell [1974] commented about this isomerisation of phytol to isophytol through "allylic rearrangement of the hydroxyl function occurring readily" in the context of sediments, probably needing certain clay mineral surfaces for the transformation [de Leeuw *et al.,* 1977]. Further, de Leeuw *et al.* also showed that this abiotic isophytol is only an early, relatively short-lived intermediate in the diagenetic conversion of chlorophyll-derived phytol to, eventually, kerogen, an insoluble, high-molecular-weight organic constituent of sedimentary

rocks that may in turn be converted to liquid and gaseous hydrocarbons (petroleum) under the influence of heat and pressure. In conclusion, while isophytol may be formed indeed as an *indirect* metabolite of chlorophyll, this only happens in recent sediments, the isophytol formed is but a transitory intermediate and is considered immobilised for practical purposes. There is also some evidence for inverse isomerisation of isophytol to phytol in plant leaf waxes [Ramachandran *et al.,* 1990]. Pending further empirical data, the importance of natural formation of isophytol through biosynthesis, biotic metabolism or abiotic transformation cannot be estimated.

Production volumes.

The industry estimate for worldwide isophytol production in the year 2002 is 35,000–40,000 tonnes. It is estimated that well over 99% of the total isophytol produced is used as an intermediate in the synthesis of vitamins E and K_1, to both of which it adds the phytyl moiety. The remaining amount (estimated at $\leq$ 0.1%) is used as such as a fragrance and cosmetics ingredient. Isophytol is a registered flavouring compound in the European Union (but not in the USA), but based on information from the flavours and fragrance industry the actual use as a flavouring compound seems to be extremely small.

Due to only few determinations of isophytol in plants and to a lack of quantitative data regarding sediments, the natural amount produced through biosynthesis, metabolism or abiotic formation cannot be estimated, even though it may well be important in comparison with industrial production.

2.2 Emissions and Environmental Exposure

Production waste streams are collected and treated in the Teranol Lalden plant, encompassing waste gas incineration, treatment of industrial wastewaters (all of which have been singly tested for biodegradability), incineration of combustible wastes including distillation residues, recycling of spent catalysts by the catalyst manufacturer with extraction and treatment of organic wastes [source: Teranol Lalden, Safety & Environmental Protection]. Minor emissions may occur into the air during cleaning operations and filling of transport containers.

Produced isophytol is only sent to professional users, either for further chemical synthesis or for incorporation into fragrance concentrates, so-called "compos" with a maximal concentration of 0.9% isophytol for perfumes and about ten times lower for creams, lotions and shampoos. These compos are then forwarded to secondary users who dilute and incorporate them in cosmetics final products. The professional first users of isophytol produced by Teranol Lalden have comparable emission controls and waste treatment organisations as the manufacturer, hence only minor losses to the environment are expected.

Final products containing isophytol are applied to the skin and hair and will either evaporate or be washed off and collect in municipal sewage or, potentially, penetrate through the skin. Any possibly ingested isophytol from flavouring will be metabolised and excreted into municipal sewage. Most isophytol from end (consumer) use is therefore expected to collect in sewage.

Regarding environmental exposure, no quantitative or qualitative monitoring data have been located for isophytol concentrations in indoors or outdoors air, sewage or surface waters or soil. Unquantified concentrations of isophytol have been reported from sediments, however, the conclusion was that this isophytol was derived from abiotic *in situ* transformation of other compounds. Biosynthesised isophytol has been reported from various plants but a global or a regional estimate of this production is not possible.

2.3 Environmental Partitioning and Fate

At 25 °C isophytol is a liquid with a poor water solubility (5.8 mg/l) [BASF, 1993], a low vapour pressure (0.00003 hPa) [Roche, internal data] and a rather small calculated Henry's law constant of $\leq 7\times10^{-4}$ atm×m^3/mol [4 values; EPISuite, SPARC, USES]; in confirmation of the latter, the modelled water-air partition coefficient is ~ 22,000. Based on a QSAR-calculated *p*K$_a$ of 18.4 [SPARC], isophytol in aqueous solutions will not be ionised at any environmental *p*H range. Two experimental *n*-octanol/water partition coefficients have been located, the first HPLC study under GLP resulting in a logP_{OW} > 6 (the upper validity limit of this test) [Rudio, 1999], while the other with a logP_{OW} of 8.8 is way above the limit and the dependability of this value cannot be assessed. For modelling purposes, it was therefore decided to use the median of nine QSAR-calculated logP_{OW} values, namely 8.1 (range 7.20–9.1) [ALOGPS, CLOGP, EPISuite, IALogP, SciFinder, SPARC, USES, XLOGP]. Three calculated organic-carbon/water partition coefficients (K_{OC}) are between 1.98×10^4 and 4.91×10^7 [EPISuite, Mackay Level III, USES], suggesting strong adsorption to dissolved or particulate organic carbon and, by extension, to sediment and soil. The measured surface tension of 28.47 mN/m [Baglay *et al.,* 1988] suggests surface-active properties of isophytol, which is made likely by the most recent daphnid ecotoxicity test, where daphnids became trapped at the surface at all isophytol concentrations but not in the controls (see chapter 4, Hazards to the Environment). Three calculated bioconcentration factors diverge widely due to the high logP_{OW} and its weighting in the regression equations used [68.1 (EPISuite); 2310 (middle value used for modelling, USES); 2,870,781 (BASF 1993 IUCLID)]. As no monitoring data have been located, environmental distribution and fate of isophytol must be modelled (see table 1). High adsorption and low water solubility are judged to be the driving forces for environmental distribution. A static EQC level I distribution model [Mackay, 1997] results in 0.006% of total isophytol partitioning to biota (fish). Nevertheless, based on the high log P_{OW} and the molecular weight of below 500, isophytol has a potential for bioaccumulation. Dynamic distribution is shown in table 2. Considering the very low measured solubility in the daphnid test medium, a distribution that tends even more to adsorption to sediment or soil may be assumed as realistic.

Table 2: Dynamic environmental distribution of isophytol using a generic fugacity model [Mackay *et al.:* Level III, Fugacity-based Environmental Equilibrium Partitioning Model, v. 2.65 (2002). Environmental Modelling Centre, Trent University, Canada].

Compartment	Continuous release, 1000 kg/h			
	100% to air	100% to water	100% to soil	33% each to air, water and soil
Air	5.73%	<0.001%	<0.001%	0.035%
Water	0.66%	6.20%	0.0038%	4.34%
Sediment	10.0%	93.8%	0.058%	65.7%
Soil	83.6%	<0.001%	99.9%	29.9%

Based on a generic fugacity model, isophytol is predicted to partition mainly to sediment and soil, depending on the original release into the environment, while water is only of secondary importance and air is a very much minor compartment.

Atmospheric compartment.

Very little isophytol will partition to the atmospheric compartment, due to the low vapour pressure and Henry's Law constant. Atmospheric fate modelling for isophytol suggests rapid physico-chemical degradation in air with a half-life for hydroxyl-radical-mediated degradation of 2.49 hours at typical •OH concentrations, while the ozone-mediated degradation has a much longer half-life of

157 hours [EPISuite, 2000]. The low volatilisation tendency combined with the high hydroxyl-radical reaction rate is the reason why the atmosphere is not considered a compartment of concern for isophytol, whereas water, sediment and soil potentially are. ***Isophytol will not significantly partition to the atmosphere; moreover, any atmospheric concentrations are expected to be rapidly degraded by hydroxyl radicals.***

Aquatic compartment.

Isophytol was tested in three ready biodegradation assays, the most recent of which missed ready biodegradability because of the 10-day-window criterion (table 3). This test under GLP [Rudio, 1999] showed a lag phase of more than 6 days until 10% biodegradation was reached, then bacterial decomposition proceeded in a flat sigmoidal curve without reaching a plateau at the end of the test (29 days, 62% degradation), suggesting that aerobic biodegradation would continue beyond the regular test duration. The two other tests attained ready biodegradability [BASF, 1989, 1993]; specifically, the 1989 test showed a lag phase of 7 days, then degradation proceeded steeply, passing 60% BOD/ThOD on day 15; however, already from day 12 the curve started to flatten until a 75% plateau was reached on day 24.

Table 3: Biodegradation test data for isophytol.

Test system	Results	Notes
Aerobic degradation		
Manometric respirometry test , OECD 301F, GLP	**62% (29 d, 100 mg/l)**	**not readily biodegradable because of 10-day window, but well inherently biodegradable; initial lag phase**
Manometric respirometry test, OECD 301F	**75% (28 days, 84 mg/l)**	**readily biodegradable; initial lag phase, then rapid degradation reaching a plateau at day 24**
MITI (I) test, OECD 301C	>60% (28 days, 100 mg/l)	readily biodegradable
Degradation with an *Arthrobacter* spec. strain	no degradation (18 h)	incubation with an *Arthrobacter* strain isolated from soil that was able to degrade squalenes
Anaerobic degradation		
Ultimate anaerobic biodegradation test, ISO 11734	**9% (93 days, 123 mg/l = 98.4 mg TOC/l)**	**not significantly anaerobically degradable; initial lag phase with slight sludge inhibition, then very slow degradation rate**

In an anaerobic biodegradation assay according to ISO 11734 [Häner, 2002], isophytol showed a lag phase of 7 days with a slight (≤ 7%) initial inhibition of the bacteria. During the following 4 weeks, degradation proceeded slowly until it re-attained the blank control baseline around day 35, from which it continued in a very slow, monotonous fashion. At 93 days the test was terminated and total degradation was determined to have reached a non-significant 9%, as measured by the production of inorganic carbon in the headspace and in the liquid medium. The viability of the digested sludge was confirmed by the positive diethylene glycol control. Based on this test, isophytol is regarded as not significantly anaerobically biodegradable; toxicity, as measured by inhibition, is low and of temporary nature.

Two of the ready aerobic tests and the anaerobic biodegradability test show a lag phase of 6–7 days before degradation sets in [BASF, 1989; Rudio, 1999; Häner, 2002]. Yamada *et al.* [1977] incubated *Arthrobacter* spec. bacteria isolated from soil with isophytol for 18 hours and later recovered the substrate unchanged. Together, these results are interpreted to reflect an appreciable

time needed by micro-organisms not previously exposed to isophytol to set up a catabolic enzyme complement tailored to this substance. On the other hand, all three aerobic biodegradation tests show good decomposition rates once the process has started and adapted bacteria are present, which implies that isophytol will degrade quickly both in the aerobic stages of sewage works and in surface waters.

In a sewage treatment model integrated in EPISuite v.3.10 [EPISuite, 2000], using the above physico-chemical data and entering 3 hours for biodegradation half-life (as recommended for moderate biodegradation according to the inbuilt EPISuite User Guide), a total removal rate of 99.98% in a sewage works is predicted for isophytol, of which 91.14% is through biodegradation and 8.8% through removal by adsorption to sludge.

Regarding abiotic degradation, aqueous hydrolysis can be excluded based on the structure. Concerning direct or indirect photodegradation, two experimental data suggest that isophytol is relatively resistant. In the first, isophytol was exposed to natural sunlight in artificial seawater in the presence of anthraquinone as a photosensitiser for 3 weeks, after which degradation had "only commenced" (without quantification), while in a parallel test the isomer phytol was "almost totally degraded" [Rontani and Giusti, 1988]. In a second experiment, jasmine absolute oil, containing 8.47% isophytol (GC, area-%) beside more than 60 other identified compounds, was irradiated with not otherwise specified high-pressure and low-pressure mercury lamps for an unspecified time under a nitrogen stream. Re-analysis of the irradiated oils showed an increase of isophytol with both light sources to 15.15% respectively 12.65%, while most other compounds decreased [Toda *et al.,* 1988]. This implies that isophytol was formed from unidentified precursors under illumination and that, moreover, the rate of photoformation of isophytol was clearly higher than a possible rate of photodegradation, which suggests relative stability.

In conclusion, isophytol is considered to be readily biodegradable in an aerobic aquatic environment, specifically in sewage works and in the aquatic compartment itself, while anaerobic biodegradation is not significant. Abiotic degradation in the aquatic compartment is considered to be non-significant.

Soil and sediment compartments

Based on the partition coefficients, any non-degraded atmospheric isophytol will distribute to soil or (via water) to sediment, while most isophytol released to water that is not degraded will collect in the sediment and practically all isophytol released to soil will remain there. No environmental monitoring data could be retrieved for the soil compartment; detection of isophytol in freshwater or marine sediments was not quantified.

Based on the aerobic biodegradation tests, isophytol is expected to degrade in soil and in the oxic sediment fraction as well. There is one report on *Arthrobacter* spec. bacteria, that were isolated from soil using a medium containing the abundant triterpene squalene, which were unable to degrade isophytol within 18 hours' exposure [Yamada *et al.,* 1977]. However, it was shown that this strain primarily cleft double bonds near the middle of long-chain (n = 24) triterpenes, but not shorter terpenes. As the incubation time was short, only 18 hours, this result is not interpreted to show non-biodegradability of isophytol in soil. Rather, it confirms the appreciable time needed by nonadapted micro-organisms to adapt to isophytol degradation. An anaerobic biodegradation test [Häner, 2002] shows that isophytol is not significantly biodegradable, hence biodegradation is not expected to be a relevant fate in anoxic sediment or during sludge digestion.

However, a more important environmental fate pathway in sediment may be through abiotic, long-term diagenetic processes. There are several reports on slow isomerisation of mainly chlorophyll-derived phytol to isophytol in both anaerobic and aerobic, freshwater and marine sediments, probably facilitated by the presence of certain minerals [Brooks and Maxwell, 1974; de Leeuw *et al.,*

1977; Didyk *et al.,* 1978; Rontani et al., 1999]. However, de Leeuw *et al.* also showed that this isophytol is only a relatively short-lived intermediate in the diagenetic transformation to high-molecular-weight kerogen. While appreciable amounts of isophytol may be formed naturally (which may be impossible to quantify), these are both transitory in character and bound in the sediment undergoing diagenesis, hence this isophytol is considered to be immobilised for practical purposes and to become definitely unavailable to the biosphere.

In conclusion, isophytol is expected to biodegrade rapidly in both soil and the oxic sediment fraction, but not significantly during sewage sludge digestion or in hypoxic or anoxic sediments; in the latter compartments, isophytol may be formed abiotically as a comparatively short-lived intermediate in the diagenetic transformation of chlorophyll-derived phytol to kerogen, insoluble high-molecular-weight hydrocarbons that are bound up in sedimentary rocks.

2.4 Human Exposure

Industrial releases of isophytol may occur from the sites of production and through use in industrial processes. In the case of the Teranol Lalden plant in Switzerland producing isophytol for the reporting company F. Hoffmann-La Roche Ltd, total synthesis of isophytol proceeds in dedicated closed systems. The same holds for the co-sponsor company in Germany. The initial formulators of isophytol produced at the Swiss plant have comparable emission controls and waste treatment facilities as the manufacturer, hence only minor losses to the environment are expected.

Exposure of workers to isophytol is possible during sampling, maintenance and cleaning operations, manual addition of new or extraction of spent catalyst and filling of storage or transport containers and vessels. Standard industrial hygiene measures, *viz.,* safety goggles, protective nitrile rubber gloves, clothing and shoes as well as local exhausts, are being routinely applied during these activities. For downstream industrial processes, *e.g.,* chemical synthesis of vitamins E and K_1 or use as fragrance and flavour ingredient, safety data sheets give professional users advice on substance properties and exposure protection. There are no recommended international, national or internal occupational exposure limits for isophytol.

Consumers, in contrast, may be directly exposed to very low levels of isophytol used as a fragrance (or flavour) compound. Based on production estimates, the global total volume for consumer uses is at most 40 tonnes *per annum*. Isophytol is listed in the European Union as both a fragrance and a flavour compound, while in the USA it is only listed as a fragrance. In perfumery, the maximal concentration of isophytol in final products is 0.2% v/v, while an average concentration in cosmetics in general (creams, lotions, shampoos etc) is extrapolated to be $\leq$ 0.02% v/v. Based on information from the European fragrance and flavours industry, isophytol is not listed as an approved flavour in the USA and utilisation in Europe is negligible, but no final use data nor concentrations for flavouring have been located. There is a current ADI (Acceptable Daily Intake) published by the United Nations for total terpenoid alcohols of 0–0.5 mg/kg bw/d [JECFA, 1999], however, it must be noted that isophytol is not expressly listed.

3 HUMAN HEALTH HAZARDS

3.1 Effects on Human Health

3.1.1 Toxicokinetics, Metabolism and Distribution

No experimental data have been located for isophytol regarding Absorption, Distribution, Metabolism, Excretion.

3.1.2 Acute Toxicity

Table 4: Summary of acute toxicity results

Route/Species	Results	Notes
oral:		
Rat	**LD_{50} > 8000 mg/kg bw** **LD_{10} > 8000 mg/kg bw**	**males and females, no deaths at 8000 mg/kg bw**
Rat	LD_{50} > 5400 mg/kg bw	
Rat	LD_{50} > 5000 mg/kg bw	males, 2/10 dead
Rat	LD_{50} > 12000 mg/kg bw LD_{10} > 12000 mg/kg bw	*Isophytol crude,* males and females, no deaths at 12,000 mg/kg bw
Mouse	**LD_{50} > 8000 mg/kg bw** **LD_{10} = 8000 mg/kg bw**	**males and females, 1/10 dead at 8000 mg/kg bw**
Mouse	LD_{50} > 8000 mg/kg bw LD_{10} > 8000 mg/kg bw	*Isophytol crude,* males and females, no deaths at 8000 mg/kg bw
inhalative:		
Rat, Mouse, Guinea pig	**NOEC ~ 0.3 mg/m^3 (calculated)**	**no effects from inhalation of an isophytol-enriched atmosphere during 8 hours**
dermal:		
Rabbit	**LD_{50} > 5000 mg/kg bw**	**occluded patches, no deaths, 2/10 animals with "skin and intestinal abnormalities" on dissection**
Guinea pig	not phototoxic	female only
other routes:		
Mouse, i.p.	**LD_{50} = 169 mg/kg bw**	

Oral

All acute oral LD_{50} values located, all from industry-internal toxicity tests, for rat and mouse are higher than 5000 mg/kg bw [Bächtold, 1973; Moreno, 1982; BASF, 1970]. Only one report states 2/10 animals dead at a dose of ≤ 5000 mg/kg bw (exact dose not stated) [Moreno, 1982], while several tests have a minimal lethal dose of 8000 mg/kg bw or higher [Bächtold, 1973]. The same holds for two results relating to "isophytol crude" [Bächtold, 1973].

Isophytol is listed in the Swiss List of Toxic Substances as toxics class 4, which normally implies an acute oral toxicity value below 5000 mg/kg bw. An enquiry referring to this classification at the Swiss Federal Office of Public Health resulted in the information that they were not aware of an acute oral toxicity value for isophytol below 5000 mg/kg bw either, but that skin irritation (see chapter 3.1.3) was also considered in establishing the present Swiss classification.

In conclusion, all acute oral toxicity tests unanimously give a high LD_{50} > 5000 mg/kg bw. No reports regarding human intoxication due to isophytol have been located.

Inhalation

Three inhalative tests in the same laboratory exposed rats, mice and guinea pigs to a non-aerosol isophytol-enriched atmosphere for 8 hours at 20 °C, by bubbling water-vapour-saturated air through a 5-cm-thick layer of isophytol into the test cages. No effects were observed [BASF, 1970].

Assuming the atmospheric isophytol partial pressure to correspond to the vapour pressure, this result translates to a calculated NOEC of 0.3 mg/m^3, possibly less as saturation may not have been reached (no analytics in the air were performed). There were no observed effects in short-term inhalative tests with isophytol.

Dermal

One dermal LD50 value is reported as > 5000 mg/kg bw [Moreno, 1982], which is congruent with the oral data. Moreover, no phototoxicity on dermal application was found [Takasago, 1999 (English translation);1982 (original)]. This finding possibly further confirms the photostability of isophytol.

Other Routes of Exposure

One intraperitoneal LD_{50} in mice was 169 mg/kg bw [BASF, 1970], which seems reasonably consistent with the oral data.

3.1.3 Irritation

Skin Irritation

Table 5: Summary or results on Irritation to the skin

Species	Results	Notes
Guinea pig	**Irritating**	**skin responses noted in an OECD sensitisation test under GLP were interpreted as signs of primary irritation rather than sensitisation**
Rabbit	Moderately to severely irritating	occluded patches, Draize scoring system, 2/10 animals with "skin abnormalities" on dissection
Rabbit	Irritating	undiluted; severe erythema and heavy oedema at 24 hours, heavy erythema and heavy flaking of skin at 8 days
Guinea pig	irritating	5-50% in acetone
Man	**not irritating**	**10% in petrolatum; occlusive, 48 h, no significant skin reactions in 27 volunteers in a maximisation test**

Three out of 4 animal tests resulted in clear to severe irritating skin reactions, the fourth was negative to ambiguous [Csato and Chubb, 1996; Moreno, 1982; BASF, 1970; Takasago, 1999 (english translation);1982 (original)]]. Specifically, in an OECD skin sensitisation test under GLP [Csato and Chubb, 1996], 6/10 control (non-induced) guinea pigs showed positive reactions to 25% v/v isophytol in ethanol and a further 2/10 control animals showed positive reactions to 12.5% isophytol in ethanol; these reactions were considered by dermatoxicologists to be signs of primary irritation caused by isophytol.

An older skin irritation test on rabbit [BASF, 1970] describes skin reactions at 24 hours after application as severe erythema after both 20-hour and 1- to 15-minute applications, with additional severe oedema in case of the longer application time; at 8 days after both long- and short-term applications, all reactions were characterised by heavy flaking of the skin, with additional heavy oedema in case of the longer application time.

The application of 10% isophytol in petrolatum in 27 human volunteers during the course of a maximisation test did not result in any significant skin reactions [Epstein, 1981].

In conclusion, based on the animal data, isophytol has a clear potential for skin irritation. In man, the irritating potential of dilutions of isophytol may be relatively minor.

Eye Irritation

In the single test located, isophytol caused transient effects described as "light reddening of the eye" at 1 hour and "light reddening and dulling of the eye" at 24 hours after a single application of 50 µl of undiluted isophytol to rabbit eyes; these reactions resolved completely and there were no observed effects at 8 days after application [BASF, 1970].

Based on this test, isophytol is regarded as a slight eye irritator.

Respiratory Tract Irritation

No specific respiratory irritation test data have been located. However, no adverse effects were observed in an 8-hour acute inhalation toxicity test with rats, mice and guinea pigs in an isophytol-enriched atmosphere [BASF, 1970].

3.1.4 Sensitisation

In a 1996 OECD 406 test under GLP, the first challenge done with isophytol at 25% v/v in ethanol caused skin reactions (discrete or patchy erythema up to moderate and confluent erythema) in 15 out of 20 animals. However, the same severness and distribution of skin reactions were also observed in 6 out of 10 control animals. A second challenge done with half of the concentration of isophytol (12.5%) resulted in a similar picture causing skin reaction in test and control animals of the same severness. Based on these data it was judged that the skin responses were of a primary irritant rather than a sensitising nature [Csato and Chubb, 1996].

An older company-internal "cross-brushing" test [BASF, 1970] with 0.5% isophytol in acetone gave an ambiguous result at first sight, based on "questionable" erythema in 5/10 induced animals and 0/3 non-induced controls; however, 1% isophytol in acetone also resulted in "questionable" erythema in 2/3 additional controls, which implies irritation rather than sensitisation.

Only one human maximisation test with a reliability of 4 was available. This test, 10% isophytol in petrolatum [Epstein, 1981] in 27 healthy, male and female volunteers, with five 48-hour induction applications to the same site on the forearm under occlusion and subsequent challenge on fresh sites after 10–14 days, produced "no significant reactions" to isophytol; the only reactions noted were attributed to sodium lauryl sulfate pretreatment.

Conclusion

While the data available on the cutaneous sensitising potential of isophytol do not allow exclusion of this effect, the effects observed in two tests are consistent with and interpreted as signs of irritation. The sensitising potential of isophytol is regarded as low to nil.

3.1.5 Repeated Dose Toxicity

Studies in Animals

Table 6: Summary of animal studies on repeated dose toxicity

Species	Results	Notes
Rat, OECD 407, GLP, 28 days	**NOEL = 250 mg/kg bw/d NOAEL= 500 mg/kg bw/d LOAEL=1000 mg/kg bw/d**	**isophytol, gavage, 28 d exposure plus 14 days post-treatment observation**
Rat, OECD 407, pretest, 7 days	NOEL = 1000 mg/kg bw/d	pretest to determine the suitability of maize/corn oil as a vehicle for the above 28-day test
Rat, OECD 415, GLP, exposure of males on average 98 (91–134) d, females 64 (52–108) d	**NOEL < 250 mg/kg bw/d NOAEL< 250 mg/kg bw/d LOAEL= 250 mg/kg bw/d**	**one-generation reprotoxicity study, gavage; effect levels based on histopathological changes in kidneys**

In a 28-day OECD repeated dose toxicity test under GLP [Strobel and Lambert, 1998] in male and female rats, with an additional 14-day treatment-free observation period for half of the vehicle control and high-dose groups, no animal died during the whole test duration. There were four groups (vehicle controls, n = 12 f + 12 m; 250 mg/kg bw/d, n = 6 f + 6 m; 500 mg/kg bw/d, n = 6 f + 6 m; 1000 mg/kg bw/d, n = 12 f + 12 m). In all four groups, body weights, body-weight gains and food consumption were in the normal range. No obvious treatment-related abnormalities were observed at dissection nor during histopathological examination; a small number of findings were within the normal range of background alterations for untreated rats of this strain and age and were not considered to be treatment-related. Oral administration of isophytol to rats for 28 days at 1000 mg/kg bw/d was associated with the following findings: fur-staining in females, including one animal that showed hypoactivity, hunched posture, weight loss and pallor; increased body weight in males; a number of clinical chemistry changes in males and females; increased liver weights in both sexes, increased kidney and spleen weights in females. Oral administration of 500 mg/kg bw/d was associated with smaller differences in a number of clinical chemistry parameters; there were no clinical signs of toxicity or significant organ weight changes. Oral administration of 250 mg/kg bw/d resulted in a minor elevation of blood calcium levels in females. The majority of clinical chemistry findings, although statistically different from the vehicle control group, were within the ranges of historical background data quoted for control animals. The toxicological significance of these findings in the absence of any corroborative histopathological changes is unclear. After a 14-day treatment-free period, the majority of changes were no longer apparent. In view of the in-life, clinical-chemistry and organ-weight findings in the four groups of animals, the NOEL was established at 250 mg/kg bw/d and the NOAEL at 500 mg/kg bw/d, while a LOAEL (that was based on still minor and reversible changes) was set at 1000 mg/kgbw/d. No unambiguous signs of overt toxicity were noted at any dose.

Additionally, in a vehicle (maize/corn oil) suitability pretest to the above 28-day study, 2 male and 2 female rats were dosed 1000 mg/kg bw/d for 7 days. This dose did not produce any observed effects.

In a one-generation OECD reproduction toxicity study under GLP [Beekhuijzen, 2002], female and male rats were orally administered isophytol in vegetable oil at daily doses of 0 (vehicle controls), 250, 500 and 1000 mg/kg bw/d for 10 weeks prior to mating in males and for at least 8 weeks in females (2 weeks prior to mating, 3 weeks gestation, 3 weeks lactation until termination of test). Exposure duration in males ranged from 91 to 134 days, with an average of 98 days, while in

females exposure range from 52 to 108 days, with an average of 64 days; these durations correspond to subchronic to chronic exposure for rats. The dose levels were selected based on the earlier OECD 28-day subchronic toxicity study. In this test there was no subsequent treatment-free period, hence a potential resolution and reversibility of observed effects cannot be assessed.

Among clinical signs, females of the 1000 mg/kg bw/d group showed an increase incidence in lethargy, hunched posture and piloerection. In males and females body weights were affected by treatment in the 1000 group, but not in the others. The absolute and relative food consumption of the females of groups 1000 and 500 was increased during part of the test period up to parturition and absolute food consumption was decreased during the lactation period. The absolute and relative food consumption of the males of groups 1000 and 500 was increased part of the time before and after mating, while the relative food consumption of the group 250 males was decreased during part of the pre-mating period.

On macroscopic examination, both absolute and relative kidney weights were significantly increased in females of group 1000 and 500, whereas in males a significant increase in the relative kidney weight was observed solely in the group 1000. On histopathological examination, females of the 1000 group showed minimal to moderate periportal hepatocyte vacuolation. In the kidneys of males and females of 500 and 1000 groups, basophilic aggregates and an increase in the incidence of basophilic tubules were noted. The following effects were consistently observed in all treatment groups and in both males and females: Dilated renal tubules and general mineralization. In addition, in the males of all treatment groups, a decrease in the incidence of hyalin droplets was found. Based on these effects, a NOAEL of < 250 mg/kg bw/d was derived. It has been noticed that no effects on kidney were observed in the 28 days repeated dose toxicity test. The reason for that is not clear but might be due to the shorter exposure time of this study in comparison to the one-generation study.

There is a current Acceptable Daily Intake (ADI) by the FAO and WHO for total terpenoid alcohols of 0–0.5 mg/kg bw/d [JECFA, 1999]; while isophytol is not expressly listed in this publication, the ADI is reasonably consistent with the above data, assuming an applied safety factor of 1000 and that isophytol will make up a negligible fraction of consumed terpenoid alcohols.

Conclusion

In conclusion, isophytol was of low toxicity to rats in a 28-day OECD repeated dose test. A NOAEL of 500 mg/kg bw/d could be derived, based on minor and reversible clinical signs observed at 1000 mg/kg bw/d. From an OECD one-generation reprotoxicity study in rats, a subchronic to chronic NOAEL < 250 mg/kg bw/d could be derived based on histological changes in the kidney.

Studies in Humans

No empirical data for human uptake of isophytol have been located nor has any report of chronic toxic effects been found. Based on occupational medicine records from the Teranol Lalden plant, no effects have been observed from occupational exposure during more than 30 years of isophytol production.

3.1.6 Mutagenicity

Table 7: Summary of results on genetic toxicity

Species	Results	Notes
Bacterial, *in vitro:*		
***Salmonella typhimurium*, TA97, TA98, TA100, TA102, TA103, TA1535**	**negative**	**up to 10,000 µg/plate, with and without metabolic (S9) activation; 10,000 µg/plate was close to the toxic concentration:** **some ambiguous results**
S. typhimurium, OECD 471, TA98, TA100	negative	liquid suspension pre-incubation assay, up to 5000 µg/plate, with and without metabolic (S9) activation
S. typhimurium, OECD 471, TA98, TA100, TA1535, TA1537	negative	standard and pre-incubation test, up to 5000 µg/plate, with and without metabolic (S9) activation
S. typhimurium, TA98, TA100 in part, TA97, TA1537	ambiguous	Ames test, unspecified concentration, with and without metabolic (S9) activation
Non-bacterial, *in vivo:*		
Mouse, OECD 474, GLP	**negative**	**micronucleus test, 2000 mg/kg (gavage, 48 h)**

Four bacterial mutagenicity tests of Ames type and one *in vivo* mouse micronucleus test are available. Of the four available *Salmonella* tests, all with and without metabolic S9-mix activation, two (including one liquid suspension pre-incubation assay) were clearly negative at up to 5,000 µg/plate [BASF, 1989, 1991], one insufficiently documented result from literature was ambiguous [Zeiger and Margolin, 2000], while the best documented assay [US National Toxicology Program, 2002] was preponderantly negative with exception of a few (5/87) equivocal and 1/87 weakly positive results, however, without any dose-response relationship being evident. Based on these tests the mutagenic potential of isophytol to bacteria is considered negative.

In confirmation, a recent OECD 474 micronucleus test in mice under GLP was unambiguously negative [Meerts, 2002]. In groups of 5 male mice each (there being no sex-related differences in the pre-test), there was no increase in the frequency of micronucleated polychromatic erythrocytes among total polychromatic erythrocytes at 24 or 48 hours after oral dosing of 2000 mg isophytol/kg bw in comparison with vehicle controls, while the positive (cyclophosphamide) controls showed a statistically significant increase in prevalence of micronucleated polychromatic erythrocytes. No data on non-bacterial *in vitro* mutagenicity assays have been located.

Conclusion

In conclusion, based on a series of bacterial in vitro and on one mammalian in vivo mutagenicity tests, isophytol is judged to have no mutagenic activity.

3.1.7 Reproduction and developmental toxicity

A one-generation reproductive toxicity study according to OECD 415 was performed under GLP [Beekhuijzen, 2002]. At the start, 96 female and 96 male rats were randomised to four test groups of 24 f and 24 m each, with the oral dose level determined by the results of the 28-day subchronic test: 0 (vehicle controls), 250 (subchronic NOEL), 500 (subchronic NOAEL) and 1000 (subchronic LOAEL) mg/kg bw/d. The test animals were dosed by gavage, the males for 10 weeks and the females for at least 8 weeks (2 weeks prior to mating, 3 weeks gestation and 3 weeks lactation) up

to termination of the study. Females were paired on a one-to-one basis with males from the same treatment group. The presence of a copulation plug marked day 0 of gestation. Females were allowed to litter normally, on day 4 after birth the size of litters was adjusted by culling and the study was terminated by killing all survivors on day 21 after birth. Various endpoints and parameters relating to health, behavioural signs, mating success, gestation period, and reproductive and developmental toxicity were recorded, including after termination of the study macroscopic examinations, organ weights and histopathology. As equivocal effects were observed during the study in the highest dose group, the study was enlarged by mating those animals that had not successfully mated with additional untreated animals, in order to ascertain their fertility or infertility.

In the study there were 8 unscheduled deaths among females, one each spontaneous in groups 0 and 500 and the rest killed *in extremis, viz.* one in group 0, three in group 250 and two in group 1000. As no dose-response relationship was present, these deaths were considered not to be due to the treatment with isophytol.

F0 (parental generation) animal parameters.

Clinical signs: Females of group 1000 showed an increase incidence in lethargy, hunched posture and piloerection. Various other incidental findings were not considered to be related to the treatment and were considered to be within the historical, normal biological variation for rats of this age and strain.

Body weights were affected by treatment in the 1000 group, but not in the others; body weights and body weight gains of the males were slightly decreased during the whole study period while the females showed a slight body weight loss during the lactation period.

Food consumption: The absolute and relative food consumtion of the females of groups 1000 and 500 was increased during at least a part of the pre-mating and post-mating period but the absolute food consumption was decreased during the complete lactation period. The absolute and relative food consumption of the males of groups 1000 and 500 was increased part of the time before and after mating while the relative food consumption of the group 250 males was decreased during part of the pre-mating period.

On *macroscopic examination,* various signs were noted in males and females from all groups, without noticeable relationship to treatment, which included enlarged liver, accessory liver lobes, fluid-filled uterus, pelvic dilation of kidneys, enlarged spleen, nodules at ovaries and uterus horn, foci on the thymus. Three females from the 250 group that had been killed *in extremis* showed foetuses in the birth canal or in one of the uterine horns; however, as no female from any of the other groups was found to have parturition problems these three were not judged to be treatment-related.

Regarding *organ weights* (see table 8), both absolute and relative kidney weights were significantly increased in females of group 1000 and 500, whereas in males a significant increase in the relative kidney weight was observed solely in the group 1000. An increased absolute and relative uterus weight was observed in the 1000 group, whereas in males decreased absolute and relative prostate weight were observed in all treated groups. A decrease in the absolute seminal vesicles weights was observed solely at 1000 mg/kg bw/d. However, for both male specific effects no correlating histopathological changes were found. All other effects on organ weight observed in females of the 250 group (liver and spleen) and males of all groups (liver) were considered to be treatment unrelated through lack of histopathological or reproductive effects or lack of recognisable dose-response relationship.

Histopathology showed consistent effects on the kidneys in the form of dilated renal tubules and renal mineralisation in all three treatment groups and in both males and females. Additionally, in males of all treatment groups, there was a decrease in the incidence of renal hyaline droplets; in both males and females of the 500 and 1000 groups, there was an increase in the incidence and severity of renal basophilic tubules and of renal basophilic aggregates. The histological changes in the kidneys from all treatment groups were characterised by the consultant histopathologist as unambiguously adverse effects. In liver, only in females of the the highest dose group a minimal to moderate periportal hepatocyte vacuolation was found.

Table 8: Body and organ weight changes

Group	**males**							
	bw		**kidney**		**liver**		**sv**	
	a	r	a	r	a	r	a	r
0	100		100	100	100	100	100	100
250	101		102	102	90	89	110	109
500	103		97	94	86	84	106	104
1000	109		97	89	91	84	119	110
	females							
	bw		**kidney**		**liver**		**uterus**	
0	100		100	100	100	100	100	100
250	99		91	93	100	98	108	110
500	93		85	91	91	85	103	105
1000	104		76	73	101	105	55	74
a = absolut; r = relative, bw = body weight, sv = seminal vesicles all values in percent derived from mean values.								

Reproductive parameters were only affected in the highest dose group of 1000 mg/kg bw/d. At this dose females showed a slightly increased mean pre-coital time, a decrease in fertility index and conception rate. The numbers of dead pups at first litter check, postnatal losses and breeding losses were significantly increased in litters of the 1000 group; due to this fact, the weaning index was enormously decreased in this group. Postnatal losses were also increased in litters of the 250 and 500 groups when compared with controls. (Percentage of postnatal loss days 0-4 post partum: Control: 2%; low dose: 7%; medium dose 8% and high dose: 39%). However, as these values with exception to the high dose group were within the historical control range, this finding was considered to be caused by chance and not due to the treatment.

Development of pups. Survival and general fitness of pups was reduced in the highest dose group of 1000 mg/kg bw/d Several pups showed pronounced signs of bad health such as very small or cold appearance, little or no milk uptake and dying. Only one pup of the 500 mg/kg bw/d group showed multiple malformations. Due to the singular nature this was not assesses as a treatment-related effect. Incidental findings consisted of a small, cold, pale or purple/blue appearance, little or no milk uptake, cannibalism, wounds on tail base or legs, red nose, thickened areas on abdominal or thoracic regions, scales or scabs on several parts of the body, alopecia, swelling of leg or dying. Macroscopic examination revealed pelvic dilation of the right kidney in one case. No relationship with the treatment could be established for these observations nor were they considered to fall within the normal biological variation for rats of this strain and age. Mean body weights of both

female and male pups of the 1000 group were significantly decreased on days 4–7 of lactation in comparison with controls.

Conclusion

In conclusion, 250 mg/kg bw/d was the LOAEL for parental systemic toxicity based on effects in kidney (dilated renal tubule; renal mineralization). 500 mg/kg bw/d was the NOAEL for maternal reprotoxic effects based on a slightly increased mean pre-coital time, a devreased fertility index and conception rate. Postnatal loss was observed at low and medium dose and a drastically increase was observed in the highest dose (1000mg/kg bw/d) where also clinical signs in the mothers appeared. A NOAEL of 500 mg/kg bw/d was derived for developmental toxicity of the pups based on clinical signs and decreased body weight during lactation period. Taken together, a daily dose of 250 mg/kg bw/d was the LOAEL for parental effects and 500 mg/kg bw/d was the NOAEL for both reproductive parameters and the development of pups.

3.1.8 Carcinogenicity

No mammalian carcinogenicity test reports have been located for isophytol. However, there is some indirect information in an older publication on the incidence of melanotic tumours in the fruit fly *Drosophila melanogaster* [Bryant and Sang, 1968]. Briefly, approximately 90% of flies of the "tu bw; +su–tu" strain develop melanotic tumours around the time of metamorphosis from larva to adult fly, which suggests that tumourigenic transformation is under hormonal control. Insect metamorphosis is determined by an antagonistic, dual hormone system (juvenile hormone *versus* ecdysone). To determine possible tumour-promoting or tumour-suppressing effects, "tu bw; +su–tu" strain larvae were fed substances with known juvenile hormone activity (such as phytol) and structurally related substances, such as isophytol which had previously been shown to have no hormonal effect. As an unexpected finding, isophytol reduced the incidence of melanotic tumours in the flies in a dose-dependent manner in comparison with untreated controls.

Conclusion

No mammalian carcinogenicity data for isophytol have been located. However, in a fruit fly model, isophytol reduced the incidence of melanotic tumours in a genetically predisposed strain.

3.1.9 Other toxicological and pharmacological effects

In an *ex vivo* rat model of physiological or pathological muscle damage [Phoenix *et al.,* 1989], as measured by the release of creatine kinase (CK) induced by treatment of excised soleus muscles with a calcium ionophore, alpha-tocopherol (vitamin E) and several structurally related compounds were tested for their potential to inhibit CK efflux. It was found that isophytol, representing the phytyl side chain of alpha-tocopherol, curbed Ca-ionophore-induced CK efflux with similar efficacy as alpha-tocopherol itself, whereas the chromanol double ring moiety of alpha-tocopherol did not reduce CK effux at all. As the muscle damage is characterised by oxidative damage to the cell membrane, the antioxidant activity of alpha-tocopherol was compared in the same publication with the activity of isophytol and the chromanol double ring in the rat model using a biomarker for non-enzymic oxidative muscle damage. The antioxidant effect of isophytol was limited in comparison with both alpha-tocopherol and chromanol. The authors also investigated enzyme-mediated oxidative muscle damage with a lipoxygenase inhibition assay, where alpha-tocopherol and isophytol were similarly effective. Phytol, the isophytol isomer, was also tested in these assays and was found to have comparable (non-enzymic and enzyme-mediated oxidative damage) respectively weaker (inhibition of CK efflux) activity than isophytol. ***Isophytol efficiently inhibited muscle damage induced by a calcium ionophore. It was protective against enzyme(lipoxygenase)-***

mediated oxidative damage to muscle cell membranes but only weakly effective as a general antioxidant, thereby suggesting specific lipoxygenase-inhibitory activity.

Retinol-binding protein (RBP) is a blood protein specific for retinol (vitamin A) transport. The relative binding of RBP to vitamin A derivatives, certain terpenes with structural similarities to parts of retinol (including isophytol) and other substances was determined [Hase *et al.,* 1976]. Isophytol had a high affinity to RBP, inhibiting retinol binding with 61% efficacy in a displacement test. ***Isophytol is a potential inhibitor of retinol-binding protein.***

Neurophysiological stimulation of jasmin absolute oil and certain identified components thereof was tested in a mouse model [Tsuchiya, 1992]. Briefly, mice were anaesthetised using sodium pentobarbital i.p. and exposed to atmospheric concentrations of jasmin absolute or its components. While both jasmin absolute and phytol significantly reduced the pentobarbital sleep time, ***isophytol had no stimulating neurophysiological effect in a mouse model,*** but it did not prolong the sleep time, either.

Several terpenes were tested for the ability to enhance percutaneous absorption of indomethacin from a gel ointment in a rat model [Takayama, 1991]. Isophytol at 1% w/w in the ointment enhanced indomethacin absorption, but the effect of isophytol was weaker than that of any other terpene. ***Isophytol was weakly active in enhancing dermal absorption of indomethacin from an ointment, however, there is no information on the absorption of isophytol itself.***

In the *in vivo* mutagenicity test [mouse micronucleus test; Meerts, 2002], isophytol at a single oral dose of 2000 mg/kg bw did not cause any decrease in the ratio of polychromatic to normochromatic erythrocytes, which reflects a lack of toxicity to erythropoiesis, in contrast to the positive cyclophosphamide controls which showed toxic effects. ***Isophytol had no toxic effects on erythropoiesis.***

3.2 Initial Assessment for Human Health

Approximately 35,000–40,000 tonnes of isophytol *per annum* are estimated to be produced worldwide through total chemical synthesis. In contrast, natural isophytol production by plants or in sediment cannot be estimated. There are no measured environmental concentrations for freshwater, seawater, soil, sediment or air.

An estimated 99.9% of worldwide isophytol production is used as an intermediate for the synthesis of vitamins E and K_1, while not more than 40 t/a are used as such in consumer products. According to a European industry estimate, over 95% of these 40 t/a of isophytol is being utilised for its fragrance properties in perfumes and cosmetics, while < 2 t/a is estimated to be used as a flavour ingredient worldwide.

At the Teranol Lalden plant in Switzerland, in view of the dedicated closed production systems, production workers will only be exposed to isophytol during filling of containers and irregular work at the installations, mostly during manual discharging of spent catalyst from the reactor, maintenance and cleaning operations. Standard occupational safety measures, both technical and organisational, are in place for those situations. There are no reports regarding occupational health effects from isophytol exposure in over 30 years of production.

Consumers, on the other hand, may be exposed to isophytol in perfumes, cosmetics and personal care products, with final concentrations at most 0.2% in perfumes, respectively at most 0.02% in cosmetics and personal care products. Concentrations as a flavouring in food or beverages cannot be estimated due to lack of data, but must be very low.

All acute oral LD_{50} values for isophytol are consistently greater than 5000 mg/kg bw, as is the only dermal value. There were no acute effects from inhalation of an isophytol-enriched atmosphere. The only intraperitoneal LD_{50} located is 169 mg/kg bw. In conclusion, isophytol is considered to be of low acute toxicity by both oral, dermal and inhalative route.

In a 28-day subchronic study, the oral NOEL was 250 mg/kg bw/d while the NOAEL was 500 mg/kg bw and even the LOAEL of 1000 mg/kg bw was based on minor and reversible changes. Based on general paternal effects in a reproductive toxicity study with longer exposure, 250 mg/kg bw/d was the LOAEL for males females while in this study no NOEL or NOAEL could be determined. Overall, 250 mg/kg bw/d is taken to be the repeat-dose LOAEL for isophytol. This would be reasonably consistent with a current ADI for total terpenoid alcohols of 0–0.5 mg/kg bw/d [JECFA, 1999] (even though isophytol is not expressly listed), assuming an integrated safety factor of 1000.

In a reproductive one-generation study, 500 mg/kg bw/d was the NOEL for both fertility and reproductive parameters and for the development of the pups. Clear adverse effects were only noted at 1000 mg/kg bw/d.

Isophytol was irritating to the skin in several animal tests, but not irritating to human volunteers as a 10% solution in petrolatum. Based on these data isophytol must be considered as having a clear irritating potential, although in solution it seems to be at most a mild skin irritant for man. Isophytol has a low eye-irritating potential as all minor effects noted shortly after application fully resolved within some days. Based on no effects reported from inhalative tests with an isophytol-enriched atmosphere, isophytol is taken to have no potential for respiratory tract irritation. The reactions observed in a cutaneous sensitisation test were interpreted to be consistent with a potential for irritation rather than to be evidence for genuine sensitisation.

Isophytol was negative or at most ambiguous in four bacterial mutagenicity tests. It also proved negative in an *in vivo* mammalian mutagenicity assay. The equivocal bacterial results are considered to be of low relevance and, overall, isophytol is regarded to have very low or no mutagenic properties. Old circumstantial literature data show no evidence for carcinogenicity.

In conclusion, isophytol has a low acute and subchronic toxicity towards mammals. The overall repeat-dose LOAEL is 250 mg/kg bw/d. It is an irritant in animal tests but the human irritation potential of dilute solutions is low, as is the sensitising potential. It is not considered mutagenic nor is there any evidence for carcinogenicity, based on circumstantial data. Further, no specific toxic modes of actions have been described. The overall toxicity of isophytol is low.

4 HAZARDS TO THE ENVIRONMENT

Isophytol has been tested in several standard acute and nonstandard ecotoxicity studies listed in table 9, beginning with the aquatic organisms.

Table 9: Summary of Ecotoxicity results of Error! Reference source not found.

Species	Results	Notes
Fish:		
***Leuciscus idus,* golden orfe (freshwater)**	**NOEC = 10,000 mg/l (loading concentration, without emulsifier)**	**DIN 38412, static, 96 h Water solubility = 5.8 mg/l**
Crustaceans:		
***Daphnia magna* (freshwater)**	**EC_0 = 0.017 mg/l EC_{50} = 0.13 mg/l EC_{100} = 0.58 mg/l**	**OECD 202 semi-static, 48 h, GLP, average measured concentrations, EC_{50} 95% CI = 0.100–0.170 mg/l**
D. magna	EC_{50} = 20.3 mg/l EC_{50} = 2.9 mg/l EC_{50} = 1.99 mg/l EC_{50} = 0.94 mg/l EC_{50} = 0.11 mg/l	84/449/EEC C.2, 48 h, nominal concentrations, tests performed with dilutions of saturated solutions with a loading concentration of 100 mg/l after different durations of stirring (with or without emulsifier), leaving to stand and centrifugation or direct use; depending on the exact preparation of the test solution, the various EC_{50} values from this study range from 0.11 to 20.3 mg/l nominal concentration.
D. magna	EC_0 = 0.08 mg/l EC_{50} = 0.65 mg/l EC_{100} > 2 mg/l	84/449/EEC, C.2, 24 h, static with Tween 80 emulsifier
	EC_0 = 0.08 mg/l EC_{50} = 0.2 mg/l EC_{100} = 0.8 mg/l	same test, 48 h
Artemia salina (saltwater)	LOEC = 500 mg/l	unspecified "weak" effects, obviously nominal concentration, 24 h
Balanus amphitrite (saltwater)	MIC = 1 µg/cm^2	minimum inhibitory concentration on surface of test vessel for settlement of larvae, 24 h
Algae:		
***Scenedesmus subspicatus* (freshwater green algae)**	**EC_{10} > 500 mg/l EC_{50} > 500 mg/l EC_{100} > 500 mg/l**	**DIN 38412, 72 h, static with Tween 80 emulsifier**
Nematoda:		
Caenorhabditis elegans	**NOEC = 15,000 mg/kg sediment (dry weight)**	**no effects in a 72-hour chronic life cycle test in artificial sediment with an ubiquitous soil and sediment nematode**
C. elegans	"only weak effects"	at unspecified concentrations
Terrestrial plants (only* in vitro *data located):		
Zea mays (maize/corn)	NOEC 680 mg/l	no toxic effects nor inhibition of chlorophyll biosynthesis in etiolated (light-deprived) leaves *in vitro,* at probably 680 mg/l nominal concentration with Tween 80 emulsifier
Carthamus tinctorius (safflower)	NOEC = 100 mg/l	no toxic effect on safflower cell cultures *in vitro* at 100 ppm with Tween 80 emulsifier in liquid culture medium
Micro-organisms:		

Activated sludge bacteria	**NOEC = 100 mg/l**	**OECD 301F, GLP, toxicity control, 28 d, nominal/loading concentration**
Activated sludge bacteria	NOEC = 1000 mg/l	ISO 8192, 30 min, nominal concentration
Pseudomonas putida (bacteria)	NOEC = 10,000mg/l	DIN 38412, 30 min, nominal concentration
Clostridium acetobutylicum (bacteria)	not inhibitory	no metabolic inhibition at unspecified concentration
Zymomonas mobilis (bacteria)	not inhibitory	no metabolic inhibition at unspecified concentration
Saccharomyces cerevisiae (yeast)	not inhibitory	no metabolic inhibition at unspecified concentration

Freshwater

In freshwater, isophytol did not show any toxic effects on fish in a static test, even at a very high loading concentration of 10,000 mg/l (the test substance was added to the test medium without any emulsifier or pretreatment, before adding the fish) over 96 hours [BASF, 1989]. As the reported nominal concentration of 10,000 mg/l is several factors of ten above the solubility limit, this test is interpreted to show no effect at saturation concentration.

Similarly, in an algal growth inhibition test over 72 hours (using Tween 80 at 10% of test article concentration as an emulsifier), no statistically significant effects were noted at 500 mg/l nominal concentration [BASF, 1988].

In contrast, several reports showing evident toxicity to daphnia have been located. An older test series [BASF, 1992] gives a comparison of the toxicities of several solutions or emulsions of isophytol prepared in different ways. With a loading concentration of 100 mg isophytol/l in the undiluted stock solution or emulsion, the nominal EC_{50} values were 20.3 mg/l by stirring for 8 hours, leaving to stand in a separation funnel for a further 17 hours and subsequently centrifugating the lower, aqueous fraction for 10 minutes at 6,000 rpm; the nominal EC_{50} was 2.9 mg/l after 15 hours of stirring without an emulsifier, leaving the emulsion to stand in a separation funnel for an additional 15 hours and using the lower aqueous fraction; the nominal EC_{50} was 1.99 mg/l using Cremophor RH40 as an emulsifier and stirring for 20 hours; the nominal EC_{50} was 0.94 mg/l using Tween 80 as an emulsifier and stirring for 20 hours and, last, the nominal EC_{50} was 0.11 mg/l after 20 hours of stirring without an emulsifier and using this emulsion directly afterwards for the test. In the oldest test available [BASF, 1988] using Tween 80 as an emulsifier, isophytol resulted in an EC_{50} of 0.65 mg/l after 24 hours, respectively in an EC_{50} of 0.2 mg/l after 48 hours.

An acute daphnid toxicity test according to OECD 202 [Migchielsen, 2002] was performed under GLP in order to clarify the daphnid toxicity. A first, static test seemed to confirm low toxicity, with no EC_{50} being reached using undiluted water accommodated fractions (WAF) at 100 mg/l loading rate; however, subsequent analysis of the test solutions showed an unexpectedly low actual isophytol concentration of 0.114 mg/l for the undiluted WAF at the start of the test and a decrease to below the limit of detection after 48 hours. Hence, a second test was run under semi-static conditions, with media exchange after 24 hours. Analysis confirmed the rapid decrease in concentration to approximately half within 24 hours, for both 24-hour media and all concentrations. The test medium was M7 Medium, where beside minor trace elements, macro-nutrients and vitamins the following hardness builders were dissolved in water previously purified by reverse osmosis: 293.8 mg/l $CaCl_2*2H_2O$, 123.3 mg/l $MgSO_4*7H_2O$, 64.8 mg/l $NaHCO_3$ and 5.8 mg/l KCl; the full composition of M7 Medium is given in the test report. In this test under GLP and with analytical confirmation, the 48-hour EC_{50} was 0.130 mg/l average concentration (95% confidence

interval 0.100–0.170 mg/l) and the EC_{100} was 0.58 mg/l. At 48 hours, it was observed that daphnids were trapped at the surface at all isophytol concentrations, but none in the blank controls. All trapped daphnids were re-immersed before recording of mobility, which showed that 39 out of 40 daphnids in the two lowest concentrations (17 and 42 mg/l average measured concentration) were mobile at 48 hours, despite 27 out of those 40 being trapped before re-immersion. It was concluded that the EC_{50} derived in this test reflects true toxicity. Additionally, as entrapment was also observed in the preliminary studies, without any concentration-related increase and without any droplets or surface film being noted, it was further concluded that the observed entrapment was induced by surface-active properties of isophytol.

Hence, daphnid toxicity is given by several 48-hour EC_{50} values from 0.11 mg/l (using an emulsifier) to 20.3 mg/l, which diverge by a factor of 200. Based on the recent OECD 202 test under GLP without emulsifier that reports actual, average measured concentrations in the medium (as opposed to the older data relating to nominal concentrations without analytical confirmations), the accepted daphnid EC_{50} is set at 0.13 mg/l.

Short- and long-term tests with activated sludge [BASF, 1989; Rudio, 1999] and *Pseudomonas* bacteria [BASF, 1988] as well as additional, unquantified data on two further bacterial species and one yeast [Bruce and Daugulis, 1991] show that isophytol is not toxic to micro-organisms including aquatic bacteria, a conclusion borne out by the bacterial mutagenicity tests where no toxicity was observed even at high concentrations [US National Toxicology Program, 2002]. The slight ($\leq$ 7%) initial inhibition of bacteria in the anaerobic degradation test [Häner, 2002] is not regarded as significant overt toxicity but as a physiological adaptation phase.

In conclusion, in freshwater isophytol is taken to be of low toxicity to fish and algae, judging from limited data, but it is toxic for daphnids. Based on the best documented, analytically supported daphnid EC_{50} value, a predicted no effect concentration (PNEC) of 0.13 µg/l is derived for freshwater, using an assessment factor of 1000 based on tests with three different trophic levels. For micro-organisms the PNEC in sewage works is set at 10 mg/l based on the toxicity control of a ready biodegradation test and using an assessment factor of 10.

Seawater

Two toxicity data for isophytol in seawater have been located in the same publication [König *et al.,* 1999]. The first test, exposing nauplius larvae of the crustacean *Artemia salina* to 0 (controls), 10, 100 and 1000 mg/l (probably nominal concentrations) of isophytol in artificial seawater for 24 hours, resulted in otherwise unspecified "weak effects" at a log-linear derived concentration of 500 mg/l. The second test described minimal inhibitory concentrations (MIC) on the surface of test vessels for the settlement of barnacle larvae, with an MIC for isophytol of 1 µg/cm^2. Based on the high $\log P_{OW}$ it is assumed that most isophytol, which was applied to the surface before adding seawater to the test vessels, remained adsorbed on the surface and that no reasonable inhibitory concentrations in the water can be derived. ***In combination, the two seawater tests suggest that in solution, isophytol has no acute effects on planktonic crustaceans up to saturation, while isophytol adsorbed to surfaces may attain inhibitory or toxic concentrations.***

Sediment and soil

As non-degraded isophytol will partition to sediment or soil, a sediment toxicity test with the nematode *Caenorhabditis elegans* was performed [Höss, 2002]. *C. elegans* occurs widely in both oxic and hypoxic to anoxic soils and sediments; it is a self-fertilising hermaphrodite that has a short reproduction time of approximately three days. The test was performed in artificial sediment spiked with isophytol at different concentrations. Young larval stages were added to the test vessels and, after three days, fixed, retrieved and examined for various endpoints or parameters: growth (length), fertility (percentage of gravid worms) and egg production (number of eggs in body). In view of the

generation time, this test qualifies as chronic and reproductive. No observed adverse effect on any of the parameters was noted up to a very high concentration of 15,000 mg isophytol per kg sediment (dry weight), taking into consideration that normally this test is only performed at concentrations of up to 5,000 mg/kg sediment. On the contrary, there was a (probably non-significant) increase in both length and egg production per worm with increasing concentrations of isophytol, confirming unreduced fitness.

In a recent publication [König *et al.,* 1999], isophytol was reported to have "only weak [but otherwise unspecified] effects" on *C. elegans*. For details there is a reference to a paper in preparation, which could not be retrieved in spite of contacting the main author. This result cannot be meaningfully utilised in the scope of the present assessment.

In a chronic test with the ubiquitous soil and sediment nematode* Caenorhabditis elegans, *isophytol was not toxic up to a maximum concentration of 15,000 mg/kg sediment (dry weight). Based on this test, the toxicity of isophytol to soil and sediment is considered to be very low.

Terrestrial plants

Only *in vitro* data for effects of isophytol on terrestrial plants have been located. In a study with etiolated (light-deprived and blanched) maize/corn leaves, several potential precursors of chlorophylls a and b and of two carotenoids were tested in a displacement assay [Costes, 1966]. Cut etiolated leaves were placed in medium with radio-labelled acetate (known to be incorporated in both types of products) alone or together with potential precursors including isophytol for 30 hours under illumination. Differences in incorporated acetate as detected by radioactivity allow calculating the uptake (or not) of precursors. It was shown that isophytol is not a significant precursor of beta-carotene, lutein or chlorophylls a or b. However, the author noted that at a nominal concentration of 680 mg/l medium using Tween 80 as an emulsifier, "this diterpene alcohol is not toxic and that the incorporation of an emulsion [of isophytol] does not restrain the infiltration of sodium acetate into the leaf parenchyma".

In a second *in vitro* study of the processes of tocopherol (vitamin E) biosynthesis by plants, potential precursors including isophytol were added at 100 ppm concentration with Tween 80 emulsifier to culture medium of a safflower cell culture [Furuya *et al.,* 1987]. The growth rates and tocopherols produced in the presence and absence of isophytol and phytol were not significantly different from controls, which implies no toxic effect at 100 mg/l nominal concentration.

In conclusion, isophytol was not toxic in two* in vitro *studies to terrestrial plants at 100 mg/l nominal concentration or higher in the medium.

Chronic toxicity

The algal, nematode and various micro-organism tests qualify as chronic and reproductive tests; in all of these tests toxicity was considered low to very low.

Further data located.

In a review article on insect juvenile hormone systems [Gilbert et al., 2000], isophytol is reported to have no juvenile hormone activity while phytol had some limited activity.

Isophytol was concluded to work as an airborne semiochemical (signal substance) for two species of rice leaf folder moths, as determined by electroantennography [Ramachandran *et al.,* 1990], working as a stronger attractant for the food specialist that feeds nearly exclusively on rice plants, as rice has been shown to produce isophytol, in contrast to the generalist species that also feeds on other plants. Isophytol possibly works as a long-range attractant for males of one of the species in locating the females' host habitat (rice plants).

Isophytol is produced by barley leaves in both epicuticular leaf waxes and within the leaf tissue. In a detailed publication, Muñoz *et al.* [1998] showed that subsequent to infestation of barley leaves by aphids (insect plant pests), the isophytol in the leaf waxes disappeared while at the same time a nearly identical concentration of the isomer phytol was recorded from the leaf waxes, which had not been present before. In contrast, the leafy-tissue isophytol disappeared without obvious metabolites subsequent to infestation. The authors concluded that ***subsequent to aphid infestation of barley leaves, leaf-wax isophytol isomerises to phytol (through unknown processes) while the leafy-tissue isophytol disappears through either dissipation, volatilisation or metabolism.***

No other reports on effects or toxicity of isophytol to other environmentally relevant species have been located.

4.1 Initial Assessment for the Environment

Isophytol is considered to be readily biodegradable in an aerobic aquatic environment. Due to its high log P_{Ow} and its molecular weight below 500, isophytol has a potential for bioaccumulation.

In acute aquatic ecotoxicity tests, isophytol consistently showed low toxicity to fish and algae with NOECs larger than 100 mg/l nominal, but a clear toxic potential to daphnids with an EC_{50} of 0.13 mg/l. Based on this lowest 50% effect concentration the aquatic PNEC is extrapolated to 0.13 µg/l using an assessment factor of 1000.

Isophytol is of low toxicity to activated sludge bacteria, although nonadapted sludge may need several days to adapt. In all tests, the NOEC was 100 mg/l or higher. Relative nontoxicity is confirmed by few additional data from tests with two bacteria and one yeast. The NOEC of isophytol for sludge micro-organisms is set at 100 mg/l, the PNEC at 10 mg/l using an assessment factor of 10.

Similarly, isophytol did not show any toxicity to the common sediment- and soil-dwelling nematode *Caenorhabditis elegans,* even at a very high concentration of 15,000 mg/kg sediment (dry weight). The soil and sediment PNEC is set at 15 mg/kg (dry weight) using an assessment factor of 1000.

Further, in two *in vitro* tests with terrestrial plants, isophytol did not cause evident signs of toxicity, with a NOEC of 100 mg/l in a safflower cell culture. Due to the *in vitro* nature of the data, no PNEC can be derived.

In conclusion, isophytol shows low toxicity to fish and algae as well as to micro-organisms, to a common sediment- and soil-dwelling nematode and to terrestrial plants (based on *in vitro* data). It is, however, toxic to daphnids. Due to its good biological degradability in water (and by extension also in soil), to the rapid predicted abiotic degradation in the atmosphere and to the transformation in sediments, respectively, no environmental concentrations that might cause toxicity are expected.

5 RECOMMENDATIONS

The chemical is currently of low priority for further work.

Human health: The only hazard identified is irritation to skin and slight irritation to eyes. Given the main use as a chemical intermediate and the low content of the substance in consumer products in the Sponsor country, the substance is considered to be of low priority for further work. Countries may desire to investigate any exposure scenarios that were not presented by the Sponsor country.

Environment: The chemical possesses properties indicating a hazard for the environment. These hazards do not warrant further work (but they should nevertheless be noted by chemical safety

professionals and users). Based on data presented by the Sponsor country, exposure to the environment is anticipated to be low.

ANNEX: FULL SIDS SUMMARY

CAS No. 505–32–8		Species	Protocol	Results
Physical-Chemical				
2.1	**Melting Point**		**NA**	**≤ –20 °C; described as increasing viscosity on chilling**
2.2	**Boiling Point**		**NA**	**313 °C (1013 hPa)**
2.3	**Density**		**double-capillary pycnometer**	**0.8458 g/cm^3 (20 °C)**
			NA	0.837–0.847 g/cm^3 (20 °C)
2.4	**Vapour Pressure**		**tensimeter** **NA**	**7.3 hPa (166.2 °C)** **0.00002982 hPa (20 °C)**
2.5	**Partition Coefficient**	**logP_{OW}**	**OECD 117, GLP**	**> 6 (35 °C)**
		logP_{OW}	HPLC	8.8 (above upper validity limit of OECD 117 method)
		logP_{OW}	**QSAR estimate**	**8.1** (median of 9 values**,** range 7.20–9.1, **used for modelling**)
2.6	**Water solubility**		**NA**	**5.8 mg/l (25 °C)**
	*p*H Value		NA	6.7 (5.8 mg/l, 25 °C)
2.62	**Surface Tension**		**capillary method**	**28.47 mN/m (20 °C)**
2.7	**Flash Point**		**DIN 51758, closed cup**	**135 °C**
2.8	**Auto-Flammability**		**DIN 51758, closed cup**	**225 °C**
2.9	**Flammability**			**not flammable according to UN transport criteria**
2.10	**Explosive Properties**		**NA**	**explosion limits in air: 0.3–3.5 % v/v**
2.11	**Oxidation/Reduction Potential**			**not applicable**
2.12	**Dissociation Constant**		**QSAR estimate**	***p*K$_a$ = 18.4 (25 °C)**

2.13	**Viscosity**		**NA**	**72.76 mPa/s (dynamic, 20 °C)**
2.14	**Additional Data**			
	Henry's Law Constant	K_H	QSAR estimate	$1.14\times10^{-6} – 6.92\times10^{-4}$ atm×m^3/mol (4 values)
	Organic-carbon/water partition coefficient, K_{OC}	K_{OC}	QSAR estimate	$1.978\times10^{4} – 4.91\times10^{7}$ (3 values)
Environmental Fate and Pathway				
3.1.1	**Photodegradation**		**irradiation of jasmin absolute oil**	**isophytol contents of jasmin absolute oil rose after irradiation, suggesting light-induced formation from unidentified precursors**
			indirect photolysis in seawater	**at most minor (not quantified) degradation after three weeks in natural sunlight + anthraquinone as a photosensitiser**
3.1.2	Stability in Water			no data located
3.1.3	Stability in Soil			no data located
3.2.1	**Monitoring Data**			**Isophytol has been determined in aerobic and anaerobic, freshwater and marine sediments, but without quantification. No monitoring data exist for other environmental compartments.**
3.3.1	**Transport**	air, soil, water, sediment, fish	QSAR Mackay EQC v.1.0 Level I model	In a Level I model, after single input of equal amounts to air, water and soil, static distributions (without taking account of reactions) are as follows: air 0.00002 % soil 97.8 % water 0.0003% sediment 2.2% suspended sediment 0.07% fish 0.006%

3.3.2	**Distribution**	air, soil, water, sediment	QSAR EPISUITE v3.10 Level III model, 2002	In a Level III model, with a constant input of 1000 kg/h each to air, water and soil and with reaction factored in, dynamic distributions are as follows: air 0.1% soil 27.2% water 3.5% sediment 69.1% persistence time 1590 hours
		air, soil, water, sediment	QSAR Mackay Level III Model v.2.65, 2002	In a Level III model, with a constant input of 1000 kg/h each to air, water and soil and with reaction factored in, dynamic distributions are as follows: air 0.04% soil 29.9% water 4.3% sediment 65.7% residence time 1473 hours
3.4	**Mode of Degradation in Actual Use**			**In sediments (both aerobic and anoxic, both freshwater and marine) isophytol is a relatively short-lived intermediate in the diagenetic abiotic transformation of phytol to high-molecular-weight compounds.**
3.5	**Biodegradation**	**aerobic**	**OECD 301F, GLP**	**62% degradation after 29 days, missed 'ready' biodegradability because of 10-day-window criterion, inherently biodegradable; initial lag phase of 6–7 days**
		aerobic	OECD 301F	75%, 28 days, readily biodegradable; lag phase of 7 days
		aerobic	OECD 301C	>60%, 28 days, readily biodegradable
		anaerobic	**ISO 11734**	**9%, 93 days, not significantly anaerobically biodegradable; lag phase of 7 days with slight inhibition, then very slow, non-significant degradation**
3.7	**Bioaccumulation**		extrapolation from $logP_{OW}$	BCF: 68.1; 2310; 2,870,781 (3 values); widely divergent because of different estimation algorithms; the old, highest value may be widely inappropriate due to the weighting of the very high $logP_{OW}$

3.8	**Additional Remarks**			
	Atmospheric degradation		**QSAR estimate, EPISUITE v3.10**	**$t_{½}$ = 2.49 hours ($^{•}$OH-mediated degradation)** **$t_{½}$ = 157 hours (O_3-mediated degradation)**
	Abiotic transformation in sediment		**various research articles**	**The Merck Index states that isophytol is a "degradation product of chlorophyll". The literature located does not support this statement on a general level, but several publications do show a slow abiotic isomerisation of phytol (derived from chlorophyll through hydrolysis) in both aerobic and anaerobic sediments, probably on certain mineral surfaces. However, this isophytol is not stable, either, but is further abiotically transformed to kerogen, high-molecular-weight sediment-bound organic substance.**
Ecotoxicology				
4.1	**Acute/Prolonged Toxicity to Fish**	***Leuciscus idus*, freshwater**	**DIN 38412**	**NOEC = 10,000 mg/l (loading concentration, no emulsifier), 96 hours, static**
4.2	**Acute Toxicity to Aquatic Invertebrates**	***Daphnia magna*, freshwater**	**OECD 202, semi-static, GLP**	**NOEC = 0.017 mg/l** **EC_{50} = 0.13 mg/l** **EC_{100} = 0.58 mg/l** **(average measured concentrations), 48 hours, semi-static protocol because of rapid decrease of measured concentrations in pre-test, EC_{50} 95% CI = 0.100–0.170 mg/l**
		Daphnia magna	84/449/EEC, C.2	EC_{50} = 20.3 mg/l EC_{50} = 2.9 mg/l EC_{50} = 1.99 mg/l EC_{50} = 0.94 mg/l EC_{50} = 0.11 mg/l (nominal concentrations; tests performed with dilutions of saturated solutions with a loading concentration of 100 mg/l after different durations of stirring with or without emulsifier, leaving to stand and centrifugation or direct use; depending on the exact preparation of the test solution, the various EC_{50} values vary widely), 48 hours, static

		Daphnia magna	84/449/EEC, C.2	24 48 hours EC_0 = 0.08 = 0.08 mg/l EC_{50} = 0.65 = 0.2 mg/l EC_{100} > 2 = 0.8 mg/l (test performed using Tween 80 as an emulsifier)
		Artemia salina, saltwater	nonstandard acute test	unspecified "weak" effects at 500 mg/l (nominal concentration) after 24 hours
		Balanus amphitrite, saltwater	nonstandard larval settlement inhibition test	minimum inhibitory concentration ≤ 1 µg/cm^2, 24 hours
4.3	**Toxicity to Aquatic Plants, eg Algae**	***Scendesmus subspicatus,*** freshwater	**DIN 38412, part 9**	**EC_{10}, EC_{50}, EC_{90} > 500 mg/l (test performed using Tween 80 as an emulsifier), 72 hours**
4.4	**Toxicity to Micro-organisms, eg Bacteria**	**activated sludge bacteria**	**OECD 301F, toxicity control, GLP**	**NOEC = 100 mg/l (loading concentration), 28 days**
		activated sludge bacteria	ISO 8192	NOEC = 1000 mg/l (nominal concentration), 30 min
		Pseudomonas putida	DIN 38412, part 27	NOEC = 10,000 mg/l (nominal concentration), 30 min
		Saccharo-myces cerevisiae, Clostridium acetobuty-licum, Zymomonas mobilis	nonstandard metabolic inhibition test	at unspecified concentrations, isophytol did not inhibit the activity of a yeast and two species of bacteria
4.5.1	Chronic Toxicity to Fish			no data located
4.5.2	Chronic Toxicity to Aquatic Invertebrates			no data located
4.6.1	**Toxicity to Sediment-Dwelling Organisms**	***Caenorhab-ditis elegans***	**chronic/life cycle test in artificial sediment**	**NOEC = 15,000 mg/kg sediment (dry weight), 72 hours; *Caenorhabditis* is an ubiquitous nematode in soils and sediments**
		C. elegans	nonstandard test	"weak" but not quantified effects

4.6.2	**Toxicity to Terrestrial Plants**	*Zea mays*	nonstandard chlorophyll biosynthesis test with maize leaves *in vitro*	[Isophytol, probably at a concentration of 680 mg/l using Tween 80 as an emulsifier] "is not toxic and ... does not restrain the infiltration of sodium acetate into the leaf parenchyma"
		Carthamus tinctorius	nonstandard cell culture test	At a concentration of 100 ppm using Tween 80 as an emulsifier in the liquid culture medium, isophytol did not show any noticeable toxicity to safflower cell cultures.
4.6.3	**Toxicity to Soil-Dwelling Organisms**	***Caenorhab-ditis elegans***	**chronic/life cycle test in artificial sediment**	**NOEC = 15,000 mg/kg sediment (dry weight), 72 hours; *Caenorhabditis* is an ubiquitous nematode in soils and sediments**
4.6.4	Toxicity to Other Non-Mammalian Terrestrial Species			no data located
4.7	Biological Effects Monitoring			no data located
4.8	Biotransformation and Kinetics	*Hordeum vulgare*	aphid infestation test	Isophytol is present in both leaf waxes and leaf tissues of barley. After aphid infestation, the isophytol disappears. There is evidence for epicuticular isophytol isomerising to phytol, while the intra-leaf isophytol disappears without identified metabolites.
		Zea mays	nonstandard chlorophyll biosynthesis test	Isophytol is not a significant precursor in the biosynthesis of beta-carotene, lutein or chlorophylls a and b.
4.9	Additional Remarks	2 species of rice leaf folder moths	nonstandard electroantenno-graphic test	Isophytol may work as a volatile semiochemical for these moths, moreover, it may serve as an attractant for males of one species
Toxicity				
5.0	Toxicokinetics, Metabolism and Distribution			no data located
5.1.1	**Acute Oral Toxicity**	**rat**	**company-internal oral toxicity test**	**NOEL = 8000 mg/kg bw** **LD_{50} > 8000 mg/kg bw** **(Roche inbred strain, males and females, gavage)**

		rat	company-internal oral toxicity test	LD_0 < 5000 mg/kg bw LD_{50} > 5000 mg/kg bw (Wistar, males, gavage; 2/10 animals found dead at a dose of 5000 mg/kg bw; LD_0 unspecified)
		rat	company-internal oral toxicity test	LD_{50} > 5400 mg/kg bw (in the original test report given as LD_{50} > 6400 mm^3/kg bw), aqueous emulsion with traganth gum
		mouse	company-internal oral toxicity test	LD_{10} = 8000 mg/kg bw LD_{50} > 8000 mg/kg bw (Roche inbred strain, males and females, gavage; 1/10 found dead at a dose of 8000 mg/kg bw)
		rat	company-internal oral toxicity test	***test substance: Isophytol crude*** LD_{10} > 12000 mg/kg bw LD_{50} > 12000 mg/kg bw (Roche inbred strain, males and females, gavage)
		mouse	company-internal oral toxicity test	***test substance: Isophytol crude*** LD_{10} > 8000 mg/kg bw LD_{50} > 8000 mg/kg bw (Roche inbred strain, males and females, gavage)
5.1.2	**Acute Inhalation Toxicity**	**rat, mouse, guinea pig**	**company-internal inhalative toxicity test**	**no effects due to inhalation of an isophytol-saturated atmosphere during 8 hours**
5.1.3	**Acute Dermal Toxicity**	**rabbit**	**company-internal dermal toxicity test**	**LD_0 > 5000 mg/kg bw** **LD_{50} > 5000 mg/kg bw** **(10 albino rabbits, occluded patches, 14 days observation; 8 animals without diagnostic findings, 2 with undescribed "skin abnormalities" and "intestinal abnormalities")**
		guinea pig	nonstandard dermal and phototoxicity test	no phototoxicity nor skin irritation observed due to 50% isophytol applied bilaterally to skin in 1.5-cm circles and subsequent one-sided UV irradiation
5.1.4	**Acute Toxicity, Other Routes**	**mouse**	**company-internal intraperitoneal toxicity test**	**LD_{50} ≈ 169 mg/kg bw** **(in the original test report given as LD_{50} ≈ 200 mm^3/kg bw), aqueous emulsion with traganth gum**

5.2.1	**Skin Irritation**	**guinea pig**	**OECD 406, *skin sensitisation,* GLP**	**In a sensitisation test, 6/10 control (non-induced) animals showed positive reactions to 25% v/v isophytol in ethanol and a further 2/10 control animals showed positive reactions to 12.5% v/v isophytol in ethanol. It was considered that these dermal responses were of an irritant rather than a sensitising nature.**
		rabbit	company-internal dermal toxicity test	At a dose of 5000 mg/kg bw, dermal reactions were scored as "moderate to severe"; at necropsy after 14 days, 2/10 animals showed unspecified "skin abnormalities".
		man	maximisation test	Isophytol at 10% in petrolatum under 48 hours occlusive application did not elicit any significant skin reactions in 27 healthy male and female volunteers
		rabbit	company-internal skin irritation test	irritating; at 24 h severe erythema and heavy oedema, at 8 days heavy flaking of skin and heavy erythema; undiluted isophytol
		guinea pig	NA	negative to ambiguous at 30% concentration
5.2.2	**Eye Irritation**	**rabbit**	**company-internal eye irritation test**	**50 µl of undiluted isophytol caused mild transient reactions at 1 and 24 hours after application, but there were no lasting effects after 8 days.**
5.3	**Sensitisation**	**guinea pig**	**OECD 406, GLP**	**not sensitising; the skin responses observed were considered to be of an irritant nature**
		guinea pig	company-internal "cross-brushing" test	ambiguous; questionable erythema in 5/10 induced animals at 0.5% in acetone, questionable erythema in 2/3 non-induced at 1% in acetone and in 0/3 non-induced at 0.5% in acetone; no confirmed sensitisation
		man	**maximisation test**	**isophytol at 10% in petrolatum was not sensitising in a maximisation test with occluded patches in 27 male and female volunteers**

5.4	**Repeated Dose Toxicity**	**rat**	**OECD 407, GLP**	**NOEL = 250 mg/kg bw/d NOAEL = 500 mg/kg bw/d LOAEL = 1000 mg/kg bw/d Wistar Crl:CD(SD)BR (VAF plus) strain, males and females, gavage, 28 days treatment plus 14 days observation; even the LOAEL effects are described as "minor and reversible"**
		rat	**OECD 415, GLP**	**NOEL < 250 mg/kg bw/d NOAEL < 250 mg/kg bw/d LOAEL = 250 mg/kg bw/d Wistar Crl: (WI) BR (outbred, SPF) rats in a one-generation reproductive toxicity test, gavage, males exposed for 91–134 (mean: 98) days, females for 52–108 (mean: 64) days; subchronic to chronic parental effect levels based on postmortem and histopathological parameters, specifically on renal effects even at the lowest tested dose that were characterised by the histopathologist as clearly adverse.**
		rat	OECD 407, pre-test	NOEL = 1000 mg/kg bw/d Crl:CD(SD)BR (VAF plus) strain, males and females, gavage, 7 days treatment; all 4 animals unremarkable
5.5.A	**Genetic Toxicity *in vitro,* Bacterial Test**	***Salmonella typhimurium* TA97, TA98, TA100, TA102, TA104, TA1535**	**bacterial reverse mutation assay, with and without S9 metabolic activation**	**"negative; ambiguous" response in 6 out of 87 test runs at concentrations up to 10,000 µg/plate**
		S. typhimurium TA1535, TA100, TA1537, TA98	OECD 471, with and without S9	negative at concentrations up to 5000 µg/plate
		S. typhimurium TA98, TA100; in part TA97, TA1535	Ames test, with and without S9	result described as "ambiguous" at undefined concentrations
		S. typhimurium TA98, TA100	OECD 471, liquid suspension assay, with and without S9	negative at up to 5000 µg/plate in a pre-incubation test

5.5	Genetic Toxicity *in vitro,* Non-Bacterial Test			no data located
5.6	**Genetic Toxicity *in vivo***	**mouse**	**OECD 474, GLP**	**negative, no increase in micronucleated polychromatic erythrocytes**
5.7	**Carcinogenicity**	***Drosophila melanogaster***	**nonstandard test with flies**	**Isophytol lowered the incidence of melanotic tumours in flies genetically predisposed to such tumours.**
5.8.1	**Toxicity to Fertility**	**rat**	**OECD 415, GLP**	**NOEL = 500 mg/kg bw/d NOAEL = 500 mg/kg bw/d LOAEL = 1000 mg/kg bw/d Wistar Crl: (WI) BR (outbred, SPF) rats in a one-generation reproductive toxicity test, gavage, males exposed for 91–134 (mean: 98) days, females for 52–108 (mean: 64) days; LOAEL based on increased mean pre-coital time, decreased fertility index and decreased conception rate.**
5.8.2	**Developmental Toxicity/Teratogenicity**	**rat**	**OECD 415, GLP**	**NOEL = 500 mg/kg bw/d NOAEL = 500 mg/kg bw/d LOAEL = 1000 mg/kg bw/d Wistar Crl: (WI) BR (outbred, SPF) rats in a one-generation reproductive toxicity test, gavage, males exposed for 91–134 (mean: 98) days, females for 52–108 (mean: 64) days; NOEL based on increased number of dead pups at first litter check, increased incidence of clinical signs, decreased body weights and postnatal losses of pups.**
5.8.3	Toxicity to Reproduction, Other Studies			no data located
5.9	**Specific Investigations**	rat	effects on physiological activity in skeletal muscles	Isophytol had cytoprotective effects similar to vitamin E on excised muscles treated with a calcium ionophore.

		mouse	OECD 474, GLP	In the micronucleus test, there was no decrease in the ratio of polychromatic to normochromatic erythrocytes, which reflects a lack of toxic effects on erythropoiesis
		rat	enhancement of percutaneous absorption of indomethacin	1% isophytol in a gel enhanced the percutaneous absorption of indomethacin; there is no information on the absorption of isophytol itself
		man	competitive binding to retinol-binding protein	Retinol-binding protein (RBP) showed a high affinity to isophytol, which therefore is a potential inhibitor of RBP
		mouse	neurophysiolo-gical stimulation	Isophytol had no stimulating effect on the central nervous system as determined using a pentobarbital-induced sleep time model, whereas phytol had a stimulating effect.
		insects	juvenile hormone activity	In an overview article on insect juvenile hormone systems, isophytol had no juvenile hormone activity, whereas phytol had limited activity.
5.10	**Exposure Experience**	**man**	**occupational medical records**	**during more than 30 years of isophytol production at the Teranol Lalden plant, Switzerland, no effects on potentially exposed workers have been noticed**
NA = Not available.				

S I D S

Dossier

Existing Chemical	ID: 505-32-8
CAS No.	505-32-8
EINECS Name	3,7,11,15-tetramethylhexadec-1-en-3-ol
EC No.	208-008-8
TSCA Name	1-Hexadecen-3-ol, 3,7,11,15-tetramethyl-
Molecular Formula	C20H40O
Producer Related Part	
Company:	Hoffmann-La-Roche AG
Creation date:	15-FEB-2002
Substance Related Part	
Company:	Hoffmann-La-Roche AG
Creation date:	15-FEB-2002
Memo:	ICCA HPVC Programme IUCLID; correct company name is F. Hoffmann-La Roche Ltd, Basel
Printing date:	06-JAN-2006
Revision date:	
Date of last Update:	**06-JAN-2006**
Number of Pages:	**132**
Chapter (profile):	Chapter: 1, 2, 3, 4, 5, 6, 7, 8, 10
Reliability (profile):	Reliability: without reliability, 1, 2, 3, 4
Flags (profile):	Flags: without flag, confidential, non confidential, WGK (DE), TA-Luft (DE), Material Safety Dataset, Risk Assessment, Directive 67/548/EEC, SIDS

1.0.1 Applicant and Company Information

Type: sponsor country
Name: Switzerland
Contact Person: Dr Georg Karlaganis **Date:** 15-FEB-2002
Street: Swiss Agency for the Environment, Forests and Landscape
Town: CH-3003 Bern
Country: Switzerland
Phone: +41 313 226 955
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Email: georg.karlaganis@buwal.admin.ch
Homepage: http://www.umwelt-schweiz.ch/buwal/eng/index.html

Flag: Critical study for SIDS endpoint
13-NOV-2002

Type: lead organisation
Name: F.Hoffmann-La Roche AG
Contact Person: Dr Louis Schnurrenberger **Date:** 15-FEB-2002
Street: Corporate Safety & Environmental Protection, 49/2.046
Town: CH-4070 Basel
Country: Switzerland
Phone: +41 616 886 638
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Email: louis.schnurrenberger@roche.com
Homepage: http://www.roche.com

22-AUG-2002

Type: cooperating company
Name: BASF AG
Contact Person: Dr Hubert Lendle **Date:** 15-FEB-2002
Street: Karl-Bosch-Str
Town: 67056 Ludwigshafen
Country: Germany
Phone: +49 621 604 4712
Telefax: +49 621 605 8043
Email: hubert.lendle@basf-ag.de

22-AUG-2002

1.0.2 Location of Production Site, Importer or Formulator

Type: manufacturer
Name of Plant: Teranol AG, Lalden
Street: PO Box 310
Town: CH-3930 Visp
Country: Switzerland
Phone: +41 279 485 733
Telefax: +41 279 486 184

13-NOV-2002

1.0.3 Identity of Recipients

1.0.4 Details on Category/Template

1.1.0 Substance Identification

IUPAC Name: 1-Hexadecen-3-ol, 3,7,11,15-tetramethyl-
Smiles Code: C=CC(C)(O)CCCC(C)CCCC(C)CCCC(C)C
Mol. Formula: C20-H40-O
Mol. Weight: 296.52

Flag: Critical study for SIDS endpoint
07-AUG-2002 (22) (97)

1.1.1 General Substance Information

Purity type: typical for marketed substance
Substance type: organic
Physical status: liquid
Purity: >= 95 - % v/v
Colour: colourless, clear or nearly clear
Odour: none or weak odour

Remark: Isophytol is a technical-grade intermediate in synthesis, hence the specification gives a minimum purity of 95%. Isophytol is described as an oily liquid at room temperature
Reliability: (1) valid without restriction
Flag: Critical study for SIDS endpoint
25-JUL-2002 (97)

1.1.2 Spectra

Type of spectra: GC

07-AUG-2002 (20) (92)

Type of spectra: IR

07-AUG-2002 (40) (41)

Type of spectra: other: Gas-phase IR

07-AUG-2002 (79)

Type of spectra: mass spectrum

07-AUG-2002 (79)

1.2 Synonyms and Tradenames

3,7,11,15-Tetramethylhexadec-1-en-3-ol

Remark: This is also the INCI Name.
23-JUL-2002 (80)

2,6,10,14-Tetramethylhexadec-15-en-14-ol

23-JUL-2002 (47)

2,6,10-Trimethyl-14-vinylpentadecan-14-ol

23-JUL-2002 (47)

Iso-phytol

23-JUL-2002 (80)

1.3 Impurities

Purity type: other: manufacturer's specifications for Isophytol Technical Grade
CAS-No: 85761-30-4
EC-No: 288-573-5
EINECS-Name: 3,7,11,15-tetramethylhexadecan-3-ol
Mol. Formula: C20-H42-O
Contents: <= 2.5 - % v/v

Remark: alternative CAS number = 20685-73-8
07-AUG-2002 (93) (97)

Purity type: other: manufacturer's specifications for Isophytol Technical Grade
CAS-No: 502-69-2
EC-No: 207-950-7
EINECS-Name: 6,10,14-trimethylpentadecan-2-one
Mol. Formula: C18-H36-O
Contents: <= 2 - % v/v

Remark: alternative CAS number = 22571-87-5
07-AUG-2002 (93) (97)

Purity type: other: manufacturer's specifications for Isophytol Technical Grade
CAS-No: 29171-23-1
EC-No: 249-484-7
EINECS-Name: 3,7,11,15-tetramethylhexadec-1-yn-3-ol
Mol. Formula: C20-H38-O
Contents: <= .5 - % v/v

07-AUG-2002 (97)

Purity type: other: manufacturer's specifications for Isophytol Technical Grade
CAS-No: 72226-32-5
EINECS-Name: 6,10,14-trimethyl-3-pentadecen-2-one
Contents: <= .3 - % v/v

07-AUG-2002 (93) (97)

Purity type: other: manufacturer's specifications for Isophytol Technical Grade
CAS-No: 69729-17-5
EINECS-Name: 6,10,14-Trimethylpentadecan-2-ol
Mol. Formula: C18-H38-O
Contents: <= .3 - % v/v

07-AUG-2002 (93) (97)

Purity type: other: manufacturer's specifications for Isophytol Technical Grade
EINECS-Name: maximum of unknown impurities (sum)
Contents: < 2 - % v/v

07-AUG-2002 (97)

Purity type: measured for specific batch

Result:

Parameter	Lot UU01113408	Specification
Appearance	clear liquid	clear liquid
Colour	colourless	colourless
Purity (GC)	97.0 % (weight)	-
Purity (GC)	98.0	> 95.0 % (area)
Impurities:		
CAS 502-69-2	0.4	<= 2.0 % (area)
CAS 29171-23-1	< 0.1	<= 0.5 % (area)
CAS 85761-30-4	1.1	<= 2.5 % (area)
other impurities, sum	0.5	<= 2.0 % (area)

Reliability: (1) valid without restriction
07-AUG-2002 (102)

1.4 Additives

1.5 Total Quantity

Quantity: ca. 35000 - 40000 tonnes produced in 2002

Remark: worldwide estimate
Source: F. Hoffmann-La Roche Ltd
16-JUL-2002

1.6.1 Labelling

1.6.2 Classification

1.6.3 Packaging

1.7 Use Pattern

Type: industrial
Category: Chemical industry: used in synthesis

Remark: Most of the isophytol produced (estimate >> 99%) is being used as an intermediate in the synthesis of two vitamins with phytyl chains. Approx. 99% of the above share is used for Vitamin E synthesis and <1% for Vitamin K synthesis.
07-AUG-2002 (49)

Type: use

Category: Cosmetics

Remark: A relatively small amount of isophytol (estimate <= 0.1% of total production) is used as such in fragrance mixtures in perfumes and cosmetics.
Concentrations in final cosmetics and personal care products are estimated at <= 0.2% in case of perfumes and at 0.02% in shampoos, lotions, creams etc.
Cosmetics containing isophytol as an ingredient of up to 5% are stated to remain stable and not to lose their moisture after prolonged storage (Japanese patent).

07-AUG-2002 (49) (64) (82)

Type: use
Category: Food/foodstuff additives

Remark: A very small amount of isophytol (estimate << 0.001% of total
production) may be used as an EU-approved flavouring substance in prepared foods. Isophytol is not food-approved in the USA.
In spite of the approved use in Europe, actual use is unknown but estimated by the flavours and fragrances industry as minuscule. No final concentrations in customer products have been located nor can they be reasonably extrapolated.

07-AUG-2002 (32) (49) (82)

1.7.1 Detailed Use Pattern

Industry category: 3 Chemical industry: chemicals used in synthesis
Use category: 41 Pharmaceuticals
Extra details on use category: No extra details necessary
No extra details necessary
Emission scenario document: not available
Fract. of tonnage for application: .999
Fract. of chemical in formulation: 1
Production: yes

Remark: used as an intermediate in the synthesis of both Vitamin E and Vitamin K

07-AUG-2002 (49)

Industry category: 5 Personal / domestic use
Use category: 15 Cosmetics
Extra details on use category: No extra details necessary
No extra details necessary
Emission scenario document: not available
Fract. of tonnage for application: .0009
Fract. of chemical in formulation: 1
Formulation: yes
Private use: yes

Remark: << 1% of total production is used as such in fragrance mixtures in perfumes and cosmetics

07-AUG-2002 (49)

Industry category: 5 Personal / domestic use

Use category: 26 Food/feedstuff additives
Extra details on use category: No extra details necessary
No extra details necessary
Emission scenario document: not available
Fract. of tonnage for application: .0001
Fract. of chemical in formulation: 1
Formulation: yes
Private use: yes

Remark: A very small amount of isophytol (estimate << 1% of total production) may be used as an EU-approved flavouring substance in prepared foods.
Reliability: (2) valid with restrictions
16-JUL-2002 (32) (49)

1.7.2 Methods of Manufacture

Orig. of Subst.: Synthesis
Type: Production

Result: Total chemical synthesis of isophytol may start from the addition of acetylene (CAS 74-86-2) to acetone (67-64-1) resulting in 3-methyl-1-butyn-3-ol (115-19-5), which is hydrated in the presence of a palladium catalyst to 3-methyl-1-buten-3-ol (115-18-4), which is reacted with either diketene or acetic acid ester to the acetoacetate and the latter thermally reacted to 2-methyl-2-hepten-6-one (110-93-0).
Alternatively, 3-methyl-1-buten-3-ol is reacted with isopropenyl methyl ether (116-11-0) to 2-methyl-2-hepten-6-one.
In a third synthetic pathway, isoprene hydrochloride is reacted with acetone in the presence of an alkaline condensating agent or in the presence of organic bases as catalysts to 2-methyl-2-hepten-6-one.
2-Methyl-2-hepten-6-one is then reacted with acetylene to dehydrolinalool (29171-20-8), to which isopropenyl methyl ether is added to make pseudoionone (141-10-6). The three double bonds are hydrated to form 6,10-dimethyl-2-undecanone (1604-34-8) which is reacted with acetylene to 3,7,11-trimethyl-1-dodecyn-3-ol (1604-35-9). Isopropenyl methyl ether is added to form 6,10-14-trimethyl-4,5-pentadecadiene-2-one (16647-10-2), which is hydrated to hexahydrofarnesyl acetone (502-69-2). This is again reacted with acetylene to 3,7,11,15-tetramethyl-1-hexadecyn-3-ol (dehydroisophytol, 29171-23-1), which is finally hydrated to isophytol.
The repeated addition of acetylene and hydration was first described by Fischer and Löwenberg (1929).
Alternatively, pseudoionone (141-10-6) may be hydrogenated to hexahydropseudoionone (1604-34-8), which is reacted with propargyl alcohol (107-19-7) to give 4,8,12-trimethyltridec-2-yn-1,4-diol (93190-74-0). The tertiary hydroxy group of this glycol is dehydrated with fused potassium hydrogen sulfate to the intermediate 4,8,12-trimethyltridec-2-yn-4-en-1-ol (93157-88-1), which (due to its instability) must be swiftly hydrogenated without prior isolation to 4,8,12-trimethyltridecan-1-ol (61973-86-2). The latter may be converted to its bromide

(88591-64-4) or chloride (88591-66-6) using phosphorus tribromide (7789-60-8) or thionyl chloride (7719-09-7), respectively. Reacting a metallic compound of this 1-halo-4,8,12-trimethyltridecane with methyl vinyl ketone (78-94-4) results in isophytol. The latter reaction is critical due to the tendency of polymerisation of the ketone (Sato et al., 1963).

03-JAN-2003 (49) (50) (91)

Orig. of Subst.: Natural origin
Type: other: abiotic formation from phytol

Result: The Merck Index states for isophytol that it is a "decomposition product of chlorophyll". Chlorophylls have a phytyl propionate side chain, hence this statement is not implausible, however, no original reference for the statement
is given. Also, no evidence for isomerisation of phytol to isophytol is presented. On the other hand, if isophytol is a decomposition product of chlorophyll indeed, then it must be formed, at least as a short-lived transitory substance, in huge amounts.

Several authors (mainly de Leeuw et al., 1977; see chapter 3.8 for more details) have shown that isophytol may indeed be formed from phytol, in both anaerobic and aerobic, marine and freshwater sediments. However, this isophytol is but a relatively short-lived, transitory intermediate in the abiotic diagenetic conversion of chlorophyll-derived phytol to, eventually, insoluble, high-molecular-weight organic compounds named kerogen, which in turn may be converted to petroleum under high temperature and pressure.

Rontani et al. (1999) found only a very small formation (0.21%) of isophytol from phytol in incubation experiments with bacteria from anaerobic marine sediments; they suggested that a minor enzymatic pathway existed for this isomerisation.

No information has been located regarding the formation of isophytol in other environmental compartments nor on concentrations respectively amounts of isophytol present at any one time in sediments.

Conclusion: Isophytol is formed abiotically from chlorophyll-derived phytol in marine and freshwater, anoxic and aerobic sediments, but only as a transitory intermediate that is completely converted in turn. No information has been located regarding the formation of isophytol in other environmental compartments.

07-AUG-2002 (27) (37) (86)

1.8 Regulatory Measures

Legal Basis: other: UN Joint FAO/WHO Expert Committee on Food Additives
Type of Meas.: ADI

Remark: Based on the title of the document referring to total terpenoid alcohols and the chemical structure of isophytol, the ADI may encompass isophytol; however, it is not

explicitly listed. Hence this ADI may not be binding.
Result: The ADI for total terpenoid alcohols in food is 0-0.5 mg/kg bw/d
07-AUG-2002 (60)

1.8.1 Occupational Exposure Limit Values

Type of limit: other: no occupational exposure limits, official or company-internal, have been located

Source: literature search
02-APR-2002

1.8.2 Acceptable Residues Levels

1.8.3 Water Pollution

Classified by: other: VwVwS of May 17, 1999 (German Directive on Substances Hazardous for Water)
Labelled by: other: officially accepted own classification according to annex 3 of VwVwS
Class of danger: 1 (weakly water polluting)

Remark: Isophytol, CAS 505-32-8 is listed as WGK 1 under substance no. 6478.
25-JUL-2002 (52)

1.8.4 Major Accident Hazards

1.8.5 Air Pollution

1.8.6 Listings e.g. Chemical Inventories

Type: OECD
Additional Info: OECD Representative List of High Production Volume Chemicals

23-JUL-2002 (93)

Type: AICS
Additional Info: Australian Inventory of Chemical Substances, June 1996 ed.

06-JAN-2006 (93)

Type: CHINA
Additional Info: Chinese Inventory of Existing Chemical Substances

23-JUL-2002 (30)

Type: DSL
Additional Info: Domestic Substances List, Supplement to Canada Gazette, part I, Jan 26, 1991

23-JUL-2002 (93)

Type: ECL
Additional Info: Korean Existing Chemicals List, Jan 1997, Serial no. KE-33599

23-JUL-2002 (93)

Type: EINECS
Additional Info: Annex, Official Journal of the European Communities, 15 Jun 1990; EINECS no. 2080088

07-AUG-2002 (44)

Type: ENCS
Additional Info: Japanese Existing and New Chemical Substances inventory, ENCS no. 2-258X

23-JUL-2002 (93)

Type: PICCS
Additional Info: Philippines Inventory of Chemicals and Chemical Substances, 2000

23-JUL-2002 (93)

Type: Poisonous Chemicals List (Switzerland)
Additional Info: Giftliste 1, 31 May 1999; toxic category 4

23-JUL-2002 (93)

Type: TSCA
Additional Info: US TSCA Jan 2002 Inventory Tape

23-JUL-2002 (59) (93)

Type: other: EU Inventory of ingredients employed in cosmetics products
Additional Info: Part II: perfume and aromatic raw materials; INCI Name: 3,7,11,15-tetramethylhexadec-1-en-3-ol

23-JUL-2002 (31)

Type: other: EU Register of flavouring substances used in or on foodstuffs
Additional Info: Isophytol, CoE no. 10233

23-JUL-2002 (32) (33)

1.9.1 Degradation/Transformation Products

1.9.2 Components

1.10 Source of Exposure

1.11 Additional Remarks

Memo: Natural occurrence (analytically confirmed)

Result: Isophytol has been identified in a number of volatile oils or extracts from plants. The following list is illustrative but not exhaustive.

Species	Family	Common name
Amaranthus mangostanus	Amaranthaceae	-
Anthemis nobilis	Asteraceae	Roman chamomile
Basella rubra	Basellaceae	Malabar spinach
Chamomilla recutita (syn. Matricaria chamomilla)	Asteraceae	German chamomile
Cistus salvifolius	Cistaceae	sage-leaf rockrose
Citrus hystrix	Rutaceae	swangi, Kaffir lime
Cochlospermum planchonii	Cochlospermaceae	-
Cochlospermum tinctorium	Cochlospermaceae	-
Daphne genkwa	Thymelaeaceae	(Chinese) daphne
Ficus carica	Moraceae	
Foeniculum vulgare	Apiaceae	(sweet) fennel
Hordeum vulgare	Gramineae	barley
Ipomoea aquatica	Convolvulaceae	water spinach
Ixeris dentata	Asteraceae	hananigana
Jasmin(i)um off. grandiflorum	Oleaceae	(Egyptian) jasmine
Jasminum sambac	Oleaceae	(sambac) jasmine
Jumellea fragrans	Orchidaceae	faham orchid
Leea guineensis	Leeaceae	-
Lotus garcinii	Fabaceae	-
Narcissus sp.	Amaryllidaceae	daffodil
Vitex cymosa	Verbenaceae	taruma guazu
Vitex polygama	Verbenaceae	taruma
Xylopia aromatica	Annonaceae	malagueta brava
Zostera marina	Zosteraceae	eelgrass
-		
Laurencia pinnatifida	Rhodophyta	(red alga)
Plocamium costatum	Rhodophyta	(red alga)
Polysiphonia denudata	Rhodophyta	(red alga)

Conclusion: The systematically broad occurrence of isophytol, from red algae to dicotyledonean and monocotyledonean flowering plants suggests that isophytol is a common, ubiquitous compound in plant biochemistry that may have a long evolutionary history.

07-AUG-2002 (1) (2) (23) (36) (39) (40) (41) (55) (61) (62) (63) (65) (66) (67) (70) (74) (78) (81) (84) (90) (94) (95) (99) (103) (106) (109)

Memo: Natural occurrence (made likely)

Result: The presence of isophytol is likely based on circumstantial data, but not analytically confirmed for the following plants:

Species	Family	Common name
Oryza sativa	Gramineae	Rice

07-AUG-2002 (85)

Memo: Natural occurrence (decomposition product of chlorophyll)

Remark: See detailed discussion in chapter 1.7.2, Methods of

Manufacture

Conclusion: No published evidence for ubiquitous formation of isophytol through degradation of chlorophyll or phytol derived from the former under aerobic conditions has been located, in disagreement with the corresponding remark in the Merck Index. However, there is good evidence for a minor degradative pathway of phytol under anaerobic conditions in sediment, resulting in isophytol, both in marine and freshwater. However, this isophytol is only a transient, relatively short-lived intermediate in the diagenetic conversion of (chlorophyll-derived) phytol to kerogen, high-molecular-weight organic carbon that is bound in sediment or rock.

No quantitative determinations are available, nor is an extrapolation to global volumes possible.

07-AUG-2002

1.12 Last Literature Search

Type of Search: Internal and External
Date of Search: 12-DEC-2002

Remark: general search covering all chapters
21-FEB-2003

1.13 Reviews

Memo: HSDB: Isophytol, CASRN 505-32-8

07-AUG-2002 (56)

2.1 Melting Point

Value: <= -20 degree C

Method: other: no data
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Remark: Internal database, data at least 30 years old, acquired by company-internal physico-chemical properties laboratory. No information on method used is available, but data from this database are used and trusted within the company.
Result: Solidification is described as increasing viscosity at -20
Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
06-JAN-2006 (49)

2.2 Boiling Point

Value: = 313 degree C at 1013 hPa

Method: other: method not stated
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Remark: Internal database, data at least 30 years old, acquired by company-internal physico-chemical properties laboratory. No information on method used is available, but data from this database are used and trusted within the company.
Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
06-JAN-2006 (48)

Value: = 335 degree C at 1013 hPa

27-DEC-1993 (19)

2.3 Density

Type: density
Value: = .8458 g/cm³ at 20 degree C

Method: other: double-capillary pycnometer
Year: 1988
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Method: The measurement of liquid density was carried out in multiplicate by using a double-capillary pycnometer calibrated with double-distilled de-gassed water. The method error as determined by measurements of acetone and of methanol was 0.02%.
Result:

Temperature, °C	Density, g/cm3
20.0	0.8458
30.0	0.8378

	40.0	0.8305
	50.0	0.8228
	60.0	0.8160
	70.0	0.8076

Test substance: The purified isophytol used for the present determination was determined to have a purity of 98.74 mol-%.

Reliability: (2) valid with restrictions
Although this was not a study under GLP or similar conditions, both preparation and careful purification of samples are described, experimental methods are briefly but concisely presented, these methods are validated against literature data and the calibration results are presented. Experimental data are listed in full. Based on these ample descriptions and internal quality control data, a reliability of 2 is assigned.

Flag: Critical study for SIDS endpoint

09-JAN-2003 (3)

Type: density
Value: = .8483 g/cm³ at 20 degree C

Method: other: method not stated
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Reliability: (4) not assignable

25-FEB-2002 (48)

Type: density
Value: = .5788 g/cm³ at 313 degree C

Method: other: method not stated
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Reliability: (4) not assignable

25-FEB-2002 (48)

Type: density
Value: .837 - .847 g/cm³ at 20 degree C

Method: other: method not stated
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Source: BASF AG Ludwigshafen
Reliability: (4) not assignable

22-FEB-2002 (19)

Type: relative density
Value: = .8439 g/cm³ at 21 degree C

Method: other: no data
Year: 1958
GLP: no

Result:

Test substance	Temperature	Relative density vs 4 °C
1, synthetic	21 °C	0.8439
2, natural extract	24.5 °C	0.8442

Test substance: Test substance 1: synthetic isophytol obtained from Light &

Co., UK, purified using column chromatography over silicic acid.
Test substance 2: natural isophytol isolated from concrete of jasmin through distillation and thin-layer chromatography.

Reliability: (4) not assignable
25-JUL-2002 (41)

2.3.1 Granulometry

2.4 Vapour Pressure

Value: = .00002982 hPa at 20 degree C

Method: other (measured): no data on method used
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Remark: Internal database, data at least 30 years old, acquired by company-internal physico-chemical properties laboratory. No information on method used is available, but data from this database are used and trusted within the company.
Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
06-JAN-2006 (48)

Method: other (measured)
Year: 1988
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Method: Vapour pressure was measured by a static method as cited [Baglay et al (1984): Khim.-Farm. Zh. 18: 1013 ff, in Russian] with a glass membrane as a null manometer. Nonvolatile compounds were introduced into the membrane camera immediately. The tensimeter was embedded in an oil or LiCl-water-solution thermostat, which allows the measurement of the temperature using a mercury thermometer with an error of ±0.1 K. Pressure was measured with a cup mercury manometer with an accuracy of ±13.3 Pa.

Result: Experimental vapour pressures are given for the range of 166.2-195.6 °C (in the original 439.35-468.75 K):

Temperature, K	°C	Vapour pressure, hPa
439.35	166.2	7.3
442.35	169.2	7.5
442.45?	169.3?	8.4?
446.45	173.3	9.5
448.35	175.2	11.0
451.05	177.9	11.5
454.55	181.4	13.1
456.65	183.5	14.7
458.35	185.2	15.3
459.95	186.8	16.5
463.95	190.8	19.3
468.75	195.6	22.0

Note. The value of 442.45 K (= 169.3 °C) may be a printing error as it is very close to the preceding temperature and

might possibly read correctly 444.45 (= 171.3 °C), whereby the indicated vapour pressure of 8.4 hPa would fit better into the series.

Test substance: Commercial isophytol was purified by drying over Na2SO4, MgSO4; K2CO3 and CaCl2, rectified through columns with efficiency equal to 50 theoretical column trays and multistage-fractionally-distilled at residual pressure varying from 6.7 to 67 Pa.
The output content of the product was determined by area normalisation of gas-liquid chromatography curves. The purified isophytol used for the present determination was determined to have a purity of 98.74 mol-%.

Reliability: (2) valid with restrictions
Although this was not a study under GLP or similar conditions, both preparation and careful purification of samples are described, experimental methods are briefly but concisely presented, these methods are validated against literature data and the calibration results are presented. Experimental data are listed in full. Based on these ample descriptions and internal quality control data, a reliability of 2 is assigned.

02-APR-2002 (3)

Value: = .002 hPa at 60 degree C

Source: BASF AG Ludwigshafen
Reliability: (4) not assignable
02-APR-2002 (19)

Value: = .07 hPa at 100 degree C

Source: BASF AG Ludwigshafen
Reliability: (4) not assignable
02-APR-2002 (19)

2.5 Partition Coefficient

Partition Coeff.: octanol-water
log Pow: > 6 at 35 degree C

Method: OECD Guide-line 117 "Partition Coefficient (n-octanol/water), HPLC Method"
Year: 1999
GLP: yes

Method: HPLC
A Hewlett-Packard HPLC Series 1050 comprising an autosampler, a high-pressure pump, a refractive index detector and a Chemstation microcomputer-based integrator and system controller was used. Technical details:

Mobile phase	acetonitrile/water 60/40, v/v
Acetonitrile	HPLC grade (Mächler AG, Reinach, Switzerland)
Water	double-distilled water
Column	250 x 4 mm, packed with Nucleosil 120-5 C18, 5 µm (Macherey-Nagel, Düren, Germany)

Flow rate 1.2 ml/min
Column temperature 35 °C
Detector temperature 30 °C
Reference substances of known retention time and logPow
Thiourea >=98%, 1.6 g/l (Merck, Darmstadt, Germany)
Aniline >=99.5%, 2.0 g/l (Merck, Darmstadt, Germany)
Methyl benzoate >=98%, 1.6 g/l (Givaudan-Roure, Vernier)
Benzophenone >=99%, 2.0 g/l (Merck, Darmstadt, Germany)
Naphthalene >=99%, 2.0 g/l (Merck, Darmstadt, Germany)
1,2,4-Trichloro-benzene >=98%, 4.0 g/l (Merck, Darmstadt, Germany)
n-Butylbenzene >=98%, 4.0 g/l (Merck, Darmstadt, Germany)
Triphenylamine >=99%, 4.0 g/l (Merck, Darmstadt, Germany)
Procedure
On the day of determination a calibration mixture was prepared by mixing 1 ml of each of the individual solutions and adding double-distilled water (5.3 ml) to obtain a solvent composition similar to the mobile phase. A solution of the test substance isophytol was prepared in the mobile phase (35.1 mg in 50 ml). After equilibration of the HPLC system, the calibration mixture was injected first, followed by the test substance solution twice and the calibration mixture again. Retention times of the reference substances were measured and averaged and the decimal logarithms of the capacity factors were calculated. The logPow of the test substance would be calculated based on the average retention time in comparison with the retention times of calibration substances.

Result: No peak corresponding to isophytol could be detected up to a retention time of 45 min, corresponding to a logPow of 7.1. Therefore, as this method is accepted as valid for logPow values up to 6.0 only, the logPow of isophytol is > 6.0.

Test substance: Isophytol from Teranol AG, Lalden/Visp, Switzerland, lot no. 9000337967, purity 97.6% (GC).

Conclusion: The logPow of isophytol is > 6.0 (upper validity limit of the HPLC method).

Reliability: (1) valid without restriction
OEDC guideline test performed under GLP, reliability 1 assigned.

Flag: Critical study for SIDS endpoint

28-FEB-2002 (88)

Partition Coeff.: octanol-water
log Pow: = 8.8

Method: OECD Guide-line 117 "Partition Coefficient (n-octanol/water), HPLC Method"

GLP: no data

Reliability: (4) not assignable

28-FEB-2002 (13)

Partition Coeff.: octanol-water

Method: other (calculated): QSAR estimate
Year: 2002
GLP: no

Result:

logPow	QSAR method	Reference	Reference no.
7.20	ALOGPS	LogP	69
7.42	XLOGP	LogP	69
7.74	SPARC	SPARC	96
8.04	IA logP	LogP	69
8.23	KOWWIN	EPISUITE	45
8.26	CLOGP	LogP	69
8.284±0.244	ACD	SciFinder	93

Flag: Critical study for SIDS endpoint
07-AUG-2002 (45) (69) (93) (96)

Partition Coeff.: octanol-water
log Pow: = 8.579

Method: other (calculated): Inkrementenmethode von Rekker mit Computerprogramm der Firma CompuDrug Ltd.
Year: 1988
GLP: no

07-AUG-2002 (14) (15)

Partition Coeff.: octanol-water
log Pow: = 9.1

Method: other (calculated)
Year: 1991
GLP: no data

Method: calculated using the Rekker method
07-AUG-2002 (25)

Partition Coeff.: water - air

Method: other (calculated): QSAR estimate
Year: 2002
GLP: no

Result:

Henry constant KH	QSAR method
1.14E-06 atm*m3/mol	EPISUITE (QSAR vap press/exp sol)
1.50E-06 atm*m3/mol	USES
1.51E-06 atm*m3/mol	Level III (value = 0.152 Pa*m3/mol)
1.09E-04 atm*m3/mol	SPARC
6.92E-04 atm*m3/mol	EPISUITE (bond estimate)

07-AUG-2002 (45) (68) (96) (108)

Partition Coeff.: soil-water

Method: other (calculated): QSAR estimate
Year: 2002
GLP: no

Result:

Koc	QSAR method
1.978E04	EPISUITE
1.690E07	USES

4.91 E07 Level III
Reliability: (4) not assignable
28-FEB-2002 (45) (68) (108)

2.6.1 Solubility in different media

Solubility in: Water
Value: = .0058 g/l at 25 degree C
pH value: = 6.7
Conc.: .0058 g/l at 25 degree C

Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
06-JAN-2006 (19)

Solubility in: Organic Solvents
Descr.: miscible

Result: miscible in benzene, ethanol, ether and other common organic solvents
Reliability: (4) not assignable
26-FEB-2002 (49) (80)

pKa: 18.4 at 25 degree C

Method: other: QSAR estimate
Year: 2002
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Conclusion: Based on a QSAR pKa of 18.4, isophytol will be present in aqueous solutions as a non-ionised substance at all environmentally and physiologically relevant pH values.
07-AUG-2002 (96)

2.6.2 Surface Tension

Test type: other: capillary method
Value: = 28.47 mN/m at 20 degree C
Concentration: 98.74 other: mol-%

Year: 1988
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Method: The liquid-gas surface tension was measured by the capillary method as described by Baglay et al. [1984: Khim.-Farm. Zh. 18: 1013 ff, in Russian] and Adamson [1979: Physical chemistry of surfaces. Mir, Moscow, in Russian]. The level of liquid in the capillary was determined by a V-630 type cathetometer with an accuracy of ±5x10E-6 m. The relative error of the surface tension experimental data determined from water, toluene and n-octane was <0.5%.
Result:

Temperature, °C	Surface tension, mN/m
20.0	28.47
30.0	28.04
40.0	27.33

50.0 26.45
60.0 25.56
70.0 24.84

Test substance: Commercial isophytol was purified by drying over NaSO4, MgSO4, K2CO3 and CaCl2, rectified through columns with efficiency equal to 50 theoretical column trays and multistage-fractionally-distilled at residual pressure varying from 6.7 to 67 Pa.
The output content of the product was determined by area normalisation of gas-liquid chromatography curves. The purified isophytol used for the present determination was determined to have a purity of 98.74 mol-%.

Reliability: (2) valid with restrictions
Although this was not a study under GLP or similar conditions, both preparation and careful purification of samples are described, experimental methods are briefly but concisely presented, these methods are validated against literature data and the calibration results are presented. Experimental data are listed in full. Based on these ample descriptions and internal quality control data, a reliability of 2 is assigned.

Flag: Critical study for SIDS endpoint

27-MAR-2002 (3)

Test type: other: no data
Value: = 29.86 mN/m at 20 degree C

Method: other: method not stated
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Reliability: (4) not assignable

25-FEB-2002 (48)

Test type: other: no data
Value: = 8.86 mN/m at 313 degree C

Method: other: method not stated
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Reliability: (4) not assignable

25-FEB-2002 (48)

Test type: other: trapping of daphnids at surface during immobilisation test
Concentration: 17 other: µg/l average measured concentration

Year: 2002
Test substance: as prescribed by 1.1 - 1.4

Result: In the final semi-static daphnid immobilisation test with medium exchange after 24 hours it showed that at all test substance concentrations daphnids tended to become trapped at the surface; however, these were first re-submerged before checking on their mobility, which resulted in clearly diminished immobility. In contrast, no single daphnia became surface-trapped in the blank control. This suggests that isophytol has appreciable surface activity even at low concentrations (17 µg/l average measured concentration

resulted in 14/20 daphnids being trapped at 48 hours).
Test substance: Isophytol from Teranol Lalden, batch no. UU02013601, purity 97.5% (GC).
Reliability: (4) not assignable
27-DEC-2002

2.7 Flash Point

Value: = 135 degree C
Type: closed cup

Method: other: DIN 51 758
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Reliability: (4) not assignable
21-FEB-2002 (19)

Value: = 169 degree C
Type: other: no data

Method: other: method not stated
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Reliability: (4) not assignable
07-AUG-2002 (48)

2.8 Auto Flammability

Value: = 225 degree C

Method: other: DIN 51 758
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Reliability: (4) not assignable
07-AUG-2002 (19)

2.9 Flammability

2.10 Explosive Properties

Result: other: explosion limits

Method: other: no data
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Remark: explosion limits in air: 0.3-3.5 % v/v
Reliability: (4) not assignable
07-AUG-2002 (19)

2.11 Oxidizing Properties

2.12 Dissociation Constant

Acid-base Const.: pKa = 18.4

Method: other: QSAR estimate
Year: 2002
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Remark: temperature for estimate = 25 °C
Conclusion: Isophytol in aqueous solutions will not be dissociated at any environmentally relevant pH.
07-AUG-2002 (96)

2.13 Viscosity

Test type: other: no data
Test procedure: no data
Value: = 72.76 mPa s (dynamic) at 20 degree C

Method: other: method not stated
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Reliability: (4) not assignable
20-FEB-2002 (48)

Test type: other: no data
Test procedure: no data
Value: = .11698 mPa s (dynamic) at 313 degree C

Method: other: method not stated
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Reliability: (4) not assignable
20-FEB-2002 (48)

2.14 Additional Remarks

Memo: Refraction index

Method: no data
Result: refraction index (20 °C) = 1.4562
Test substance: Commercial isophytol was purified by drying over NaSO4, MgSO4, K2CO3 and CaCl2, rectified through columns with efficiency equal to 50 theoretical column trays and multistage-fractionally-distilled at residual pressure varying from 6.7 to 67 Pa.
The output content of the product was determined by area normalisation of gas-liquid chromatography curves. The purified isophytol used for the present determination was determined to have a purity of 98.74 mol-%.
Reliability: (4) not assignable

23-JUL-2002 (3)

Memo: Refraction index

Method: no data
Result:

Test substance	Temperature	Refraction index
1, synthetic	20 °C	1.4570
2, natural extract	25 °C	1.4540

Test substance: Test substance 1: synthetic isophytol obtained from Light & Co., UK, purified using column chromatography over silicic acid.
Test substance 2: natural isophytol isolated from concrete of jasmin through distillation and thin-layer chromatography.
Reliability: (4) not assignable
10-APR-2002 (41)

Memo: Optical rotation

Method: no data
Result:

Test substance	Optical rotation alpha(D), without solvent
1, synthetic	± 0°
2, natural extract	+ 7.23°

Test substance: Test substance 1: synthetic isophytol obtained from Light & Co., UK, purified using column chromatography over silicic acid.
Test substance 2: natural isophytol isolated from concrete of jasmin through distillation and thin-layer chromatography.
Reliability: (4) not assignable
10-APR-2002 (41)

Memo: Stability

Result: Based on safety laboratory tests, isophytol is stable under normal conditions, However, it is susceptible to degradation or reaction in the presence of oxidising agents or acids. Further, it is thermically stable up to 200 °C.
Reliability: (2) valid with restrictions
17-APR-2002 (49)

Memo: Hazardous reactions

Remark: Gefährliche Reaktionen: Exotherme Reaktion mit Säuren.
(Hazardous reactions: exothermic reactions with acids)
Source: BASF AG Ludwigshafen
Reliability: (4) not assignable
17-APR-2002 (19)

3.1.1 Photodegradation

Type: other: photodegradation in synthetic seawater in the presence of a photosensitiser
Light source: Sun light
Rel. intensity: 1 based on Intensity of Sunlight
Conc. of subst.: .56 mg/l
INDIRECT PHOTOLYSIS
Sensitizer: other: anthraquinone
Deg. products: yes
1604-34-8 216-509-8 6,10-dimethylundecan-2-one
928-68-7 213-179-7 6-methylheptan-2-one

Year: 1988
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: 100 µl of substrate (isophytol and phytol, respectively) plus "a few microlitres of an acetone solution of anthraquinone (Fluka, purum)" were added to 150 ml of synthetic seawater in a Pyrex flask, yielding a substrate concentration of approximately 0.56 mg/l using a density of 0.8458 kg/l for isophytol. Flasks were then irradiated by natural sunlight [in Japan] for 3 weeks. As controls, identical flasks were kept in the dark for the same time. No information is given on light intensity or temperature. After 3 weeks, the medium was extracted with chloroform (water:chloroform ratio 2:1 v/v), chloroform extracts were dried on calcium chloride, filtered and concentrated. Compounds in the concentrates were identified by GC and GC-MS (details given in paper).
Result: Whereas phytol was "considerably" respectively "almost totally degraded" after three weeks, with 4 identified metabolites, "degradation of isophytol has just commenced" after 3 weeks. Degradation was not quantified.
The only two metabolites of isophytol showing in the GC spectra are 6,10-dimethylundecan-2-one and 6-methylheptan-2-one, both evidencing removal of the terminal allylic carbon in 1-position, the methyl group in 3-position with concomitant formation of a carbonyl group from the hydroxy function and single or double removal of a terminal 2-methyl-butyl group from the opposite end of the original isophytol molecule.
Test substance: "Isophytol from Tokyo Chemical Industry", no other information
Conclusion: In seawater isophytol is shown to degrade very slowly by indirect photodegradation in the presence of photosensitisers.
Reliability: (4) not assignable
25-JUL-2002 (87)

Type: other: irradiation of jasmin absolute oil
Light source: other: High-Pressure Mercury Lamp and Low-Pressure Mercury Lamp

Method: other (measured)
Year: 1988
GLP: no
Test substance: other TS: jasmin absolute oil containing 8.47% (GC peak area)

isophytol beside more than 60 other identified compounds

Method: Natural jasmin absolute oil containing 8.47% (GC peak area) isophytol beside more than 60 other identified and quantified compounds was irradiated under otherwise undefined High-Pressure Mercury Lamps (HPML) and Low-Pressure Mercury Lamps (LPML) for an unspecified time under a nitrogen stream. Both the native oil and the irradiated oils were analysed by GC (details given in paper).

Result: While native jasmine oil contained 8.47% (GC, area-%) isophytol, LPML-irradiated oil contained 12.65% and HPML-irradiated oil contained 15.15%.
Most other identified ingredients decreased on irradiation, however, parallel increases subsequent to both LPML and HPML irradiation were found for 3 other compounds and increases subsequent to either LPML or HPML, but not to both, were found for 4 other ingredients.

Conclusion: Isophytol may be formed from unidentified other ingredients in natural jasmine absolute oil under irradiation.
In case isophytol itself should be unstable under irradiation, the light-induced formation of isophytol from other precursors has a higher rate than the degradation in the case of the jasmine oil composition. Hence isophytol is regarded as relatively stable to photodegradation.

Reliability: (4) not assignable
07-MAY-2002 (103)

3.1.2 Stability in Water

3.1.3 Stability in Soil

3.2.1 Monitoring Data (Environment)

Type of measurement: other

Remark: no data available
Source: Literature search
19-JUL-2002

3.2.2 Field Studies

3.3.1 Transport between Environmental Compartments

Type: fugacity model level I
Media: other: air-water-soil(-biota-sediment-suspended sediment)
Method: other: Mackay EQC model v1.0
Year: 2002

Method: Input basic data

Molecular mass	297	g/mol
Melting point	-20	°C
Vapour pressure	0.0022	Pa
Solubility (water)	5.8	g/m3

	logPow	8.078	
	(amount for Level I: 100,000		kg)
Result:	Air	0.00002	%
	Water	0.0003	%
	Soil	97.8	%
	Sediment	2.172	%
	Suspended sediment	0.068	%
	Biota (fish)	0.00552	%
Flag:	Critical study for SIDS endpoint		

07-AUG-2002 (72)

3.3.2 Distribution

Media: air - biota - sediment(s) - soil - water
Method: Calculation according Mackay, Level III
Year: 2002

Result:

Compartment	Mass, %
Air	0.131
Water	3.51
Soil	27.2
Sediment	69.1

(1000 kg/h each to air, water and soil)
Persistence 1590 h (EPISUITE v3.10)
Per cent reacted 90.1 (EPISUITE v3.10)

Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint

06-JAN-2006 (45)

Media: air - biota - sediment(s) - soil - water
Method: Calculation according Mackay, Level III
Year: 2002

Result:

Compartment	Mass, %
Air	0.04
Water	4.3
Soil	29.9
Sediment	65.7

(1000 kg/h each to air, water and soil)
Persistence 1473 h (Level III Model v2.65)

Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint

06-JAN-2006 (68)

3.4 Mode of Degradation in Actual Use

3.5 Biodegradation

Type: aerobic
Inoculum: other bacteria: activated sludge from a biological wastewater treatment plant (Aïre, City of Geneva, Switzerland) receiving predominantly domestic sewage, with some industrial wastewater
Concentration: 102 mg/l related to Test substance
Contact time: 29 day(s)
Degradation: = 62 % after 29 day(s)
Result: other: inherently biodegradable, missed ready biodegradability

because of 10-day-window criterium
Kinetic: 7 day(s) = 13 %
14 day(s) = 38 %
21 day(s) = 51 %
28 day(s) = 60 %
29 day(s) = 62 %
Control Subst.: Benzoic acid, sodium salt
Kinetic: 3 day(s) ca. 50 %
20 day(s) ca. 90 %
Deg. product: not measured

Method: OECD Guide-line 301 F "Ready Biodegradability: Manometric Respirometry Test"
Year: 1999
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Method: The test was run in a respirometer, model Sapromat D1 by JM Voith GmbH, Heidenheim, Germany.
All water used for the study was deionised.
Stock mineral solutions were made up according to the OECD Guideline 301F and added to the water in the correct amounts to make the test medium, the pH of which was measured and adjusted if necessary using phosphoric acid or potassium hydroxide.
As the inoculum, fresh activated sludge from the predominantly domestic sewage works of Aïre (City of Geneva, Switzerland) was collected in the morning, washed three times in the mineral medium with centrifugation at 1000 g for 10 min, discarding the supernatant and re-suspension in mineral medium. The washed sludge was kept under aerobic conditions until use in the test the same day. Two samples of known volume of suspended sludge were evaporated, dried at 105-100 °C and weighed to determine the sludge dry weight and to be able to standardise the sludge concentration in the test vessels to 30 mg dry weight/l.
Test substance samples were weighed (25 mg) and added directly to 250-ml test flasks in duplicate. Then, adjusted sludge (30 mg dry weight/l) was added. The test article concentration was analytically confirmed. In parallel, two test flasks containing only standardised sludge, two flasks containing 100 mg sodium benzoate as a reference substance and two flasks containing 100 mg test substance/l plus 100 mg sodium benzoate/l as a toxicity/oxygen consumption inhibition conrol were prepared. Temperature of the Sapromat was kept at 22±1 °C by thermostat, the initial and end-of-test pH values were measured.
All 8 test flasks were installed in the Sapromat and the automatic oxygen consumption meters were linked up.
Oxygen demand was determined daily for every single flask. The oxygen demand of the 2 blank flasks were deducted from that of the experimental flasks (2 with test substance, 2 with reference substance, 2 with combination of both) to reflect substance-related biochemical oxygen consumption.
The biochemical oxygen consumption for every single flask was tabulated, the respective averages for the parallel flasks was also presented in a graph. The per cent biodegradation (after deduction of blank values) was computed as the quotient of biochemical to theoretical oxygen demands and tabulated for days 7, 14, 17, 21, 28 and

29. Further, the per cent biodegradation of isophytol was presented as a graph.

Result: Biodegradation (average of both flasks) of isophytol started slowly, remaining under 4% until day 4. It then reached 10% during day 6 and climbed steadily until reaching 60% on day 28, respectively 62% on day 29, when the test was stopped. The degradation of the reference substance, sodium benzoate, started without delay, passing 10% during day 1, and continued until reaching a plateau at approximately 80% on day 10. Afterwards it only rose very slowly.
The toxicity control run with both isophytol and sodium benzoate showed at least as high a biochemical oxygen demand as any of the single substences for every measurement. It started quickly and, on reaching the sodium benzoate plateau, climbed approximately in parallel with the isophytol biochemical oxygen demand curve.

Test substance: Isophytol rect., lot no. 9000337967 from Teranol, purity 97.6%.

Conclusion: Isophytol was well degradable in this OECD study. However, due to an initial lag phase and to the degradation rate rising at a steady but relatively slow rate thereafter, the 10-day-window criterion for ready biodegradability was not fulfilled. Hence, based on the present test isophytol would be characterised as inherently (but not quite readily) biodegradable.
Isophytol showed no inhibition of the biodegradation of the reference substance in the toxicity control test.

Reliability: (1) valid without restriction
OECD protocol, GLP study.

Flag: Critical study for SIDS endpoint

19-JUL-2002 (89)

Type: aerobic
Inoculum: other: activated sludge from a municipal sewage treatment plant
Concentration: 84 mg/l related to Test substance
Contact time: 28 day(s)
Degradation: = 75 % after 28 day(s)
Result: readily biodegradable
Kinetic:

6 day(s)	= 0 %
7 day(s)	= 4 %
8 day(s)	= 14 %
15 day(s)	= 60 %
24 day(s)	= 75 %

Control Subst.: Aniline
Deg. product: not measured

Method: OECD Guide-line 301 F "Ready Biodegradability: Manometric Respirometry Test"
Year: 1989
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Method: Isophytol was tested according to OECD 301F in a Sapromat apparatus, at a concentration of 84 mg/l isophytol in two parallel runs. Sludge concentration was 30 mg/l (dry sludge), aniline at 100 mg/l served as a positive control.

Result: After a lag phase of 6 days, biodegradation of isophytol started on day 7, passed 10% on day 8 and passed 60% on day 15, but even around day 12 the degradation curve started to

flatten slightly and degradation proceeded more slowly until 73% were reached on day 22 and the plateau of 75% on day 24, where no more degradation was seen until the end of the test on day 28.

Conclusion: After a lag phase of several days, isophytol was readily biodegradable in a BOD/ThOD test and reached a plateau of 75% degradation.

Reliability: (2) valid with restrictions
While this test was not performed under GLP, it was performed in a professional industry environmentnal laboratory, there is a test report detailing procedures and giving daily average % BOD&ThOD degradation rates as well as a graph of the degradation. Only the batch number of isophytol is not listed, but probybly available from the original lab journal. Based on this ample documentation, reliability is set at 2.

Flag: Critical study for SIDS endpoint

07-AUG-2002 (18)

Type: aerobic
Inoculum: activated sludge
Degradation: > 60 %
Result: readily biodegradable

Method: OECD Guide-line 301 C "Ready Biodegradability: Modified MITI Test (I)"
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Reliability: (4) not assignable

27-MAR-2002 (5)

Type: aerobic
Inoculum: Arthrobacter sp. (Bacteria)
Contact time: 18 hour(s)
Degradation: = 0 % after 18 hour(s)

Year: 1977
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Method: A strain of the soil bacterium Arthrobacter sp. that was able to grow in a medium containing the linear triterpenoid squalene as the sole carbon source was tested for its ability to degrade other linear terpene and squalene variants including isophytol.
Arthrobacter sp. were suspended in 50 ml of an 0.1-M sodium phosphate buffer (ph 7.0) to give an optical density of 10 at 600 nm. The cell suspension was mixed with substrate (0.6% v/v) in 500.ml flasks and incubated on a reciprocating shaker at 30 °C for 18 h. The reaction mixture was then extracted with dichloromethane and dried over anhydrous sodium sulfate. After evaporation of the solvent the residue was analysed by thin-layer chromatography.

Result: Both isophytol and phytol were not degraded by an Arthrobacter strain adapted to squalene as the sole carbon source. These substrates were recovered unchanged from the reaction mixture. The substrate specificity is critical, as also squalane, a completely reduced form of squalene, and cholesterol, lanosterol, cyclised triterpenes, isophytol,

phytol, nerolidol, digeranyl and geranylfarnesyl were not cleaved by this Arthrobacter strain. Successful cleavage of other structures (triterpenes) was mostly observed at double bonds near the middle of the molecules.

Conclusion: The fact that linear reduced triterpenes and shorter linear terpenes (including isophytol and phytol) were not cleaved by this Arthrobacter strain suggests that chain length and structure of the terminal part of the substrate molecule affect the enzyme system that attacks the central part of the substrate molecule.
Squalene- and fatty-acid-adapted Arthrobacter strains cannot degrade isophytol.

Reliability: (4) not assignable
07-AUG-2002 (110)

Type: anaerobic
Inoculum: anaerobic sludge
Concentration: 121.6 mg/l related to Test substance
Contact time: 93 day(s)
Result: other: barely anaerobically biodegradable
Control Subst.: Diethylene glycol
Kinetic: 41 day(s) = 82 %

Method: other: ISO11734
Year: 2002
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Method: An Ultimate Anaerobic Degradation test was performed according to ISO Guideline 11734.
Briefly, three replicate isophytol flasks, three incolum blank flasks and two diethylene glycol positive control flasks were run in parallel. The flasks were 1222-ml glass bottles closed with hermetically sealing butyl rubber stoppers with ports and a manometer attached. The flasks contained a test solution volume of 800 ml, made up of digested sludge from the digester of the biological step of the municipal sewage works ARA Werdhölzli in Zürich, Switzerland, at 2 g/l (dry matter) in the final mixture, with defined mineral salts according to ISO 11734 (details in report) in de-aerated water and either isophytol at a loading concentration of 98.4 mg total organic carbon (TOC)/l (= 121.6 mg/l isophytol) as the only organic carbon source for the test flasks; 45.6 mg TOC/l (= 100.9 mg/l diethylene glycol) for the control flasks; or nothing else for the inoculum controls. The flasks were filled with the de-aerated medium and substances as above, the headspace was filled with nitrogen gas and stoppered.
Test flasks were incubated at 25±2 °C in the dark and agitated once a day except on weekends. Determination of anaerobic biodegradation was made by precisely measuring the pressure in the headspace using a MP340A measuring device by EIRELEC Ltd, bleeding of the excess biogas volume and determining the inorganic carbon (IC) in the excess biogas with a Shimadzu 5050 TOC-Analyzer. Based on IC concentration, headspace volmue and pressure, the amount of IC produced since the last measurement can be calculated and summed up. The IC produced by the inoculum blank serves as a baseline and is subtracted from the test and control values. IC divided by TOC gives the degradation at a time point. At

the end of the test, the remaining IC in the aqueous phase is also determined and added to the headspace IC to give the final degradation.

Result: Degradation kinetics:

	day	%degradation (baseline = inoculum blank)	
Isophytol	0	0	
	6	-7	(slight inhibition, lag phase)
	34	0	
	55	2	
	93	4	(headspace IC only)
	93	9	(total IC including liquid)
Diethylene glycol (positive control)	0	0	
	3	11	
	13	66	
	41	82	(plateau)
	55	83	

The negative degradation phase at the beginning shows an initial inhibition of the (non-adapted) digested sludge to isophytol. After 6 days, slow degradation sets in until on day 34, an identical amount of IC has been produced as in the inoculum control. From this point degradation continues at a very slow rate, rising above the inoculum blank, until attaining a total including the inorganic carbon in the liquid medium of 9% on day 93, when the test was stopped. The positive control showed rapid degradation of diethylene glycol, reaching a plateau on day 41.

Conclusion: Isophytol is not significantly anaerobically biodegradable within 93 days. It shows an initial lag phase of 7 days during which the digested sludge bacteria were inhibited in comparison with controls.

Reliability: (2) valid with restrictions
While BMG Engineering Ltd are not GLP-certified, they adhere to quality assurance system SN EN 45001. The test report is concise and detailed, with all single basic data, measurements, calculations and graphs given, hence reliability was set 2.

Flag: Critical study for SIDS endpoint

03-JAN-2003 (57)

3.6 BOD5, COD or BOD5/COD Ratio

3.7 Bioaccumulation

BCF: = 68.1

Method: other: QSAR estimate
Year: 2002
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Result: Based on logPow = 8.078
Equation used for BCF estimate:
logBCF = -1.37 logPow + 14.4 + correction
Correction used: alkyl chains (8+ CH2 groups) = -1.500
Estimated logBCF = 1.833 <=> BCF = 68.10

07-AUG-2002 (45)

BCF: = 2310

Method: other: estimated value
Year: 2002
GLP: no
Test substance: as prescribed by 1.1 - 1.4

07-AUG-2002 (108)

BCF: = 2870781

Method: other: estimated value
Year: 1994
GLP: no
Test substance: as prescribed by 1.1 - 1.4

07-AUG-2002 (4) (6)

3.8 Additional Remarks

Memo: Abiotic atmospheric degradation with hydroxyl radicals and ozone

Method: QSAR estimate
Result: OH-radical-mediated atmospheric degradation
overall OH rate constant = 51.55E-12 cm3/molecule*s
half-life = 0.207 days (12-hour day, 1.5E06 ·OH/cm3)
half-life = 2.490 hours
Ozone-mediated atmospheric degradation
overall O3 rate constant = 0.175E-17 cm3/molecule*s
half-life = 6.549 days (at 7E11 O3-molecules/cm3)
half-life = 157 hours
07-AUG-2002 (45)

Memo: Abiotic formation and conversion in sediments

Result: The Merck Index states for isophytol that it is a "decomposition product of chlorophyll". Chlorophylls have a phytyl propionate side chain, hence this statement is not implausible, however, no original reference for the statement
is given. Also, no evidence for isomerisation of phytol to isophytol is presented. On the other hand, if isophytol is a decomposition product of chlorophyll indeed, then it must be formed, at least as a short-lived transitory substance, in huge amounts.

A pathway for natural formation of isophytol in water or sediment under anoxic conditions was postulated by Didyk et al. (1978). Briefly, chlorophyll-alpha loses its central magnesium ion through sediment-catalysed demetallation to form pheophytin-alpha, which subsequently hydrolyses to the chlorin and phytol moieties, the latter of which isomerises to isophytol. Didyk et al. cite the unpublished 1974 PhD thesis of PW Brooks from the University of Bristol (UK), stating in the legend to figure 3 that Brooks had identified 3,7,11,15-tetramethylhexadec-1-en-3-ol, isophytol, (among several other compounds) as a product from 14C-radiolabelled phytol incubated in sediments.

Brooks and Maxwell (1974) incubated freshwater lacustrine sediment cores with radiolabelled phytol in the dark for periods of up to 8 weeks. In their subsequent analysis of fractions isolated through radio-thin-layer-chromatography and radio-gas-chromatography (details available) they identified one major radiolabelled metabolite with a mass spectrum identical to phytol in GC-MS, "suggesting that it is an isomer". The same compound was also isolated from natural untreated sediment cores. The authors further stated that the "phytol isomer ... is thought to be the structural isomer shown (fig. 5) [= isophytol], allylic rearrangement of the hydroxyl function occurring readily".

de Leeuw et al. (1975) incubated phytol in the laboratory in artificial sediment under different conditions (phytol + montmorillonite + water, under air or under vaccum, at 20 °C or 60 °C, during 2-140 days) and subsequently analysed the benzene/methanol extract using GC-MS. Additionally, they incubated radiolabelled 14C-phytol in a recent sediment core for 70 days; incubation products were extracted with chloroform and separated with TLC, using radio-TLC for the identification of radio-active bands; separates were analysed by radio-GC and GC-MS. Last, sediments from freshwater Lonnekermeer and marine sediment samples from Walvis Bay and from the Deep Sea Drilling Project were extracted with isopropanol/hexane and subsequently analysed by GC-MS (all analytical details available in paper). In the first incubation series using montmorillonite, isophytol was determined in the extracts of the following conditions: ++ (not quantified) from 140 days at 20 °C under air; + from 3 days at 60 °C under air; and + from 2 days at 60 °C under vacuum. No isophytol was found from the longer incubations at 60 °C, but a whole list of other, mostly longer-chained compounds. No isophytol was determined from the incubation with radio-labelled phytol nor from the natural sediments. de Leeuw et al. concluded that phytol may isomerise to isophytol under various conditions, both oxic and anoxic; that isomerisation may need or proceed faster with (clay) mineral surfaces; and that isophytol is only a relatively short-lived transitory intermediate in the abiotic diagenetic conversion of phytol (from chlorophyll) to high-molecular-weight, insoluble organics subsumed under the name kerogen, from which petroleum may form under pressure or high temperature.

Rontani et al. (1999) followed the biodegradation pathways of (E)-phytol in artificial seawater, subsequent to incubation with an aerobic and an anaerobic-denitrifying bacterial culture isolated from marine sediment from the French Mediterranean coast. Degradation intermediates and products were identified by GC-MS (details in paper). Further, they also analysed fresh sediment cores for previously identified metabolites. In the biodegradation experiments, no isophytol was detected in aerobic flasks while in the anaerobic flasks, approximately 0.21% isophytol (relative to degraded phytol) was found. The formation of isophytol was attibuted by the authors to the involvement of a reversible enzyme-catalysed allylic re-arrangement of (E)-phytol, analogous to a published (Foss & Harder, 1997)

pathway for the transformation of linalool to geraniol. Rontani et al. (1999) did not detect isophytol in the natural marine sediments they analysed.

Conclusion: Isophytol can be formed from phytol in anoxic and aerobic, marine and freshwater sediments, probbybly or preferentially in the presence of certain clay minerals. However, this isophytol is but a transient, relatively short-lived intermediate in the further diagenetic conversion of phytol.

Flag: Critical study for SIDS endpoint

07-AUG-2002 (24) (27) (37) (42) (86)

AQUATIC ORGANISMS

4.1 Acute/Prolonged Toxicity to Fish

Type: static
Species: Leuciscus idus (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** no
NOEC: 10000 -
LC0: 10000 -
LC50: > 10000 -
LC100: > 10000 -
Limit Test: no

Method: other: DIN 38412, Determination of the effects of substances in water on fish
Year: 1982
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Method: Test system
All-glass aquaria, filled with 10 l of reconstituted freshwater (294.0 mg/l CaCl2 * 2 H2O; 123.3 mg/l MgSO4 * 7 H2O; 63.0 mg/l NaHCO3; 5.5 mg/l KCl, in demineralised water), with continuous aeration with oil-free air, test temperature 20±1 °C, 16 h light, 8 h dark.
Test organisms
Leuciscus idus (golden orfe) from Fischzucht Paul Eggers, Hohenwested, Germany, arrived at test institution 7 weeks before start of test. At start of test, fish were 6.7 (5.7-7.2) cm long and had a mass of 2.9 (1.5-4.4) g. Food was withdrawn 1 day before and through the test.
Test procedure
Test vessels were filled with test medium and aerated 3 days before start of exposure. For the two doses tested, based on a pre-test, isophytol was directly added tto the tanks, without an emulsifier and without additional stirring; the first loading concentration was 5,000 mg/l and the second 10,000 mg/l; no dosing was made to the negative control tank, a positive control run with fish from the same delivery had been made one week before the isophytol test with chloroacetamide and resulted in an LC50 of 32 mg/l. Subsequent to dosing, ten fish per tank were added to the high and low concentration and to the negative control. The fish were observed at 1, 4 24, 48, 72 and finally at 96 hours after dosing.
Result: No deaths occurred in both isophytol concentrations (5,000 and 10,000 mg/l loading rate) nor in the negative controls. No behavioural effects were noted durng several observations.
Conclusion: In a static test wihout emulsifier over 96 hours, isophytol caused no observable effects in golden orfe up at nominal concentrations of 5,000 and 10,000 mg/l. THE NOEC was 10,000 mg/l nominal concentration, which indicates that at natural solubility isophytol is not toxic to the fish.
Reliability: (2) valid with restrictions
Not GLP, but a detailed and well-documented test according to an official guideline, hence reliability was set 2.
Flag: Critical study for SIDS endpoint

22-JUL-2002 (11)

4.2 Acute Toxicity to Aquatic Invertebrates

Type: semistatic
Species: Daphnia magna (Crustacea)
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:** yes
NOEC: = .017 - measured/nominal
EC50: = .13 - measured/nominal
EC100: = .58 - measured/nominal
Limit Test: no

Method: OECD Guide-line 202
Year: 2002
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Method: Test species
Daphnia magna Straus, freshly hatched animals, less than 24 hours old, from the laboratory's own breeding culture.
Test medium
M7 ISO medium prepared with reverse-osmosis ultra-pure water according to Elendt B-P (1990): Selenium deficiency in crustecea; an ultrastructural approach to antennal damage in Daphnia magna Straus. Protoplasma 154: 25-33. Full details in test report.
Preparation of test solutions
For the tests, water acommodated fractions (WAF) were prepared by magnetically stirring 100 mg of isophytol per litre of medium for 96 hours, without any additional emulsifier. The mixture was left to stabilise for 16 hours, then part of the aqueous phase was siphoned out of the stirring flask and left to stand in a separation funnel. After another short settling period, the water phase from this funnel was collected as the WAF and used for preparation of the final test concentrations by subsequent dilutions with test medium. Stirring and stabilisation was then continued to obtain in the same manner the test solutions for the second 24-hour test period. The final test solutions were all clear, without precipitates nor a surface film. The blank control medium was treated in the same manner, but without additon of test substance.
Test concentrations
Targeted test concentrations, based on non-GLP pretests, were 30, 70, 160, 360 and 800 µg/l, plus a blank (0 µg/l) medium control. Test concentrations decreased over time, as expected. The measured concentrations, based on fresh at 0 hours, old at 24 hours, fresh at 24 hours and old at 48 hours, were averaged by computing first the geometric mean of the initial and final concentrations for the two subsequent media and then determining the arithmetic average of those. Average measured concentrations were 17 µg/l (30 µg/l target), 42 (70) µg/l, 104 (160) µg/l, 249 (360) µg/l and 580 (800) µg/l. The single initial and final concentrations were comparable, measured concentrations decreased by 40-70% during 24 hours. All analytical determinations were made on the same day as the sample was collected.
Analytics
A GC method was set up and validated, based on the standard GC

method from Teranol, Lalden. The test system was validated regarding repeatability, stability, linearity and limit of detection. Full details are given in the annexe to the test report. Samples from the semi-static test (0.5 or 3 ml volume) were made up with medium to 3 ml, then vortex-mixed with 3 ml acetone and 3 ml hexane (the latter containing 1 mg/l heneicosane as an internal standard) for 15 seconds. Injection originated from the upper, organic layer.
Test procedure
Vessels: 100 ml, all-glass
Number of daphnia: 20 per concentration
Loading: 10 daphnids per vessel containing 80 ml medium
Photoperiod: 16-hour-light, 8-hour-dark cycle
Feeding during test: none
Aeration during test: none
Test medium change: after 24 hours
Introduction of daphnia: immediately after preparation of test solutions
Measurements and recordings
Immobility at 24 hours and 48 hours. As it was noted that daphnids were trapped at the surface at all test concentrations (but not in the controls), these were first gently re-submerged before their swimming reaction to disturbance was checked.
pH and dissolved oxygen was measured at the beginning, after 24 hurs of exposure and at the end of the test for all concentrations and controls. Temperature was measured continuously in a separate temperature control vessel.
Statistics
The EC50 value was calculated at 48 hours from the probits of percentages of affected daphnids and the logarithms of the corresponding test substance concentrations using the maximum likelyhood estimation method (Finney DJ (1971): Probit analysis. Cambridge University Press, 3rd ed).

Result: In the static range-finding test, 4/10 daphnids were immobilised at 48 hours. Based on this result, a first, static test using a water-accommodated fraction (WAF) prepared at 100 mg/l resulted in no more than 40% immobility after 48 hours, either; however, analytical results then showed that measured concentrations in the WAF were unexpectedly low with 0.114 mg/l at 100 mg/l nominal at the start of the test, which decreased to below detection level after 48 hours.
In the subsequent final semi-static test with medium exchange after 24 hours the NOEC was 0.017 mg/l average, 1/20 daphnid was immobilised at 0.42 mg/l average concentration, 7/20 at 0.104 mg/l average, 16/20 at 0.249 mg/l average and 20/20 at 0.580 mg/l average. Hence, this semi-static test resulted in a 48-hour EC50 of 0.130 mg/l average concentration (100-170 mg/l, 95% confidence interval), with the NOEC at 0.017 mg/l average and the EC100 at 0.580 mg/l average.
It also showed that at all test substance concentrations daphnids tended to become trapped at the surface; these were first re-submerged before checking on their mobility, which resulted in clearly diminished immobility. In contrast, no single daphnia became surface-trapped in the blank control. The fact that daphnids at all substance concentrations became trapped without any concentration-related increase observed, while none did so in the blank controls, evidences an appreciable surface activity of isophytol.

Test substance: Isophytol from Teranol Lalden, batch no. UU02013601, purity

97.5% (GC).

Conclusion: In a recent semi-static OECD 202 test with analytical confirmation of actual exposures, the EC50 for daphnids was 0.130 mg/l average concentration (95% confidence interval 0.100-0.170 mg/l) while the NOEC was 0.017 mg/l and the EC100 was 0.580 mg/l average concentration.

Reliability: (1) valid without restriction
GLP OECD study.

Flag: Critical study for SIDS endpoint

20-FEB-2003 (76)

Type: static
Species: Daphnia magna (Crustacea)
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:** no
EC0: < .01 - 1.56 calculated
EC50: = .11 - 20.3 calculated
EC100: = 5 - 100 calculated
Limit Test: no

Method: Directive 84/449/EEC, C.2 "Acute toxicity for Daphnia"
Year: 1992
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Method: A static test with daphnia was performed in a professional company-internal environmental laboratory according to an international guideline. Juvenile daphnia (own bred) were exposed to different solutions or emulsions of isophytol at various concentrations for 48 hours; all stock solutions were prepared at 100 mg/l loading or nominal concentration (see Results). The number of immobile daphnia in the various dilutions were used to compute LC50 value by log-probit transformation.

Result: This test report comprises several series of differently prepared stock solutions of 100 mg/l loading or nominal concentration of isophytol each, respectively dilutions thereof. EC values after 48 hours are given in the following table; all concentrations are taken to be nominal as there is no information on analytics performed:

Preparation of stock solution	EC0	EC50	EC100, mg/l
1) 20 h stirring, no emulsifier, used instantly	<0.001	0.11	5
2) Tween 80 (100 mg/l), 20 h stirring	0.1	0.94	10
3) Cremophor RH40 (100 mg/l), 20 h stirring	0.1	1.99	10
4) 15 h stirring, no emulsifier, 15 h left to stand in separation funnel, used lower fraction	0.78	2.9	100
5) 8 h stirring, no emulsifier, 17 h left to stand in separation funnel, centrifugate lower fraction for 10 min at 6,000 rpm, used lower fraction	1.56	20.3	>100

Conclusion: The comparison shows differences in toxicity with a factor up to 200, depending on the preparation of the stock solution. The range of EC50s is not explained, nor is it explicable in a simple manner. Considering the nature of isophytol, as an oily liquid of limited water solubility, it is possible that due to stirring, possibly also due to emulsifiers, minuscule droplets form that may later re-aggregate to larger drops over time. Daphnia may adsorb to these droplets and become immobilised physically or be exposed to much higher local concentrations of the test substance. However, other possibilities, eg, partitioning of isophytol out of the aqueous compartment onto surfaces through adsorption or rapid degradation, were not discussed. Additionally, some synergistic toxic effect of the emulsifiers cannot be excluded. This test series is nearly impossible to interpret.
Reliability: (4) not assignable
Not GLP, but a detailed and well-documented test according to an official guideline, with a lot of additional information. On the other hand, the results are difficult to interpret, hence reliability was set to 4.
06-JAN-2006 (16)

Type: static
Species: Daphnia magna (Crustacea)
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:** no
EC0: = .08 -
EC50: = .2 -
EC100: = .8 -
Limit Test: no

Method: Directive 84/449/EEC, C.2 "Acute toxicity for Daphnia"
Year: 1984
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Method: Tested using Tween 80 as an emulsifier (at one-tenth of substance concentration), 10 daphnia per nominal concentration, concentrations tested in duplicate, following an international guideline.
Result: The detailed immobilisation data were used to derive an EC50 on log-probit paper.

Time, hours	EC0	EC50	EC100, mg/l nominal concentration
24	0.08	0.65	>2.0
48	0.08	0.2	0.8

The detailed data on the single test vessels show that there is a clear increase in immobilisation over time, as reflected in the decreased EC50 and EC100 values.
Conclusion: In this test with Tween 80 emuslifier, isophytol had a daphnid EC50 of 0.2 mg/l nominal concentration at 48 hours. This value is somwhat lower but still within one dimension of other EC50 values derived using emulsifiers.
Reliability: (4) not assignable
19-JUL-2002 (17)

Type: static
Species: Artemia salina (Crustacea)
Exposure period: 24 hour(s)
Unit: mg/l **Analytical monitoring:** no data

LOEC : = 500 -

Year: 1999
GLP: no data
Test substance: other TS: 3,7,11,15-tetramethylhexadec-1-en-3-ol, no data on source or purity

Method: Artemia assays were performed according to Meyer et al. (1982). Briefly, commercial brine shrimp eggs (Living World, Elmwood Park, NJ, USA) were hatched in artificial seawater (Instant Oceans seawater salt, Aquarium Systems, Mentor, OH, USA, in double-distilled water). For tests, 48 hours after setting the eggs to hatch, 10 artemia nauplii each were pipetted to test vessels containing 5 ml of artificial seawater. A drop of dry yeast extract (Red Star products) was added as food to each vial. Test substances were added at 0 (controls), 10, 100 or 1000 mg/l concentration to the test vials, tests were run in 5 replicates each. The vials were maintained under constant illumination at room temperature. After 24 hours' test duration, surviving nauplii were counted with a magnifying glass and the percentage of survivors recorded for every concentration and replicate. LC50 determinations were done using probit analysis or else using logit transformation and best-fit-line linear regression.
Remark: Isophytol had previously been detected in dichloromethane extracts of the marine red alga Plocamium costatum. It was subsequently tested in an assay described by Meyer at al. (1982) with the holoplanktonic marine to hypersaline crustacean Artemia salina. Only the result of this test is briefly mentioned, for details there is only a reference to a paper in preparation.
Result: Unspecified "weak effects" against Artemia at a concentration of 500 mg/l (in the original: 0.5 mg/ml).
Conclusion: Isophytol is relatively nontoxic or at most moderately toxic against Artemia brine shrimp in acute tests.
Reliability: (4) not assignable
Pending receipt of detailed results reliability is set 4.
06-JAN-2006 (66) (75)

Type: static
Species: other aquatic crustacea: Balanus amphitrite (barnacle) larvae
Exposure period: 24 hour(s)
Unit: **Analytical monitoring:** no

Year: 1999
GLP: no data
Test substance: other TS: 3,7,11,15-tetramethylhexadec-1-en-3-ol, no data on source or purity

Method: The potential of isophytol for deterrence of Amphitrite larvae settlement was tested according to de Nys et al. (1996). Briefly, isophytol was dissolved in pure (99.7%) ethanol at various concentrations and an aliquot of 0.5 ml of this ethanol solution was added to treatment Petri dishes of 9 cm2 surface area; then, the solvent was left to evaporate. Concentrations of isophytol were selected to result in test substance concentrations of 0.01 to 10.0 µg/cm2 Petri dish surface. 4 ml of sterile-filtered (0.22 µm) seawater was added to each vessel. Both solvent (ethanol

only) and untreated controls were run in parallel. All treatments and all controls were run in triplicate. Adult Balanus amphitrite, kept in the laboratory, served as a broodstock for nauplii larvae which were collected and reared on Skeletonema costatum algae until they reached the cypris stage. Settlement tests were conducted by adding 25-35 mature free-swimming cypris larvae to the test vessels and incubating for 24 hours at 28 °C in a 15/9-hour light/dark cycle. After 24 hours the test was terminated by addition of 3 drops of 40% formaldehyde and subsequently filtering non-settled cyprids from the dish. Both settled and non-settled larvae were counted. The endpoint is the number of settled larvae per total number of larvae added. The raw data were analysed by analysis of variance followed by Tukey's multiple comparison test.

Remark: Isophytol had previously been detected in dichloromethane extracts of the marine red alga Plocamium costatum. It was subsequently tested in an antifouling assay using barnacle larvae. Barnacles are specialised crustaceans that as larvae are planktonic, while the adults are sessile, often fouling ship and marine construction surfaces.

Result: The algal extract deterred barnacle larvae from settling on substrate at concentrations of 100 and 10 µg/cm2, while pure isophytol was significantly deterrent at concentrations of 10 and 1 µg/cm2:

Test material	concentration (µg/cm2)	barnacle settling rate (average number +/- SD)	
seawater control		83 +/- 6.6	
seawater/solvent control		75 +/- 7.6	
Plocamium extract	100	0	*
Plocamium extract	10	39 +/- 4.4	*
Plocamium extract	1	69 +/- 11.4	
Plocamium extract	0.1	76 +/- 16	
Plocamium extract	0.01	78 +/- 13.1	
Plocamium extract	0.001	68 +/- 15.8	
Isophytol	10	13 +/- 3.1	*
Isophytol	1	23 +/- 7.5	*
Isophytol	0.1	54 +/- 17	
Isophytol	0.01	43 +/- 7.6	

The minimal inhibitory concentration for isophytol was calculated as <= 1 µg/cm2.

Conclusion: Isophytol has the potential to inhibit the settlement of barnacle larvae and it is therefore a potential deterrent for biofouling by barnacles.

Reliability: (2) valid with restrictions
While the toxicity results are presented in a brief fashion only in the paper by König, Wright and de Nys (1999), the experimental procedure is detailed in a previous paper by de Nys et al. (1996). As one author links both method and results papers, the reliability is regarded as 2.

06-JAN-2006 (38) (66)

4.3 Toxicity to Aquatic Plants e.g. Algae

Species: Scenedesmus subspicatus (Algae)
Endpoint: other: biomass and growth rate
Exposure period: 72 hour(s)
Unit: mg/l **Analytical monitoring:** no data

EC10: > 500 -
EC50: > 500 -
EC100 : > 500 -
Limit Test: no

Method: other: DIN 38412 part 9, Determination of the inhibitory effect on substances in water on green algal growth
Year: 1988
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Method: The toxicity of isophytol to Scenedesmus subspicatus was tested according to an accepted German standard guideline. Briefly, Scenedesmus algae from an in-house stock were exposed to isophytol emulsified with Tween 80 (10% of respective substance concentration) at the following nominal concentrations in quadruplicate, 0.1, 1, 10, 100, 1000 and 0 (control, without Tween 80) mg isophytol/l, during 72 hours at 23±2 °C. Every 24 hours the concentration of algal cells was determined fluorometrically. The pH was measured at the beginning and at the end of the test. Additionally, a potential inhibition of the photosynthetic capacity of the algae in the control and highest-concentration solutions was determined through spectrophotometric scanning from 300 to 780 nm at the end of the test. Biomass and growth rates were averaged for the four parallel flasks and inhibitions rates were determined by computer program and plotted.
Result: Both biomass and growth rate of the algae were not statistically significantly inhibited at 500 mg/l, with EbC10 and ErC10 values > 500 mg/l. Further, there was no inhibition of photosynthetic capacity.
Conclusion: Isophytol was not inhibitory to freshwater algae at nominal concentrations above 500 mg/l using Tween 80 as an emulsifier. Further, even the highest isophytol test concentration was not inhibitory on photosynthetic activity of the algae.
As all the average cell counts from the four replicates are given for all test conconetrations over time, it is not clear why the report only gives an EC10 and EC50 > 500 mg/l nominal copncentration, but not 1000 mg/l as no inhibitory effects are evident.
Reliability: (2) valid with restrictions
Not GLP, but a detailed and well-documented test according to an official guideline, with a lot of additional information, hence reliability was set 2.
Flag: Critical study for SIDS endpoint
07-AUG-2002 (17)

4.4 Toxicity to Microorganisms e.g. Bacteria

Type: aquatic
Species: other bacteria: activated sludge of a predominantly domestic sewage treatment plant (Aïre, Geneva, Switzerland)
Exposure period: 29 day(s)
Unit: mg/l **Analytical monitoring:** no
NOEC: = 100 - measured/nominal

Method: other: OECD Guideline 301F, Toxicity control
Year: 1999

GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Method: The test was run in a respirometer, model Sapromat D1 by JM Voith GmbH, Heidenheim, Germany.
All water used for the study was deionised.
Stock mineral solutions were made up according to the OECD Guideline 301F and added to the water in the correct amounts to make the test medium, the pH of which was measured and adjustd if necessary using phosphoric acid or potassium hydroxide.
As the inoculum, fresh activated sludge from the predeminantly domestic sewage works of Aïre (City of Geneva, Switzerland) was collected in the morning, washed three times in the mineral medium with centrifugation at 1000 g for 10 min, discarding the supernatant and re-suspension in mineral medium. The washed sludge was kept under aerobic conditions until use in the test the same day. Two samples of known volume of suspended sludge were evaporated, dried at 105-100 °C and weighed to determine the sludge dry weight and to be able to standardise the sludge concentration in the test vessels to 30 mg dry weight/l.
Test substance samples were weighed (25 mg) and added directly to 250-ml test flasks in duplicate. Then, adjusted sludge (30 mg dry weight/l) was added. The test article concentration was analytically confirmed. In parallel, two test flasks containting only standardised sludge, two flasks containing 100 mg sodium benzoate as a reference substance and two flasks containing 100 mg test substance/l plus 100 mg sodium benzoate/l as a toxicity/oxygen consumption inhibition conrol were prepared. Temperature of the Sapromat was kept at 22±1 °C by thermostat, the initial and end-of-test pH values were measured.
All 8 test flasks were installed in the Sparomat and the automatic oxygen consumption meters were linked up.
Oxygen demand was determined daily for every single flask. The oxygen demand of the 2 blank flasks were deducted from that of the experimental flasks (2 with test substance, 2 with reference substance, 2 with combination of both) to reflect substance-related biochemical oxygen consumption.
The biochemical oxygen consumption for every single flask was tabulated, the respective averages for the parallel flasks was also presented in a graph. The per cent biodegradation (after deduction of blank values) was computed as the quotient of biochemical to theoretical oxygen demands and tabulated for days 7, 14, 17, 21, 28 and 29. Further, the per cent biodegradation of isophytol was presented as a graph.
Remark: Neither isophytol nor the reference substance, sodium benzoate, were determined analytically, but only the biochemical oxygen demand after subtraction of the biochemical oxygen demand for the blank (sludge only) control.
Result: In the toxicity control of the OECD 301F ready biodegradability test, the degradation as measured by biochemical oxygen demand proceeded quicker at every single daily determination point for 100 mg isophytol/l plus 100 mg sodium benzoate/l than for either 100 mg isophytol/l alone or for 100 mg sodium benzoate/l alone.
Test substance: Isophytol rect., lot no. 9000337967 from Teranol, purity

97.6%.
Conclusion: Up to the tested nominal concentration of 100 mg/l, isophytol showed no inhibition of activated sludge activity.
Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
17-APR-2002 (89)

Type: aquatic
Species: activated sludge
Exposure period: 30 minute(s)
Unit: mg/l **Analytical monitoring:** no
NOEC: = 1000 -
EC50: > 1000 -
EC80 : > 1000 -

Method: other: Test for Inhibition of Oxygen Consumption by Activated Sludge, ISO 8192
Year: 1989
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: The potential respiration inhibition of activated sludge due to isophytol was tested according to an accepted ISO standard that is essentially identical to OECD guideline 209. Briefly, activated sludge from a municpal sewage works wasrinsed and suspended at a concentration of 1 g/l (dry matter). The baseline oxygen consumption of this dilution was compared to the oxygen consumption on the test flaks, where 1000 mg/l isophytol had been added for a test duration of 30 minutes.
Result: Both the EC20, EC50 and EC80 after 30 minutes were > 1000 mg/l (loading rate). There was no respiration inhibition up to 1000 mg/l, which is therefore the NOEC in this test.
Conclusion: Even at high concentrations, isophytol was not inhibitory on sewage sludge micro-organisms as measured by oxygen consumption. Hence, no disruption of the degradation capability of activated sludge is to be expected subsequent to considerate discharge into sewage works.
Reliability: (2) valid with restrictions
Not GLP, but a well-documented test performed according to an official guideline in a professional industry laboratory, hence reliability was set 2.
Flag: Critical study for SIDS endpoint
06-JAN-2006 (18)

Type: aquatic
Species: Pseudomonas putida (Bacteria)
Exposure period: 30 minute(s)
Unit: mg/l **Analytical monitoring:**
NOEC: = 10000 -
EC10: > 10000 -
EC50: > 10000 -
EC90 : > 10000 -

Method: other: Pseudomonas-Atmungs-Hemmtest, DIN 38412 Teil 27, in Vorber., Bestimmung der Hemmwirkung von Abwasser auf die Sauerstoffzehrung von Pseudomonas putida (effect of substances in water on the oxygen consumption of P. putida)
Year: 1988
GLP: no data

Test substance: as prescribed by 1.1 - 1.4

Source: BASF AG Ludwigshafen
Reliability: (4) not assignable
06-JAN-2006 (17)

Type: other: laboratory tests with three micro-organisms
Species: other bacteria: Saccharomyces cerevisiae (Fungi), Clostridium acetobutylicum (Bacteria), Zymomonas mobilis (Bacteria)

Year: 1991
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: In order to select biocompatible solvents for extractive biocatalysis, potential solvents were screened using two criteria, a QSAR-derived n-octanol/water partition coefficient and metabolic activity test data by exposing three different micro-organisms to non-specified concentrations of solvents.
Result: Test results are given as graphs only. For both Saccharomyces cerevisiae, Clostridium acetobutylicum and Zymomonas mobilis, there was no remarkable change of metabolic activity with undefined concentrations of compounds having a predicted logPow of 9.1, including isophytol, phytol and Eutanol G.
Conclusion: At undefined concentrations, isophytol does not inhibit the metabolic activity of a yeast, S. cerevisiae, and two bacteria, C. acetobutylicum and Z. mobilis.
Reliability: (4) not assignable
27-MAR-2002 (25)

4.5 Chronic Toxicity to Aquatic Organisms

4.5.1 Chronic Toxicity to Fish

4.5.2 Chronic Toxicity to Aquatic Invertebrates

TERRESTRIAL ORGANISMS

4.6.1 Toxicity to Sediment Dwelling Organisms

Species: other: Caenorhabditis elegans (Nematoda), common soil and sediment invertebrate
Endpoint: other: growth, egg production, fertility
Expos. period: 72 other: hours
Unit: mg/kg sediment dw
NOEC: = 15000 - measured/nominal

Year: 2002
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Method: Test institution
Ecossa, Ecological Sediment and Soil Assessment, is a company founded by Dr Sebastian Höss in Munich, Germany. Dr

Höss did his PhD on sediment testing using nematodes, he co-developed the published protocol for this test (see reference Traunspurger et al., 1997) and he has years of experience with this type of testing.
Test animals
Caenorhabditis elegans is a common soil and sediment nematode that feeds on bacteria. Caenorhabditis are mostly (>99.9%) self-fertilising hermaphrodites, only <0.1% are males capable of fertilising hermaphrodites. The animals pass through 4 juvenile stages with molts to reach adult stage, self-fertilise and develop eggs in their body. At room temperature a full reproductive cycle takes about 72 hours. They can be easily grown and maintained as stock cultures on Petri dishes on agar plates with a bacterial lawn for food. They can be selected and synchronised to obtain juveniles of the first stage (J1), which were used in the tests. Test animals were fed on cultures of the bacterium Escherichia coli (OP50 strain).
Artificial sediment
An artificial sediment containing 30% dry sediment mix and 70% M9-medium (mostly water) was used for the test. Briefly, quartz sand, calcitic sand, kaolin, dolomite sand, ground sphagnum peat, iron(III) oxide and aluminium(III) oxide (all sources listed in report) were mixed in adequate proportions to result in an artificial sediment mix made up of 44% sand fraction, 48% silt fraction and 8% clay fraction and containing 2% organic substances.
Media
M9-medium was made up of 6 g Na2HPO4/l, 3 g KH2PO4/l, 5 g NaCl/l, 0.25 g MgSO4*7H2O/l and 1 ml/l of a cholesterol stock solution, consisting of 5 g cholesterol in 1 l of absolute ethanol. M9-medium was made up to 1 l using distilled water.
Food medium for E. coli bacterial culture consisted of 10 g peptone from casein/l, 5 g yeats extract/l and 10 g NaCl/l, made up with water.
NGM agar for E. coli bacterial culture consisted of 2.5 g peptone from casein/l, 17 g agar/l and 13 g NaCl/l; after mixing, autoclaving and cooling to approx. 55 °C, the follwing aliquots of sterile solutions are added: 1 ml cholesterol stock solution (see above), 1 ml 1M CaCl2 solution, 1 ml 1M MgSO4 solution and 25 ml 1MKH2PO4 solution, the latter adjusted to pH 6 using KOH.
Test setup
Test substances were dissolved in 96% ethanol in concentration series and 0.01 ml of the respective stock solution was thoroughly mixed with 0.75 g wet artificial sediment in the test vessels (Nunc polystyrene multiwells). Spiked sediments were left for 24 hours to allow equilibration of test substance between aqueous and solid phases. Before the start of the assay, 0.25 ml of bacterial suspension in double-concentrated M9-medium was added to each test well as food for the nematodes. After that, 10 juvenile worms of stage J1 were added by pipette to each well. Every test concentration including a vehicle control was run in triplicate for the range-finding test and in quintuplicate for the main test. The multiwell plates were incubated for 72 hours on a shaker at ±20 °C. Then, to stop the test, nematodes were heat-killed by warming the plates to approx. 55 °C, which makes them stretch, and stained with

Rose Bengal dye. Nematodes were extracted from the sediment by centrifugation in a density gradient and parameters for the endpoints were determined under a microscope at x100 and x400 magnification.
Endpoints
Parameters for the endpoints were as follows. Growth: length in µm; egg production: number of eggs in body; fertility: percentage of gravid worms (worms with >= 1 egg).
Statistics
One-way ANOVAS were carried out with the mean values of the replicates of the main test. In order to obtain NOEC and LOEC values, post-hoc tests according to Dunnett were performed additionally. For the determination of ECx values, dose-response curves (% inhibition vs control) were fitted to the respective data using a sigmoidal model.

Result: The range-finding pretest had shown no effect up to 5000 mg/kg sediment (dry weight). The main test was performed using concentrations of 0 (control); 5000; 10,000 and 15,000 mg isophytol/kg sediment (dry weight). At 10,000 mg/kg there was a slight, non-dignificant reduction in fertility with 96.7% gravid worms as compared to controls; however, at 15,000 mg/kg fertility was again 100%, suggesting that the slight decline was not due to a toxic effect. Moreover, there was a probably non-significant increase in both length and egg production per worm with increasing concentrations of isophytol. Overall, there were no observed adverse effects on growth, egg production and fertility in the different concentrations up to and including 15,000 mg/kg sediment (dry weight).

Test substance: Isophytol from Teranol Lalden, Lot no. UU02013601, purity 98.0% (GC).

Conclusion: Even at a very high loading of 15000 mg isophytol per kg artificial sediment (dry weight), no effects on growth, egg production or fertility were observed. In view of the short reproduction time of Caenorhabditis, a very common sediment- and soil-dwelling nematode, this test also qualifies as a chronic and reproductive study.

Reliability: (2) valid with restrictions
While the protocol is not an accepted OECD guideline and the institution is not GLP-approved, Dr Höss co-developed and refined the protocol, has a lot of experience with this type of testing which he does as a contract lab and presented a detailed report with all single basic data for the different concentrations tested (5 dishes with 10 animals each per concentration in the main test) plus full statistics for the whole test. Based on a clear protocol, careful documentation, testing in quintuplicate and full statistics, the report is judged to be of reliability 2.

Flag: Critical study for SIDS endpoint

03-JAN-2003 (58) (104)

Species: other: Caenorhabditis elegans (Nematoda), common soil and sediment invertebrate

Endpoint: other: no data

Method: other: no data

Year: 1999

GLP: no data

Test substance: other TS: 3,7,11,15-tetramethylhexadec-1-en-3-ol, no data on source or purity

Remark: Isophytol had previously been detected in dichloromethane extracts of the marine red alga Plocamium costatum. It was subsequently tested in an assay with the ubiquitous soil- and sediment-dwelling nematode C. elegans.
Result: There is a very brief note that isophytol "had only weak" [but not quantified] effects. For details there is a reference to a paper in preparation.
Reliability: (4) not assignable
07-MAY-2002 (66)

4.6.2 Toxicity to Terrestrial Plants

Species: other terrestrial plant: etiolated leaves of maize/corn (Zea mays, Poaceae)
Endpoint: other: incorporation of radiolabelled acetate as a biosynthetic building block of carotenoids and chlorophylls
Expos. period: 1 day(s)
Unit: mg/l
NOEC: = 680 -

Year: 1966
GLP: no
Test substance: other TS

Method: In order to elucidate the precursors of carotenoids and chlorophylls a and b, isophytol, phytol, geranyl geraniol and geranyl linalool were tested in a displacement assay with maize/corn (Zea mays, Poaceae) leaves. As both carotenoids and chlorophylls are formed in chloroplasts, maize plants were etiolated (blanched through keeping in the dark), then maize leaves were cut, placed in a medium containing both one of the above terpenoid alcohols and 14C-labelled acetate and irradiated for 30 hours, restarting biosynthesis of the pigments. After the test period, leaves were fixed by immersion in liquid nitrogen, extracted in acetone, the extract separated through chromatography and the formation of pigments (beta-carotene, lutein, chlorophylls a and b) determined and quantified through spectroscopy and chromatography (details in paper).
Based on the known fact that acetate is incorporated during regular biosynthesis of these pigments, the relative activity respectively suitability of tests compounds as precursors was determined by measuring the relative incorporation of radiolabelled acetate, standardised against pigment content: If in the same concentration of the respective pigment less acetate-14C is incorporated in comparison with acetate-only controls, this means that the test substance administered must have been incorporated instead.
Result: While it was shown that isophytol is not a significant precursor of beta-carotene, lutein or chlorophylls a or b, neither the uptake of radiolabelled acetate nor the biosynthesis of the above pigments were significantly reduced in the presence of isophytol in the medium (concentration not stated but probably 680 mg/l in medium with Tween 80 as an emulsifier). The authors note that "cet alcool diterpénique n'est pas toxique et que l'incorporation d'une émulsion ne freine pas l'infiltration de l'acétate de

sodium dans le parenchyme foliaire" (this diterpene alcohol is not toxic and that the incorporation of an emulsion [of isophytol] does not restrain the infiltration of sodium acetate into the leaf parenchyma).
Test substance: "du phytol et de l'isophytol Light, purifiés sur acide silicique" (isophytol and phytol Light, purified over silicate), no other information.
Conclusion: In a test measuring uptake of radiolebelled acetate into maize leaves, the presence of emulsified isophytol did not inhibit the uptake of acetate nor the formation of pigments.
Reliability: (4) not assignable
06-JAN-2006 (34)

Species: other terrestrial plant: cell cultures of the safflower, Carthamus tinctorius
Endpoint: other
Expos. period: 14 day(s)
Unit: mg/l
NOEC: = 100 -

Year: 1987
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: In order to study the processes of tocopherol (vitamin E) production by plants, cell cultures were established from safflower (Carthamus tinctorius, Asteraceae) callus. Potential tocopherol precursors were added to the liquid culture medium containing 1-week-old cultures at a concentration of 100 ppm in 20% Tween 80 as a solvent, cultures were then incubated for a further 2 weeks (details on culture media in paper). A vehicle control culture was administered only the appropriate amount of Tween 80. At the end of the test, the growth rate by mass was determined as well as alpha, beta, gamma, delta and total tocopherols as mg/100 g dry weight.
Result: The growth rates of isophytol- and phytol-treated cultures were very close to and probably not significantly different from the one for vehicle controls. However, there is no statistical evaluation of the results.
Test substance: Isophytol and phytol from Kuraray Co. Ltd, Japan. No further information on test substances.
Conclusion: At a concentration of 100 ppm in the liquid culture medium, isophytol did not show any noticeable toxicity to safflower cell cultures.
Reliability: (4) not assignable
06-JAN-2006 (51)

4.6.3 Toxicity to Soil Dwelling Organisms

Type: other: artificial sediment
Species: Caenorhabditis elegans (Worm (Nematoda), soil dwelling)
Endpoint: other: growth, egg production, fertility
Exposure period: 72 hour(s)
Unit: other: mg/kg artificial sediment (dry weight)
NOEC: = 15000 - measured/nominal

Year: 2002
GLP: no

Test substance: as prescribed by 1.1 - 1.4

Remark: Same test as in 4.6.1, Toxicity to Sediment-dwelling organisms, please see there for deatils.
Test substance: Isophytol from Teranol Lalden, Lot no. UU02013601, purity 98.0% (GC).
Conclusion: Even at a very high loading of 15000 mg isophytol per kg artificial sediment (dry weight), no effects on growth, egg production or fertility were observed. As Caenorhabditis is a very common sediment- and soil-dwelling nematode and as the substrate had an organic matter and particle size content comparable to sandy soils, this test is also judged to be predictive and useful for the soil compartment. Moreover, based on the short reproduction time for the nematodes and the endpoints chosen, it qualifies as a chronic and reproductive study.
Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
06-JAN-2006 (58)

Type: other: no data
Species: Caenorhabditis elegans (Worm (Nematoda), soil dwelling)
Endpoint: other: no data

Method: other: no data
Year: 1999
GLP: no data
Test substance: other TS: 3,7,11,15-tetramethylhexadec-1-en-3-ol, no data on source or purity

Remark: Isophytol had previously been detected in dichloromethane extracts of the marine red alga Plocamium costatum. It was subsequently tested in an assay with the ubiquitous soil- and sediment-dwelling nematode C. elegans.
Result: There is a very brief note that isophytol "had only weak" [but not quantified] effects. For details there is a reference to a paper in preparation, however, this could not be retrieved.
Reliability: (4) not assignable
22-MAY-2002 (66)

4.6.4 Toxicity to other Non-Mamm. Terrestrial Species

4.7 Biological Effects Monitoring

4.8 Biotransformation and Kinetics

Type: plant
Deg. product: yes
150-86-7 205-776-6 3,7,11,15-tetramethylhexadec-2-en-1-ol

Method: Plants and aphid infestation
Barley plants were grown from seeds (Hordeum vulgare L. cv. Aramir) on vermiculite at 25 °C with 14/10-h light/dark periods and were irrigated twice weekly with a Hoagland solution. Six days after sowing the experimental plants were

infested with 15 adults of the aphid Schizaphis graminum Rondani biotype C while control plants were left undisturbed. After another six days, both experimental and control plants were extracted as follows.
Isolation and extraction of epicuticular compounds
Epicuticular leaf waxes were obtained by immersion of the shoots in CH2Cl2 for 20 s. Extracts were filtered, dried with anhydrous sodium sulfate evaporated under vacuum and dissolved in 25 ml boiling methanol. Solutions were cooled to 20 °C to precipitate the major part of alkanes and fatty constituents. After centrifuging, the supernatants were concentrated and placed directly to Sephadex LH-20 (column 35X2 cm) with petrol-CHCl3-methanol (1:1:0.3 v/v/v) as the mobile phase. Fractions of approx. 10 ml were collected and monitored by thin-layer chromatography (TLC) procedures.
Isolation and extraction of tissue compounds from leaves
Fresh leaves from infested and non-infested plants were cut in small pieces. The material was extracted twice with either ethyl acetate or methanol for 48 h at room temperature. After filtering and drying over anhydrous sodium sulfate as above, the extracts were subjected to repeated column chromatography on silica gel (5-40 mm) using mixtures of hexane-ethyl acetate-methanol and hexane-ethyl acetate, respectively. These extracts were separated by TLC.
Acetylation of samples
Each fraction was acetylated with 5 ml acetic anhydride and 2 ml pyridine at 60 °C for 48 h. Excess reagents were eliminated by washing 3 times with water, adjusted to acidic pH and the extracted with CHCl3. After drying, residues dissolved in acetone and filtered. 5.5 µl heneicosane was added as an internal standard to the plants extracts immediately after homogenisation. Peak areas were integrated. Quantification was performed at least 3 times to ensure reproducibility.
GC-MS
Compounds were identified by their GC-MS fragmentation patterns. A Hewlett-Packard GC (HP-5972 series II) coupled to a mass-selective detector (8Ms 5972) was used for separation and detection.The GC-Ms was operated in the electron-impact mode at 70 eV. Helium was used as a carrier gas at 1 ml/min. Injection was performed in splitless mode (valve time 1 min) at an injection volume of 1 µl. Scan mode from 50 to 500 Da was used to identify the compounds. A 30 m X 0.5 mm inner diameter phased-silica capillary column coated with phenylmethyl silicone phase HP 5-MS (film thickness 25 µm) was used. The temperature program was 200 °C for 3 min, then an increment of 6 °/min up to 275 °C, at which the temperature was kept stable for 15 min.
Identification of compounds
Retention times and mass spectra of unknown compounds were compared with those of authentic material or from literature data. Mass spectra of the samples were entered into a VG Analytical data system 2000 on a Digital PDP 8 computer, together with literature spectra, for automatic computerised identification.

Result: Isophytol was detected at a concentration of 0.83% (area-%) in epicuticular leaf waxes of non-aphid-infested barley. It was not detected in the same leaf waxes of aphid-infested barley; however, in the latter, nearly the identical concentration (0.81%) of phytol was detected, which was not

present on the surface of non-infected leaves.
Isophytol was detected at a concentration of 1.40% (area-%) in methanol extracts of leaves of non-aphid-infested barley. It was not detected in the same methanol leaf extracts of aphid-infested barley; however, in these methanol extracts no phytol was detected, nor any other compound of the same concentration.
No isophytol was detected in ethyl acetate extracts of non-aphid-infested nor of infested barley leaves.

Conclusion: Isophytol is present in the epicuticular wax and within the leaf tissue of barley. Subsequent to infestation by aphids the isophytol disappears; while the epicuticular-wax isophytol may isomerise to phytol, which is found at similar concentrations in infested plants, the leafy-tissue isophytol seems to either dissipate, volatilise or be metabolised without obvious metabolites.

Reliability: (2) valid with restrictions
Detailed description of methods and results including analytical identification of compounds.

Flag: Critical study for SIDS endpoint

07-MAY-2002 (78)

Type: plant
Deg. product: not measured

Method: In order to study the processes of tocopherol (vitamin E) production by plants, cell cultures were established from safflower (Carthamus tinctorius, Asteraceae) callus. Potential tocopherol precursors were added to the liquid culture medium containing 1-week-old cultures at a concentration of 100 ppm in 20% Tween 80 as a solvent, cultures were then incubated for a further 2 weeks (details on culture media in paper). A vehicle control culture was administered only the appropriate amount of Tween 80. At the end of the test, the growth rate by mass was determined as well as alpha, beta, gamma, delta and total tocopherols as mg/100 g dry weight. Tocopherols were identified and quantified by TLC and HPLC.

Result: Whereas phytol enhanced total tocopherol content approximately 5 times in comparison with vehicle controls, with relative enhancement the highest for gamma and delta tocopherols, the effect of isophytol was to slightly decrease the tocopherol content; this decrease is assumed to be not significant, however, there is no statistical evaluation of the reported effects.

Test substance: Isophytol and phytol from Kuraray Co. Ltd, Japan. No further information on test substances.

Conclusion: In a plant cell culture assay, the addition of isophytol to the culture medium did not enhance tocopherol production by the cells, in contrast to phytol which showed a strong amplification of tocopherol production. Based on this test, isophytol is not an important or potent precursor of tocopherols.

Reliability: (4) not assignable

07-MAY-2002 (51)

Type: plant
Deg. product: not measured

Method: In order to elucidate the precursors of carotenoids and chlorophylls a and b, isophytol, phytol, geranyl geraniol and geranyl linalool were tested in a displacement assay with maize/corn (Zea mays, Poaceae) leaves. As both carotenoids and chlorophylls are formed in chloroplasts, maize plants were etiolated (blanched through keeping in the dark), then maize leaves were cut, placed in a medium containing both one of the above terpenoid alcohols and 14C-labelled acetate and irradiated for 30 hours. After the test period, leaves were fixed by immersion in liquid nitrogen, extracted in acetone, the extract separated through chromatography and the formation of pigments (beta-carotene, lutein, chlorophylls a and b) determined and quantified through spectroscopy and chromatography (details in paper).
Based on the known fact that acetate is incorporated during regular biosynthesis of these pigments, the relative activity respectively suitability of tests compounds as precursors was determined by measuring the relative incorporation of radiolabelled acetate, standardised against pigment content: If in the same concentration of the respective pigment less acetate-14C is incorporated in comparison with acetate-only controls, this means that the test substance administered must have been incorporated instead.

Result: Neither isophytol nor phytol significantly changed the rate of radiolabelled acetate incorporation in beta-carotene or lutein. Similarly, isophytol did not significantly change the rate of radiolabelled acetate incorporation in chlorophylls a or b. In contrast, phytol strongly reduced the rate of radiolabelled acetate incorporation in both chlorophyll a and b.

Conclusion: Isophytol is not a significant precursor in the biosynthesis of the carotenoids beta-carotene and lutein nor of chlorophylls a and b. In contrast, phytol was shown to be an important precursor of both chlorophylls a and b.

Reliability: (4) not assignable

07-AUG-2002 (34)

Type: plant
Deg. product: yes

Remark: See detailed discussion in chapter 1.7.2, Methods of Manufacture

Conclusion: No published evidence for ubiquitous formation of isophytol through degradation of chlorophyll or phytol derived from the former under aerobic conditions has been located, in disagreement with the corresponding remark in the Merck Index. However, there is good evidence for a minor degradative pathway of phytol under anaerobic conditions in sediment, resulting in isophytol, both in marine and freshwater.

Reliability: (4) not assignable

25-JUL-2002 (27)

4.9 Additional Remarks

Memo: Biological effects: Effect on rice leaf folder moths

Method: Male and female rice leaf folder moths, the food specialist Marasmia patnalis and the generalist Cnaphalocrocis medinalis from the same family, were used to record electroantennograms in the presence of defined volatile chemicals of plant origin. Moths were fixed in pipets with paraffin wax and recording electrodes were inserted into the base of one antenna. The antennae were bathed in a stream of activated-charcoal-filtered air delivered by a stainless-steel tube positioned 2 cm from the moth. Stimuli of 91 volatile plant substances of defined purity were given through 1-ml volumes of air saturated with the respective substance for 1 second. Positive (1-hexanol) and negative (heptane) standards were administered in the same manner after every 5 experimental stimulations.

Result: The response as measured by electroantennogram of two sympatric rice leaf folder moths to 91 volatile plant chemicals was similar to all compounds except three monoterpenes and two sesquiterpenes, including isophytol. The response of the food specialist Marasmia patnalis, that feeds on relatively few plants, most notably rice, to these substances was higher in comparison with the generalist Cnaphalocrocis medinalis. Further, while in M. patnalis both males and females showed similar electroantennographic reactions to isophytol, in C. medinalis the males showed a much stronger (but not statistically significant) reaction than the females.

Test substance: Isophytol of >99% purity from Target Synthesis Extraction, BP100, F-33107 Therigrace, France.

Conclusion: The general electroantennographic response of two rice moths to isophytol suggests that isophytol may work as a volatile plant-derived semiochemical for (at least) these moths, allowing them in conjunction with other semiochemicals to locate certain plant species for food or mating/egg-laying purposes.
The pronounced difference in reaction to isophytol in fodd-generalist C. medinalis males and females, with higher albeit non-significant reaction males, may additionally hint that isophytol, among other positive substances, may serve as a long-range attractant for males locating the females' host habitat.

Reliability: (2) valid with restrictions

17-APR-2002 (85)

5.0 Toxicokinetics, Metabolism and Distribution

5.1 Acute Toxicity

5.1.1 Acute Oral Toxicity

Type: LD50
Species: rat
Strain: other: Roche inbred strain
Sex: male/female
Vehicle: no data
Doses: no data on dose range, but highest tested dose was 8000 mg/kg bw
Value: > 8000 mg/kg bw

Method: other: former Roche gavage oral toxicity test
Year: 1973
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Method: As usual for this internal Roche testing scheme, groups of 5 or 10 animals per dosage were used. Administration was by gavage. Observation was either 5 or 10 days after administration, then the test animals were killed and dissected. Statistics were computed if applicable. Controls were historical with the same rat strains.
Result: LD10 > 8000 mg/kg bw (24 h)
LD50 > 8000 mg/kg bw (24 h)
LD90 > 8000 mg/kg bw (24 h)
Conclusion: Based on the LD10 value and the dosage group size (5 or 10), no rat from the highest-dosage group (8000 mg/kg bw) died within 24 hours of administration.
Reliability: (2) valid with restrictions
While this test is reported only in very abbreviated form, the acute toxicity group led by the author of the report performed large series of highly standardised toxicity tests in the late 1960s, 1970s and early 1980s. Serial testing in a dedicated facility assures dependably regular animal keeping, test substance administration, laboratory protocol and reporting. Therefore these internal data are regarded as valid and dependable.
Flag: Critical study for SIDS endpoint
10-JAN-2003 (28)

Type: LD50
Species: rat
Value: > 5400 mg/kg bw

Method: other: BASF-Test
Year: 1970
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Method: This test was performed 1970 in the toxicology laboratory of a chemcials company, with own-bred rats. As is typical for

older industry test reports, this one is relatively short, without detailed description of procedures. Briefly, an unstated number of rats were dosed by gavage isophytol at concentrations between 1% and 30% in aqueous emulsions with traganth gum. Post-dosing observation time was 7 days. The LD50 was calculated following Litchfield-Wilcoxon.

Result: The statistical oral LD50 in rats was > 6,400 mm3/kg bw, probably the highest tested dose repsectively concentration, which on the original test report is converted to > 5,400 mg/kg bw using a relative density of 0.844. Apathy and dispnoea are listed as symptoms.

Conclusion: In an industry-internal test the acute oral rat LD50 for isophytol was > 5,400 mg/kg bw.

Reliability: (4) not assignable

22-JUL-2002 (12)

Type: LD50
Species: rat
No. of Animals: 10
Vehicle: no data
Doses: at least 5000 mg/kg bw
Value: > 5000 mg/kg bw

Method: other: no data
Year: 1982
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: Application
Ten healthy male Wistar albino rats were dosed orally by gavage with 5000 mg/kg bw isophytol.
Observations
Observations for mortality and/or systemic effects were made 3-4 hours after dosing and once daily thereafter for 14 days. Then surviving animals were killed and gross necropsy was carried out on all animals.
(cited from the RIFM-FEMA Database entry)

Result: 2 out of 10 test animals were found dead during the observation period. Principal toxic signs are reported as diarrhoea and oily fur. Necropsy is summarised as: "survivors - 6 normal, kidney abnormalities; death - lung, kidney, stomach, thoracic cavity and intestinal abnormalities". There is no further or more detailed description of findings in the abstract of the report.

Conclusion: The acute oral LD50 for isophytol in male rats is greater than 5000 mg/kg bw. The acute oral LD0 is below 5000 mg/kg bw, but unspecified in this report.

Reliability: (4) not assignable
It must be noted that on the one-page abstract of the test report received, neither strain nor sex nor dosages nor any methodological information is given, but only the bare results, in contrast to the RIFM database printout where more information is available. The reliability of this result is therefore judged to be 4.

30-JUL-2002 (77)

Type: LD50
Species: mouse
Strain: other: Roche inbred strain

Sex: male/female
Vehicle: no data
Doses: no data on dose range, but highest tested dose was 8000 mg/kg bw
Value: > 8000 mg/kg bw

Method: other: former Roche gavage oral toxicity test
Year: 1980
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Method: As ususal for this internal Roche testing scheme, groups of 5 or 10 animals per dosage were used. Administration was by gavage. Observation was either 5 or 10 days after administration, then the test animals were killed and dissected. Statistics were computed if applicable. Controls were historical with the same mouse strains.
Result: LD10 = 8000 mg/kg bw (24 h), = 8000 mg/kg (10 d)
LD50 > 8000 mg/kg bw (24 h), > 8000 mg/kg (10 d)
LD90 > 8000 mg/kg bw (24 h), > 8000 mg/kg (10 d)
Conclusion: Based on the LD10 value and the dosage group size, which must have been 10 in this case, one mouse from the highest-dosage group (8000 mg/kg bw) died within 24 hours of administration.
Reliability: (2) valid with restrictions
While this test is reported only in very abbreviated form, the acute toxicity group led by the author of the report performed large series of highly standardised toxicity tests in the late 1960s, 1970s and early 1980s. Serial testing in a dedicated facility assures dependably regular animal keeping, test substance administration, laboratory protocol and reporting. Therefore these internal data are regarded as valid and dependable.
22-JUL-2002 (29)

Type: LD50
Species: rat
Strain: other: Roche inbred strain
Sex: male/female
Vehicle: no data
Doses: no data on dose range, but highest tested dose was 12000 mg/kg bw
Value: > 12000 mg/kg bw

Method: other: former Roche gavage oral toxicity test
Year: 1980
GLP: no
Test substance: other TS: Isophytol crude

Method: As ususal for this internal Roche testing scheme, groups of 5 or 10 animals per dosage were used. Administration was by gavage. Observation was either 5 or 10 days after administration, then the test animals were killed and dissected. Statistics were computed if applicable. Controls were historical with the same rat strains.
Result: LD10 > 12,000 mg/kg bw (24 h), > 12,000 mg/kg bw (10 d)
LD50 > 12,000 mg/kg bw (24 h), > 12,000 mg/kg bw (10 d)
LD90 > 12,000 mg/kg bw (24 h), > 12,000 mg/kg bw (10 d)
Conclusion: Based on the LD10 value and the dosage group size (5 or 10), no rat from the highest-dosage group (12000 mg/kg bw) died

within 24 hours nor within 10 days of administration.
Reliability: (2) valid with restrictions
While this test is reported only in very abbreviated form, the acute toxicity group led by the author of the report performed large series of highly standardised toxicity tests in the late 1960s, 1970s and early 1980s. Serial testing in a dedicated facility assures dependably regular animal keeping, test substance administration, laboratory protocol and reporting. Therefore these internal data are regarded as valid and dependable.
17-APR-2002 (29)

Type: LD50
Species: mouse
Strain: other: Roche inbred strain
Sex: male/female
Vehicle: no data
Doses: no data on dose range, but highest tested dose was 8000 mg/kg
Value: > 8000 mg/kg bw

Method: other: former Roche gavage oral toxicity test
Year: 1980
GLP: no
Test substance: other TS: Isophytol crude

Method: As ususal for this internal Roche testing scheme, groups of 5 or 10 animals per dosage were used. Administration was by gavage. Observation was either 5 or 10 days after administration, then the test animals were killed and dissected. Statistics were computed if applicable. Controls were historical with the same mouse strains.
Result: LD10 > 8000 mg/kg bw (24 h), > 8000 mg/kg (10 d)
LD50 > 8000 mg/kg bw (24 h), > 8000 mg/kg (10 d)
LD90 > 8000 mg/kg bw (24 h), > 8000 mg/kg (10 d)
Conclusion: Based on the LD10 value and the dosage group size (5 or 10), no mouse from the highest-dosage group (8000 mg/kg bw) died within 24 hours nor within 10 days of administration.
Reliability: (2) valid with restrictions
While this test is reported only in very abbreviated form, the acute toxicity group led by the author of the report performed large series of highly standardised toxicity tests in the late 1960s, 1970s and early 1980s. Serial testing in a dedicated facility assures dependably regular animal keeping, test substance administration, laboratory protocol and reporting. Therefore these internal data are regarded as valid and dependable.
17-APR-2002 (29)

Type: LD0
Species: mouse
Strain: other: NMRI BR mice (SPF)
Sex: male/female
No. of Animals: 6
Vehicle: other: maize/corn oil
Doses: 2000 mg/kg bw
Value: = 2000 mg/kg bw

Method: other: dose range-finding test to an in vivo micronucleus test
Year: 2002
GLP: yes

Test substance: as prescribed by 1.1 - 1.4

Result: In a range-finding test, 3 young adult male and 3 young adult female mice were dosed with 2000 mg isophytol/kg bw in maize/corn oil. There were no deaths nor any negative effects within the scheduled observation period of 72 hours.
Reliability: (2) valid with restrictions
This pre-test was performed under GLP, but it was technically not an acute toxicity test, hence reliability 2.
01-JUL-2002 (73)

5.1.2 Acute Inhalation Toxicity

Type: other: IRT (respiratory toxicity test)
Species: rat
Strain: no data
Sex: no data
No. of Animals: 12
Doses: isophytol-enriched air, no analytical concentration given
Exposure time: 8 hour(s)

Method: other: BASF-Test
Year: 1970
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Method: Clean air was first saturated with water vapour at 20 respectively at 100 °C and subsequently bubbled through a 5-cm-high column of isophytol. In both tests, 12 animals each were exposed to this isophytol-laden air and observed for a duration of 8 hours. Then, the animals were killed and dissected.
Remark: Assuming full saturation of the air, with partial pressure of gaseous isophytol corresponding to the vapour pressure, the negative result corresponds to a NOEC of 0.3 mg/m3. This has been calculated as gaseous isophytol but not as test substance in the form of an aerosol.
Result: No effects and no deaths were observed during and after the exposure of 8 hours to isophytol-enriched air, both after at 20 and 100 °C. In the first group, no behavioural effects were observed nor was there anything remarkable at dissection. In the second (100-°C-saturated) group, initial escape attempts were noted, but no other observations during the exposure or at dissection.
Conclusion: No effects due to inhalation of isophytol-saturated air were observed over 8 hours.
Reliability: (2) valid with restrictions
This test was performed in a professional industry toxicology laboratory. While the report is very brief by today's standards there is a short overview of the setup, number of animals, pretreatment of the air and description of deaths (0/12 in both groups) was well as behavioural and dissection observations, hence reliability was accepted as 2.
Flag: Critical study for SIDS endpoint
06-JAN-2006 (12)

Type: other: IRT (respiratory toxicity test)
Species: mouse

Strain: no data
Sex: no data
No. of Animals: 12
Doses: isophytol-saturated air, no analytical concentration given
Exposure time: 8 hour(s)

Method: other: BASF-Test
Year: 1970
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Method: Clean air was bubbled through a 5-cm-high layer of isophytol, the atmosphere was saturated with steam at 20 °C. 12 test animals were exposed to this isophytol-laden air and observed for a test duration of 8 hours.
Remark: Assuming full saturation of the air, with partial pressure of gaseous isophytol corresponding to the vapour pressure, the negative result corresponds to a NOEC of 0.3 mg/m3. This has been calculated as gaseous isophytol but not as test substance in the form of an aerosol.
This test provides supportive data to the first inhalative test listed.
Result: No effects were observed during and after the exposure period of 8 hours.
Conclusion: No effects due to inhalation of isophytol-saturated air were observed over 8 hours.
Reliability: (2) valid with restrictions
This test was performed in a professional industry toxicology laboratory. While the report is very brief by today's standards there is a short overview of the setup, number of animals, pretreatment of the air and description of deaths (0/12 in both groups) was well as behavioural and dissection observations, hence reliability was accepted as 2.
Flag: Critical study for SIDS endpoint
06-JAN-2006 (12)

Type: other: IRT (respiratory toxicity test)
Species: guinea pig
Strain: no data
Sex: no data
No. of Animals: 12
Doses: isophytol-saturated air, no analytical concentration given
Exposure time: 8 hour(s)

Method: other: BASF-Test
Year: 1970
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Method: Clean air was bubbled through a 5-cm-high layer of isophytol, the atmosphere was saturated with steam at 20 °C. 12 test animals were exposed to this isophytol-laden air and observed for a test duration of 8 hours.
Remark: Assuming full saturation of the air, with partial pressure of gaseous isophytol corresponding to the vapour pressure, the negative result corresponds to a NOEC of 0.3 mg/m3.
Result: No effects were observed during and after the exposure period of 8 hours.
Conclusion: No effects due to inhalation of isophytol-saturated air were

observed over 8 hours.

Reliability: (2) valid with restrictions
This test was performed in a professional industry toxicology laboratory. While the report is very brief by today's standards there is a short overview of the setup, number of animals, pretreatment of the air and description of deaths (0/12 in both groups) was well as behavioural and dissection observations, hence reliability was accepted as 2.

19-FEB-2003 (12)

5.1.3 Acute Dermal Toxicity

Type: LD50
Species: rabbit
Strain: no data
Sex: no data
No. of Animals: 10
Vehicle: other: none
Doses: at least 5000 mg/kg bw
Value: > 5000 mg/kg bw

Method: other: no data
Year: 1982
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: Application
Ten healthy albino rabbits received one dermal application of test material. The test material was applied to clipped, intact or abraded abdominal skin under occluded patches for 24 hours of contact.
Observation
Observations for mortality and/or systemic effects were made daily for 14 days following application. Dermal reactions were scored on days 1, 7 and 14 after application using the Draize scoring system. On day 14 after application, test animals were killed and gross necropsy was performed on all animals.
(cited from the RIFM-FEMA Database entry)

Result: At a limit dose of 5000 mg/kg bw there were no deaths over the observation period. As the principal toxic sign, "few faeces" were observed. Skin reactions are described as "moderate/severe", without further details. At necropsy, 8 animals were without diagnostic findings while 2 showed "treated skin and intestinal abnormalities"; these abnormalities are not described in detail.

Conclusion: The acute dermal LD50 for isophytol is greater than 5000 mg/kg bw. At 5000 mg/kg bw there were no deaths at all in the 10 test animals.
Observations during 14 days after application and necropsy showed skin abnormalities at treated sites in 2 out of 10 animals and some changes to the function and structure of the digestive tract, all of which were judged to be related to isophytol. However, with the exception of a short description of the observation of "skin abnormalities" and "few faeces" there are no further details available.

Reliability: (4) not assignable
It must be noted that on the one-page abstract of the test

report received, neither strain nor sex nor dosages nor any methodological information is given, but only the bare results, in contrast to the RIFM database printout where more information is available. The reliability of this result is therefore judged to be 4.

Flag: Critical study for SIDS endpoint

07-AUG-2002 (77)

Type: other: dermal toxicity and phototoxicity
Species: guinea pig
Strain: Hartley
Sex: female
No. of Animals: 5
Vehicle: other: acetone
Doses: 50%, 30%, 10% or 5% isophytol in acetone applied to skin in circles of 1.5 cm diameter each

Year: 1999
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: 5 female Hartley guinea pigs, weighing 310-415 g at start of the experiment, were used for the test. The hair on the back of the animals was cut using an electric hair clipper and an electric shaver. 4 hours after depilation, the test article was applied in concentrations of 50%, 30%, 10% or 5% dissolved in acetone on circles of 1.5 cm diameter each on the depilated area of the back of the animals. A total of 8 such spot applications was made, one concentration each on the left and right side of the animals.
Immediately after application, one side of the animals was covered with aluminium foil. The other side was irradiated with a bank of 6 ultraviolet lights (model FL-40 BLB lamps, 40-watt tubes supplied by Toshiba co., Japan; emission spectrum 320-400 nm). The lighting installation had previously been equipped with window glass filters in order to eliminate radiation below 320 nm- The distance from the light sources to the skin was 10 cm. Irradiation was continued for 70 min.
The irradiated and non-irradiated test sites were observed for skin reactions 24 and 48 hours after irradiation. The intensity of skin reactions was graded from 0 to 8 according to criteria and the scoring system for assessment by Draize. The scores for erythema and oedema at 24 hours post-irradiation were totalled for the 5 guinea pigs and this total was divided by 5 to give the primary irritation index.

Result: No phototoxicity was determined in this test from the primary irritation indices, however, neither the single values nor the average of the latter are stated. No dermal toxicity was reported. Further, there was no difference observed between the irradiated and the non-irradiated sides.

Conclusion: No phototoxicity nor dermal toxicity was observed in this test, however, it must be noted that there was no difference between irradiated and non-irradiated sites. Based on the available data there is no evidence for phototoxicity of isophytol.

Reliability: (2) valid with restrictions
Excerpt (1999 English translation, confirmed by study

director of Takasago Safety Assessment Laboratory to reflect correctly the Japanese original from 1982) of the original report. Detailed description of animals, method and grading, reliability judged as 2.

19-FEB-2003 (100)

5.1.4 Acute Toxicity, other Routes

Type: LD50
Species: mouse
Strain: no data
Sex: no data
Vehicle: other: water with traganth gum
Doses: 1-30% aqueous emulsions
Route of admin.: i.p.
Exposure time: 168 hour(s)
Value: ca. 169 mg/kg bw

Method: other: BASF-Test
Year: 1970
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Method: This test was performed 1970 in the toxicology laboratory of a chemcials company, with own-bred mice. As is typical for older industry test reports, this one is relatively short, without detailed description of procedures. Briefly, an unstated number of mice were dosed by intraperitoneal injection isophytol at concentrations between 1% and 30% in aqueous emulsions with traganth gum. Post-dosing observation time was 7 days. The LD50 was calculated following Litchfield-Wilcoxon.
Result: The acute i.p. LD50 for isophytol in aqueous/traganth gum emulsion is given as "ca. 200 mm3/kg bw", amended in the report as ca. 169 mg/kg bw based on a relative density of 0.844. Behavioural reactions of the mice are described as a stagger, trembling and dispnoea. On dissection, intra-abdominal adhesions and incorporations of substance were noted.
Conclusion: This test was performed in a professional industry toxicology laboratory. While the report is very brief by today's standards there is a short overview of the setup, application, symptoms and statistics, hence reliability was accepted as 2.
Reliability: (2) valid with restrictions

22-JUL-2002 (12)

5.2 Corrosiveness and Irritation

5.2.1 Skin Irritation

Species: guinea pig
Concentration: 12.5 %
Exposure: Occlusive
No. of Animals: 30
Vehicle: other: see methods
Result: irritating

Method: other: OECD Guideline 406, Skin Sensitisation
Year: 1996
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Method: Animals and housing
In total, 30 female albino Dunkin Hartley guinea pigs (20 test and 10 control animals) were used. Animals were supplied by D. Hall, Darley Oaks, Burton-on-Trent, UK. On delivery they were in a weight range of 300-350 g and healthy on external inspection. Animals for the range-finding study were acclimatised for 5 days while the animals for the main test were acclimatised for 19 days. Animals were housed in groups of 5 in stainless steel cages and were identified by the number of the cage to which they were allotted and within each cage by ear tattoo.
The animal rooms were air-conditioned with a temperature in the range of 20-23 °C and relative humidity in the range of 36-68% during acclimatisation and study periods. Fluorescent lighting gave a controlled 12-h-light (06:00-18:00)/12-h-dark cycle.
The animals were fed pelleted SQC FD1 guinea pig diet with added vitamin C (Special Diets Services, Witham, Essex, UK) and mains drinking water ad libitum. Certificates of analysis for both diet and drinking water are held on file at Quintiles.
Test procedures
1) Intradermal injection range-finding study
A ranging study was performed in 1 animal which was pretreated with 4 intradermal injections of isophytol in a 1:1 mixture of Freund's Complete Adjuvant (FCA) and water. After 7 days' delay, 0.1-ml aliquots of 50%, 25%, 10%, 5%, 1% and 0.5% v/v concentrations of isophytol in light liquid paraffin were injected intradermally into the flanks of the guinea pig. The animal was examined on the day of dosing and then daily for another 5 days; the response at each injection site was noted. From the results of this range-finder it was concluded that 1% isophytol v/v in light liquid paraffin would not provoke an unacceptable irritant response and this concentration was selected for use in the main study.
2) Topical irritancy ranging study
The potential of isophytol to cause skin irritation was assessed with a topical ranging study using 4 animals in a weight range of 387-404 g that had been previously treated with 1:1 FCA/water as above. The concentrations used were undiluted and 50%, 25% and 12.5% isophytol in ethanol. 4 patches of Whatman No 3 filter paper, 2x2 cm, were each saturated with a different test concentration, affixed to the clipped fur on the back and flanks of each of the 4 animals, covered with Blenderm surgical tape as an occlusive barrier and the patches held in place for 24 h by encircling the trunk of each animal with Elastoplast adhesive bandage. 24h and 48 h after removing the patches and dressings the animals were examined under a standard light source (artificial daylight for the assessment of colour) and any reaction at the treated sites was assessed. From this pre-test it was concluded that the 50% concentration was the minimum irritant concentration and was suitable for the

topical induction stage of the main test, while 25% was non-irritant and therefore suitable for use in the challenge stage of the main study.
3) Main study induction
30 animals were selected and randomly allocated to a group of 20 test and 10 control animals using a stratified bodyweight procedure. Animals were in a weight range of 477-564 g on day 1 of the main study. The dorsal area between the shoulders of each animal was clipped free of fur and 3 pairs of intradermal injections were made in this area. The dosing volume was 0.1 ml and each pair of injections consisted of
a) 50% FCA/water, 1% isophytol in light paraffin and 1% isophytol in 1:1 FCA/water for the test group and
b) 50% FCA/water, light liquid paraffin and 50% light liquid paraffin in FCA/water in the controls.
24 hours after intradermal induction all test and control animals showed moderate irritation at the injection sites.
6 days after intradermal induction the injection area was clipped free of fur. The epicutaneous induction of sensitisation was conducted under occlusion with 50% v/v isophytol in ethanol. Whatman No 3 filter papers, 2x2 cm, were each saturated with 50% v/v isophytol in ethanol, affixed to the clipped fur on the back and flanks of the animals, covered with Blenderm surgical tape as an occlusive barrier and the patches held in place for 48 h. Control animals were painted with 0.5 ml of 10% w/v sodium lauryl sulfate to mimic the response to isophytol expected in test animals, then treated only with ethanol-saturated papers and occluded for 48 h.
24 hours after removal of the patches, all control and test animals exhibited moderate skin irritation at the treated site.
4) Main study challenge
Two weeks after epidermal induction, the challenge was completed by epicutaneous application of isophytol in the highest non-irritating concentration, ie, 25% v/v in ethanol (as determined in the range-finding phase of the study) under occlusive dressing as above on the left flank of both test and control animals, while the right flank was treated with ethanol alone. Patches and dressings were removed after 24 hours. Cutaneous reactions, ie, erythema and eschar as well as oedema formation were evaluated at 24 and 48 hours after removal of the dressing.
As positive reactions to 25% v/v were noted also in the control animals, this concentration was found to be irritating. Therefore, a re-challenge was conducted 7 days later using 12.5% v/v isophytol in ethanol on the left flank and ethanol only on the right as above. As in the initial challenge, skin reactions were evaluated 24 and 48 hours after removal of dressings.

Result: First challenge
15 of the 20 test animals exhibited positive skin reactions after the first challenge with 25% v/v isophytol in ethanol. However, 6 of the 10 control animals also responded positively to challenge with 25% v/v isophytol. None of the test or control animals exhibited positive responses to ethanol alone.
Re-challenge
As these results suggested that 25% v/v isophytol in ethanol

produced an irritant response, a re-challenge was conducted 7 days later with 12.5% v/v isophytol in ethanol. 7/20 test animals exhibited positive responses following the re-challenge application, giving a response incidence of 35%. 2/10 control animals also exhibited positive reactions, giving a response incidence of 20%. None of the test or control animals exhibited positive responses to ethanol alone.

Conclusion: Based on the results of this test, it was considered that the skin responses elicited by isophytol are of a primary irritant nature rather than indicating sensitisation in the guinea pig. It can be assumed therefore that accidental or occasional exposure to isophytol as such may potentially give rise to irritant skin reactions in man; the risk of cutaneous sensitisation is relatively low, however, it cannot be entirely excluded.

Reliability: (2) valid with restrictions
In an OECD 406 skin sensitisation study under GLP, skin irritation due to isophytol was made highly likely. This test was not a proper irritation study, however, it was a skin study conducted under GLP at a reliable contract laboratory under the monitoring of a dermal toxicology specialist. Hence, the reliability is regarded as 2.

Flag: Critical study for SIDS endpoint

22-JUL-2002 (35)

Species: rabbit
Concentration: undiluted
Exposure: no data
Result: irritating
EC classificat.: irritating

Method: other: BASF-Test
Year: 1970
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Method: In a 1970 test in a professional industry toxicology laboratory, an unstated number of rabbits were applied an unstated volume of undiluted isophytol for 1, 5 and 15 minutes on the shaven skin of the bakc as well as for 20 hours on the back and for 20 hours on the ear. Skin reactions were graded 24 hours and 8 days after application.

Result: At 24 hours after application, symtoms are described as follows: very heavy erythema on all sites and for all exposure times, additionally heavy oedema and transverse fold formation on the skin of the back after 20 hours exposure. At 8 days after exposure, there was heavy scaling of all skin sites and all exposure times, additionally heavy erythema on the skin of the back after 20 hours exposure.

Conclusion: Application of undiluted isophytol to rabbit skin for different exposure times elicited reactions consistent with irritation, both at 1 and 8 days after application. Isophytol must be regarded as having a potential for skin serious irritation.

Reliability: (2) valid with restrictions
This test was performed 1970 in a professional industry toxicology laboratory. While test procedures are given in very brief fashion, the results are given in a table that lists reactions after 1, 5 and 15 minutes as well as 20

hours exposure the skin on the back (the latter also the ear) at 24 hours and 8 days post-exposure, with grading of reactions in five categories. Based on these data the test is judged to be of validity 2.

Flag: Critical study for SIDS endpoint

22-JUL-2002 (7)

Species: guinea pig
Concentration: 50 %
Exposure: Open
Exposure Time: 24 hour(s)
No. of Animals: 5
Vehicle: other: acetone
PDII: 1.8
Result: irritating

Year: 1982
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: In a combined acute dermal irritation and phototoxicity test, five female Heartley albino guinea pigs (Japan SLC, Inc, 310-415 g at start of experiment) had the hair on the back removed with an electric clipper and shaver. 4 hours after depilation, isophytol at concentrations of 5%, 10%, 30% and 50% dissolved in acetone was applied on a circle of 1.5 cm diameter in the depilated area on both sides of every animals, so that a total of 8 such applications were made, two of each concentration and one each on both sides. Immediately after application, one side of the animal was covered with aluminium foil. The other was irradiated with a bank of 6 ultraviolet lights (model FL-40 BLB lamps, 40-watt tubes, emission 320-400 nm; Toshiba Co, Japan) that habe been equipped with a window glass filter to eliminate radiation below 320 nm. The distance from the light source to the skin was 10 cm, irradiation was continued for 70 min. Covered and uncovered treatment sites were observed and graded for skin reactions at 24 and 48 hours after irradiation. The intensity of reactions were scored according to Draize (1959). Scores for erythema and oedema at the 24-hour reading were averaged to give the primary irritation index.

Result: Isophytol dose-dependently irritated both the irradiated and the non-irradiated guinea pig skin:

Isophytol concentration (%)	5	10	30	50
Primary irritation index	0.4	0.9	1.6	1.8
Irritation rate	3/5	4/5	5/5	5/5

The authors state that "phototoxicity was not determined, but there were no differences in the reaction between the UV-radiated site and the non-radiated site".

Source: RIFM (Research Institute of Fragrance Materials) Monograph on Isophytol, version 30 Nov 2001.

Conclusion: Isophytol was concentration-dependently irritating to guinea pig skin, however, there was no indication for phototoxicity.

Reliability: (2) valid with restrictions
Excerpt (1999 English translation, confirmed by study director of Takasago Safety Assessment Laboratory to reflect correctly the Japanese original from 1982) of the original report. Detailed description of animals, method and grading,

reliability judged as 2.
19-FEB-2003 (100)

Species: rabbit
Concentration: other: probably undiluted
Exposure: Occlusive
Exposure Time: 24 hour(s)
No. of Animals: 10
Vehicle: other: probably none, undiluted test article
Result: irritating
EC classificat.: irritating

Year: 1982
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Method: Application
In the course of an acute dermal toxicity test, 10 healthy albino rabbits received one dermal application of test material of 5 g/kg bw. The test material was applied to clipped, intact or abraded abdominal skin and kept under occluded patches for 24 hours of contact.
Observations
Observations for mortality and/or systemic effects were made daily for 14 days following application. Dermal reactions were scored on days 1, 7 and 14 after application using the Draize scoring system. On day 14 after application, test animals were killed and gross necropsy was performed on all animals.
Result: Dermal reactions according to the Draize scoring system are described as "moderate to severe".
At necropsy, 8 animals were without diagnostic findings while 2 had "skin abnormalities" at treated sites, which are not described in detail.
Conclusion: Isophytol, applied to intact or abraded skin probably as the pure test material at a dose of 5000 mg/kg bw, was a moderate to severe skin irritant as determined by Draize score and as confirmed at necropsy in 2/10 animals by skin abnormalities at treated sites.
Reliability: (4) not assignable
Reliability may in fact be better than 4, but no details on observed reactions in single animals are available, hence reliability score 4 was assigned.
30-JUL-2002 (77)

Species: human
Concentration: 10 %
Exposure: Occlusive
Exposure Time: 48 hour(s)
No. of Animals: 27
Vehicle: petrolatum
Result: not irritating

Year: 1981
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: In a pretest for a human maximisation (sensitisation) test, a closed-patch test was performed on 27 healthy male and female human volunteers. The test materials including

isophytol were pretested on all subjects in order to determine whether sodium lauryl sulfate pretreatment was required. Isophytol was diluted to 10% concentration in petrolatum and a patch of this solution was applied to normal back skin for 48 hours under occlusion.
After 48 hours exposure, the patches were taken off and skin reactions were scored.

Result: The author states in his synopsis that isophytol [as a 10% solution in petrolatum, according to the RIFM Database entry] did not elicit any¨significant skin reactions after 48 hours of occlusive application in 27 human volunteers. However, approximately one-third of the subjects were slightly irritated by the sodium lauryl sulfate pretreatment.

Conclusion: At a concentration of 10% in petrolatum, isophytol is not a human skin irritant.

Reliability: (4) not assignable
Reliability may be better than 4 but there are details missing regarding dose administered and vehicle in the synopsis received, hence reliability 4 was assigned.

30-JUL-2002 (46)

5.2.2 Eye Irritation

Species: rabbit
Concentration: undiluted
Dose: .05 ml
Result: slightly irritating

Method: other: BASF-Test
Year: 1970
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Method: Undiluted isophytol was applied in a single dose of 50 mm3 to the surface of the eyes of an unspecified number of rabbits.
Reactions were observed and graded at 1 hour, 24 hours and 8 days after application.

Remark: The dose of 0.05 ml applied in this test in 1970 differs from the default dose in the current OECD guideline of 0.1 ml.

Result: The following effects were observed: light reddening at 1 hour and light reddening and light dulling of the eye at 24 hours post-application. There were no lasting effects after 8 days. Control rabbits that were dosed physiological saline did not show any remarkable reactions at any time.

Conclusion: While undiluted isophytol may produce some symptoms of eye irritation in the short term, these recede over time. No remaining effects were noted 8 days after application. Based on these results, isophytol is regarded of having a slight potential for eye irritation.

Reliability: (2) valid with restrictions
This test was performed 1970 in a professional industry toxicology laboratory. While test procedures are given in very brief fashion, the results are given in a table that lists reactions after 1 and 24 hours and 8 days post-exposure, with grading of reactions in five possible categories. Based on these data the test is judged to be of validity 2.

Flag: Critical study for SIDS endpoint
06-JAN-2006 (7)

5.3 Sensitization

Type: Guinea pig maximization test
Species: guinea pig
Concentration 1st: Induction 1 % intracutaneous
2nd: Induction 50 % occlusive epicutaneous
3rd: Challenge 25 % occlusive epicutaneous
No. of Animals: 30
Vehicle: other: see methods
Result: ambiguous
Classification: not sensitizing

Method: OECD Guide-line 406 "Skin Sensitization"
Year: 1996
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Method: Animals and housing
In total, 30 female albino Dunkin Hartley guinea pigs (20 test and 10 control animals) were used. Animals were supplied by D. Hall, Darley Oaks, Burton-on-Trent, UK. On delivery they were in a weight range of 300-350 g and healthy on external inspection. Animals for the range-finding study were acclimatised for 5 days while the animals for the main test were acclimatised for 19 days. Animals were housed in groups of 5 in stainless steel cages and were identified by the number of the cage to which they were allotted and within each cage by ear tattoo.
The animal rooms were air-conditioned with a temperature in the range of 20-23 °C and relative humidity in the range of 36-68% during acclimatisation and study periods. Fluorescent lighting gave a controlled 12-h-light (06:00-18:00)/12-h-dark cycle.
The animals were fed pelleted SQC FD1 guinea pig diet with added vitamin C (Special Diets Services, Witham, Essex, UK) and mains drinking water ad libitum. Certificates of analysis for both diet and drinking water are held on file at Quintiles.
Test procedures
1) Intradermal injection range-finding study
A ranging study was performed in 1 animal which was pretreated with 4 intradermal injections of isophytol in a 1:1 mixture of Freund's Complete Adjuvant (FCA) and water. After 7 days' delay, 0.1-ml aliquots of 50%, 25%, 10%, 5%, 1% and 0.5% v/v concentrations of isophytol in light liquid paraffin were injected intradermally into the flanks of the guinea pig. The animal was examined on the day of dosing and then daily for another 5 days; the response at each injection site was noted. From the results of this range-finder it was concluded that 1% isophytol v/v in light liquid paraffin would not provoke an unacceptable irritant response and this concentration was selected for use in the main study.
2) Topical irritancy ranging study
The potential of isophytol to cause skin irritation was assessed with a topical ranging study using 4 animals in a

weight range of 387-404 g that had been previously treated with 1:1 FCA/water as above. The concentrations used were undiluted and 50%, 25% and 12.5% isophytol in ethanol. 4 patches of Whatman No 3 filter paper, 2x2 cm, were each saturated with a different test concentration, affixed to the clipped fur on the back and flanks of each of the 4 animals, covered with Blenderm surgical tape as an occlusive barrier and the patches held in place for 24 h by encircling the trunk of each animal with Elastoplast adhesive bandage. 24h and 48 h after removing the patches and dressings the animals were examined under a standard light source (artificial daylight for the assessment of colour) and any reaction at the treated sites was assessed. From this pre-test it was concluded that the 50% concentration was the minimum irritant concentration and was suitable for the topical induction stage of the main test, while 25% was non-irritant and therefore suitable for use in the challenge stage of the main study.
3) Main study induction
30 animals were selected and randomly allocated to a group of 20 test and 10 control animals using a stratified bodyweight procedure. Animals were in a weight range of 477-564 g on day 1 of the main study. The dorsal area between the shoulders of each animal was clipped free of fur and 3 pairs of intradermal injections were made in this area. The dosing volume was 0.1 ml and each pair of injections consisted of
a) 50% FCA/water, 1% isophytol in light paraffin and 1% isophytol in 1:1 FCA/water for the test group and
b) 50% FCA/water, light liquid paraffin and 50% light liquid paraffin in FCA/water in the controls.
24 hours after intradermal induction all test and control animals showed moderate irritation at the injection sites. 6 days after intradermal induction the injection area was clipped free of fur. The epicutaneous induction of sensitisation was conducted under occlusion with 50% v/v isophytol in ethanol. Whatman No 3 filter papers, 2x2 cm, were each saturated with 50% v/v isophytol in ethanol, affixed to the clipped fur on the back and flanks of the animals, covered with Blenderm surgical tape as an occlusive barrier and the patches held in place for 48 h. Control animals were painted with 0.5 ml of 10% w/v sodium lauryl sulfate to mimic the response to isophytol expected in test animals, then treated only with ethanol-saturated papers and occluded for 48 h.
24 hours after removal of the patches, all control and test animals exhibited moderate skin irritation at the treated site.
4) Main study challenge
Two weeks after epidermal induction, the challenge was completed by epicutaneous application of isophytol in the highest non-irritating concentration, ie, 25% v/v in ethanol (as determined in the range-finding phase of the study) under occlusive dressing as above on the left flank of both test and control animals, while the right flank was treated with ethanol alone. Patches and dressings were removed after 24 hours. Cutaneous reactions, ie, erythema and eschar as well as oedema formation were evaluated at 24 and 48 hours after removal of the dressing.
As positive reactions to 25% v/v were noted also in the

control animals, this concentration was found to be irritating. Therefore, a re-challenge was conducted 7 days later using 12.5% v/v isophytol in ethanol on the left flank and ethanol only on the right as above. As in the initial challenge, skin reactions were evaluated 24 and 48 hours after removal of dressings.

Result: First challenge
15 of the 20 test animals exhibited positive skin reactions after the first challenge with 25% v/v isophytol in ethanol. However, 6 of the 10 control animals also responded positively to challenge with 25% v/v isophytol. None of the test or control animals exhibited positive responses to ethanol alone.
Re-challenge
As these results suggested that 25% v/v isophytol in ethanol produced an irritant response, a re-challenge was conducted 7 days later with 12.5% v/v isophytol in ethanol. 7/20 test animals exhibited positive responses following the re-challenge application, giving a response incidence of 35%. 2/10 control animals also exhibited positive reactions, giving a response incidence of 20%. None of the test or control animals exhibited positive responses to ethanol alone.
Reactions of single test animals:

Concentration	25%	25%	12.5%	12.5%
Time	24 h	48 h	24 h	48 h
Test group:	1	1	0	0
	0	0	0	0
	1	1	1	1
	1	2	0	0
	2	2	0	0
	1	1	0	0
	2	2	1	0
	0	0	0	0
	2	2	2	2
	0	0	0	0
	2	1	0	0
	1	0	0	0
	0	0	2	1
	1	1	2	2
	2	1	0	0
	0	0	0	0
	1	1	0	0
	2	2	2	2
	2	1	1	1
	1	1	0	0
Control group:	0	0	2	2
	0	0	0	0
	1	2	0	0
	0	0	0	0
	1	1	0	0
	1	1	0	1
	2	2	0	0
	0	0	0	0
	2	2	0	0
	2	2	0	0

0 = no reaction
1 = discrete or patchy erythema

2 = moderate or confluent erythema
3 = intense erythema and swelling

Conclusion: Based on the results of this test, it was considered that the skin responses elicited by isophytol are of a primary irritant nature rather than indicating sensitisation in the guinea pig. It can be assumed therefore that accidental or occasional exposure to isophytol as such may potentially give rise to irritant skin reactions in man; the risk of cutaneous sensitisation is relatively low, however, it cannot be entirely excluded.
Reliability: (1) valid without restriction
Flag: Critical study for SIDS endpoint
02-OCT-2003 (35)

Type: Skin painting test
Species: guinea pig
Concentration 1st: Induction 10 % active substance open epicutaneous
2nd: Challenge .5 % active substance open epicutaneous
No. of Animals: 10
Vehicle: other: acetone

Method: other: BASF-Test
Year: 1970
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Method: Induction
Ten "white" guinea pigs were used as test animals, a further six served as controls. In the test group, the skin on both flanks was shevd, after 4 hours the bare skin was degreased with diethyl ether and the test substance was applied by thrice brushing a solution (of either 10% in acetone in one animal or 1% in acetone on the other nine animals) in cross shape on the left flank with cotton wool dipped in the solution.
Challenge
After 14 days, both flanks were shaved again, degreased after 4 hours and then challenged by single cross-brushing with a 0.5% solution of isophytol in acetone on the right flank. In parallel, 6 non-induced control animals were prepared identically and cross-brushed once with 0.5% isophytol (3 animals) or 1% isophytol in acetone (3 animals).
Skin reactions were graded 12 hours after challenge respectively control application.
Result: Subsequent to induction, both the 10% and 1% application resulted in gross skin scaling on the area of application. On challenge, 5/10 induced animals showed a reaction graded as "questionable erythema" in the area of the challenge application. 2/3 controls in the 1% group showed reactions of the same grade while 0/3 controls of the 0.5% group showed no reactions.
Conclusion: While first-time (induction) application of 1% and 10% isophytol in acetone resulted in gross scaling of the skin, challenge application on the other flank 14 days later elicited a questionable erythema in 5/10 animals. On the other hand, also 2/3 non-induced control animals showed questionable erythemy after first-time application of a 1% solution in acetone while there were no observed reactions

in all three non-induced controls that were applied 0.5% isophytol in acetone.
The test report concludes that while both a 10% and a 1% solution of isophytol ion acetone causes gross skin scaling on repeat application, no unambiguous evidence for skin sensitisation was found in this study.

Reliability: (2) valid with restrictions
Short but detailed report from a professional industry toxicology laboratory, with methods, results and conclusion, rated validity 2.

22-JUL-2002 (8)

Type: other: maximisation test
Species: human
No. of Animals: 27
Vehicle: petrolatum
Result: not sensitizing

Year: 1981
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: The following is a literal citation from the Synopsis received:
"Subjects
27 healthy male and female volunteers were screened and they all completed the study.
Method
The materials were pretested on all subjects in order to determine wheter sodium lauryl sulfate (SLS) pretreatment was required. A patch of each material [there were three other substances beside isophytol in this study] was applied to normal sites on the backs for 48 hours under occlusion. No significant evidence of irritation was observed and all subjects were pretreated with SLS.
Maximisation procedure (modified after JID 47: 393-409, 1966)
The material was applied under occlusion to the same site on the volar aspets of the forearms of all subjects for five alternate-day 48-hour periods. Patch sites were pretreated for 24 hours with 5% aqueous SLS under occlusion for the initial patch only. Following a ten to fourteen day rest period challenge patches of the material were applied under occlusion to fresh sites for 48 hours. Challenge applications were preceded by 30-minute applications fo 5% aqueous SLS under ollcusion on the left side whereas the test material was applied without SLS pretreatment on the right side. A fifth side challenged with petrolatum served as a control."
Note: Based on the RIFM Database entry, the isophytol concentration was 10% in petrolatum.

Result: "Results. Datasheets with final tabulation are enclosed [in the Synopsis]. In this study approximately one-third of the subjects were slightly irritated by the SLS pretreatment. No other significant reactions were seen."

Conclusion: "Preparations 81-10-14 [=isophytol; and others tested at the same time] produced no reactions that were considered either irritant or allergic in the 27 subjects tested."

Reliability: (4) not assignable
A "Synopsis" of the study was made available by RIFM. Many

details (that are given in the RIFM Database entry) are missing from this short report, such as concentrations applied and vehicle; these were taken from the RIFM Database entry. However, as they cannot be corroborated with the sources available, reliability was judged to be 4, even though it may in fact be better.

Flag: Critical study for SIDS endpoint
30-JUL-2002

5.4 Repeated Dose Toxicity

Type: Sub-chronic
Species: rat **Sex:** male/female
Strain: other: Cr1:CD(SD)BR (VAF plus)
Route of administration: gavage
Exposure period: 28 days
Frequency of treatment: once daily
Post exposure period: 14 days
Doses: 250, 500 or 1000 mg/kg bw/d
Control Group: yes, concurrent vehicle
NOAEL: = 500 mg/kg bw
LOAEL: = 1000 mg/kg bw
NOEL : = 250 mg/kg bw

Method: OECD Guide-line 407 "Repeated Dose Oral Toxicity - Rodent: 28-day or 14-d Study"
Year: 1997
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Method: Animals
In all, 44 male and 44 female Cr1:CD(SD)BR (VAF plus) rats, approximately 28 days old at arrival, were delivered to the testing facility on Jan 24, 1997; all animals were supplied by Charles River UK, Margate, UK. All animals were found to be healthy by external examination on arrival. They were acclimatised for 13 days prior to the start of dosing. Towards the end of this acclimatisation period, the animal were re-examined and confirmed to be suitable for experimental use. On the first day of dosing, males weighed 154-186 g and females weighed 122-164 g.
Housing and keeping
The experimental room used, internal designation E9, was air-conditioned and recorded temperatures were in the range of 19-24 °C throughout. Relative humidity ranged from 36 to 49%. Fluorescent lighting was controlled automatically with a 12-h light (06:00-18:00) and dark cycle.
The animals were housed groups of six per sex. Groups were housed in grid-bottomed stainless-steel cages (TR18, Modular Systems, Kent, UK), suspended over cardboard-lined litter trays.
The animals had free access to SQC Rat and Mouse Maintenance Diet No. 1, Expanded (Special Diets Services, Whitham, UK) and mains tap water from polypropylene bottles. Each batch of diet was accompanied by a certificate of analysis from the supplier, detailing nutritional composition and levels of specified contaminants, specifically heavy metals, aflatoxins and insecticides. The tap water is analysed periodically for microbiological purity and levels of metals

and halogenated hydrocarbons (Hyder Environmental, Bridgend, UK). All of these analytical data were judged to be unlikely to influence the outcome of the study.

Test article formulation

Isophytol was formulated for dosing as solutions using maize (corn) oil of BP grade. Separate formulations were prepared daily for each dose level. As a confirmation of concentrations, samples of each formulation including the vehicle only control prepared on day 1 of weeks 1 and 4 of dosing were sent to the sponsor and analysed.

Treatment groups

Nine days before start of treatment, all animals were weighed and the required number of animals selected by excluding those at the extremes of the weight range. Test animals were then randomised to treatment and control groups using a stratified-bodyweight procedure. At the start of the study, the range of weight variation did not exceed ±20% of the appropriate mean value in all groups. After allocation to treamtent groups, each animal was uniquely identified by a subcutaneous transponder. Each cage of animals was albelled with a card, coloured according to group and detailing study number, licensee, treatment start date, group, cage number, treatment dose and animal number per sex.

Group	No. males	No. females	Dose level (mg/kg bw/d)
1	12	12	0 (vehicle controls)
2	6	6	250
3	6	6	500
4	12	12	1000

Dosing and duration

All animals were dosed once daily by gavage with a rubber catheter and a disposable syringe. A constant dose of 50 ml solution/kg bw was used, individual doses were adjusted according to the most recently recorded bodyweight. Control animals (group 1) received the vehicle only.

All animals were dosed for 28 consecutive days. 6 males and 6 females from each group were killed on day 29. The remaining 6 animals per sex in groups 1 (controls) and 4 (1000 mg/kg bw/d) were further maintained without test substance administration for another 14 days, after which they were also killed.

Observations

All animals were examined twice daily for mortality and morbidity. All visible signs (including behavioural) of reaction to treatment were recorded daily. All animals were weighed at the start of the study and then twice weekly up to the day of necropsy. The amount of food consumed by each cage of animals was recorded weekly throughout the treatment and treatment-free periods.

Clinical laboratory studies

Blood and urine samples were obtained from the first 6 males and females in each group during week 4 of treatment. Further samples were taken from the remaining animals towards the end of week 2 of the treatment-free period. Haematological examinations, coagulation tests, blood chemistry determinations and urinalysis were performed (details available). In samples from the treatment-free period those parameters where treatment-related changes were suspected were re-examined in both sexes.

Terminal observations

At the end of the treatment and treatment-free period, the respective animals were killed by CO2 asphyxiation. Dissections were completed in two days respectively one day for the remaining treatment-free animals. All animals were weighed, examined externally, the abdominal cavity opened, exsanguinated, macroscopically examined and the following selected organs excised and weighed: adrenals, brain, epididymides, heart, kidneys, liver, ovaries, spleen, testes, thymus. Tissue samples were taken and fixed for histology from 22 organs and sites (details available), including all gross lesions.
Statistical analysis
Sexes were analysed separately. Observations included bodyweights, bodyweight gains over the treatment period, food consumption, absolute and relative organ weights as well as clinical pathology data. The data were subjected to ANOVA. If between-group differences in variance were detected, pairwise tests versus controls were performed using Williams' test. Statistical significance was declared at two-sided 5% level and also noted at 1% and 0.1% levels. Haematological, biochemical and urinalytical results were analysed non-parametrically.

Result:

Mortalities
There were no mortalities during this study.
Clinical observations
Fur-staining was recorded in a number of females given 1000 mg/kg bw/d. One female from this group also showed hypoactivity, hunched posture, weight loss and pallor. These signs were considered related to the treatment. All other signs noted were considered not to be related to the treatment.
Body weight and food consumption
Both bodyweights and bodyweight gains were normal for rats of this age and strain in all four groups. There were no obvious treatment-related effects on food consumption.
Haematology
At the end of the 4-week treatment, increased white blood cell (WBC) count was noted for the high-dose females. In the high-dose males the group mean prothrombin time was slightly reduced. At the end of the treatment-free period, the WBC count in females remained marginally higher than in controls but fell within the quoted background ranges and was considered to have recovered. The prothrombin time in males was comparable with controls.
Blood chemistry
Small but statistically significant increases in alanine aminotransferase (ALT) were observed in mid-dose males and in both sexes at high dosage. Groups mean cholesterol levels in females demonstrated an apparent dose-related increase. Calcium levels were also elevated in low-dose females and in both sexes at mid and high dose. At the end of the treatment-free period there were no significant differences from controls regarding those parameters re-examined.
Urinalysis
After 4 weeks of treatment urine volumes were increased, with a corresponding decrease in specific gravity, in mid-dose males and in both sexes at high dosage. At the end of the treatment-free period the group mean urine volume in both sexes at high dosage was higher than in associated

controls, again with a corresponding decrease in specific gravity.
Organ weights
Absolute and relative liver weights were increased in both sexes at a dose of 1000 mg/kg bw/d. Absolute kidney weights were increased in females at all dose levels, although when related to bodyweight this increase was only significant at the high dose. Absolute spleen weights were increased in both sexes in all dose groups; when related to bodyweight, spleen mass showed an increase for females only at the high dose. The dead bodyweights of males from treated groups were significantly higher than controls, which was considered to be the reason for the apparent increased absolute spleen weights and also a reduction in bodyweight-related brain weights seen in both sexes at high dose.
At the end of the treatment-free period the liver weights of females had dropped to significantly below the comparable control levels. The liver weights of males from the high-dose group had regained levels similar to controls. Other, minor changes noted were considered not to be related to treatment.
Dissection, histopathology
No obvious treatment-related abnormalities were observed at dissection. No treatment-related effects were recorded during histopathology. A small numer of findings were within the normal range of background alterations for untreated rats of this strain and age and were considered not to be related to treatment.

Conclusion: Oral administration of isophytol to rats for 28 days at a dose of 1000 mg/kg bw/d was associated with the following findings: furstaining in females, including one animal that showed hypoactivity, hunched posture, weight loss and pallor; a number of clinical chemistry changes in males and females; increased liver weights in both sexes; increased kidney and spleen weights in females.
Administration of 500 mg/kg bw/d was associated with smaller differences in a number of clinical chemistry parameters. There were no clinical signs of toxicity or significant organ weight changes.
The majority of clinical chemistry findings, although statistically different from control animals, were within the ranges of historical background data quoted for control animals. The toxicological significance of these findings in the absence of any corroborative histopathological changes is unclear.
After a 14-day treatment-free period, the majority of findings were no longer apparent.
In view of the in-life, clinical-chemistry and organ-weight findings in animals, this study established the NOEL to be 250 mg/kg bw/d and the NOAEL to be 500 mg/kg bw/d, while a LOAEL (based on still minor and reversible changes) was set at 1000 mg/kg bw/d. No unambiguous signs of overt toxicity were noted at any dose.

Reliability: (1) valid without restriction
Flag: Critical study for SIDS endpoint
19-FEB-2003 (98)

Type: Sub-chronic
Species: rat **Sex:** male/female
Strain: other: Wistar Crl: (WI) BR (outbred, SPF)

Route of administration: gavage
Exposure period: males: mean 98 days, range 91-134 days
, females: mean 64 days, range 52-108 days
Frequency of treatment: once daily
Post exposure period: none
Doses: 250, 500 and 1000 mg/kg bw/d
Control Group: yes, concurrent vehicle
NOAEL: < 250 mg/kg bw
LOAEL: = 250 mg/kg bw

Method: other: OECD 415, One-generation reproductive toxicity test
Year: 2002
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Method: In the course of a one-generation reproductive toxicity test, four groups of 24 male and 24 female Wistar rats were exposed by daily gavage to 0 (vehicle controls, maize/corn oil), 250, 500 or 1000 mg isophytol/kg bw/day; these doses were selected based on the results of a previous 28-day subchronic gavage study. The duration of exposure was on average 98 (range 91-134) days for the males and on average 64 (range 52-108) days for females. Animals were observed and weighed regularly, on scheduled termination maroscopic and histopathological findings were recorded. Detailed methods are described in chapter 5.8.1, Toxicity to Fertility.

Result: Mortalities
There were 8 unscheduled deaths out of a total of 96 main parental animals; all 8 animals were females. Two were spontaneous deaths, one each in the 0 and the 500 mg/kg bw/d groups after 45 respectively 33 days of treatment; no evident cause of death was noted. The other six animals were all killed in extremis for humane reasons, one from the 0 (control) group, three from the 250 group and two from the 1000 group. The three from the 250 group were killed because of severe delivery difficulties, the other three for extreme signs of bad health. Dissection showed no consistent picture for the eight animals, with exception of the three with delivery problems, and there was no dose-response relationship present. Therefore, these deaths were considered not to be related to the treatment with the test substance.
The percentages of postnatal losses during days 1-4 post partum were as follows: controls 2%, low-dose 7%, medium-dose 8% and high-dose 39%.

Toxicological findings in the survivors
The following toxicologically relevant findings were noted for the parental, F0-generation animals:
At 1000 mg/kg bw/d, there was an increase in lethargy, hunched posture and piloerection in females; slightly decreased body weights in males and slight body weight loss during lactation in females; decreased food consumption during lactation in females; decreased terminal body weight, absolute and relative prostate weight, absolute seminal vesicles weight and incresed relative kidneys weight in males as well as increased absolute and relative kidneys and uterus weight in females; an increase in the incidence and severity of renal basophilic aggregates in males and females, an increase in the incidence and severity of renal basophilic tubules in males and females, dilated renal tubules in males and females, renal

mineralisation in males and females, a decrease in the incidence of renal hyaline droplets in males and a decrease in the incidence of periportal hepatocyte vacuolation in females.
At 500 mg/kg bw/d, there was an increase in absolute and relative kidneys weight in males and females; an increase in the incidence and severity of renal basophilic aggregates in males and females, an increase in the incidence and severity of renal basophilic tubules in males and females, dilated renal tubules in males and females, renal mineralisation in males and females and a decrease in the incidence of renal hyaline droplets in males.
At 250 mg/kg bw/d, there were dilated renal tubules in males and females, renal mineralisation in males and females and a decrease in the incidence of renal hyaline droplets in males.

Weight table:

Males, mg/kg bw/d	body a	kidney a	kidney r(%)	liver a	liver r(%)	semin.ves. a	semin.ves. r(%)
0	546	3.52	0.65	18.41	3.38	2.851	0.529
250	538	3.46	0.64	20.41	3.78	2.601	0.487
500	529	3.64	0.69	21.29	4.01	2.683	0.508
1000	500	3.62	0.73	20.20	4.04	2.387	0.479
Females, mg/kg bw/d	**body a**	**kidney a**	**kidney r(%)**	**liver a**	**liver r(%)**	**uterus a**	**uterus r(%)**
0	352	2.41	0.69	17.33	4.94	0.537	0.155
250	356	2.65	0.74	17.69	4.94	0.497	0.141
500	378	2.85	0.76	20.41	5.41	0.522	0.148
1000	338	3.17	0.94	16.55	4.87	0.969	0.209

a = absolute weights in grams; r = relative weights in per cent of body weight; semin.ves. = seminal vesicles.

The kidneys were processed for histology; based on scrutiny of the slides, the attendant histopathologist considered the observed changes unambiguously adverse in nature. In contrast to the earlier 28-day toxicity study, the present reprotoxicity study was not conceived to detect reversibility of effects through a subsequent treatment-free period.

Test substance: Isophytol from Teranol, Lalden, Switzerland, batches UU01113408 (purity by GC 97.0 weight-% respectively 98.0 area-%) and UU02013601 (purity by GC 97.5 weight-% respectively 98.0 area-%).

Conclusion: In the course of a one-generation reproductive toxicity study, with daily exposure by gavage during an average of 98 (range 91-134) days in males and an average of 64 (range 52-108) days in females, out of a total of 96 main test animals, two died spontaneously during the study while six were killed in extremis for humane reasons. There were no obvious systematic findings in these eight animals nor was there any dose-response relationship. Therefore, these deaths were considered not to be related to the treatment with isophytol. Based on toxicological and histopathological examinations, however, no NOEL or NOAEL could be established. At the lowest dose of 250 mg isophytol/kg bw/d, the FO-generation rats exhibited renal changes that were judged by the

histopathologist to be unambiguous adverse effects. Based on this study, with a subchronic to chronic exposure duration in rats, 250 mg/kg bw/d was the LOAEL.

Reliability: (1) valid without restriction
While the present reprotoxicity study was not strictly speaking a chronic toxicity study, it was performed according to an OECD protocol in an experienced contract laboatory under GLP quality assurance. The parental animals were fully documented regarding treatment, body weightchanges, behaviour, gross anatomy and histopathology over the treatment duration of minimally 52 days in one female to maximally 132 days in one male, with the average treatment time for females being 64 days and for males being 98 days. Therefore, the data from this study are regarded as highly dependable and are assigned a reliability rating of 1.

Flag: Critical study for SIDS endpoint

06-JAN-2006 (21)

Type: Sub-chronic
Species: rat **Sex:** male/female
Strain: other: Cr1:CD(SD)BR (VAF plus)
Route of administration: gavage
Exposure period: 7 days
Frequency of treatment: once daily
Post exposure period: 1 day
Doses: 1000 mg/kg bw /d
Control Group: no
NOEL : = 1000 mg/kg bw

Method: other: pretest to 28-day OECD 407 subchronic study
Year: 1997
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Method: In order to determined the suitability of maize (corn) oil as a vehicle for a planned 28-day subchronic study, 2 male and 2 female rats were administered 1000 mg isophytol in maize oil per kilogram body weight per day by gavage once daily for 7 days. Animals were observed daily, bodyweights were recorded on days 1, 5 and 8 and food consumption was recorded over the treatment period as a whole.

Result: All animals were unremarkable throughout the treatment period. All animals gained weight over the 8-day study period. The food consumed by each cage of animals was considered to be normals for animals of this age and strain.

Conclusion: Isophytol did not produce any observed adverse effects over 7 days' administration by gavage at a dose level of 1000 mg/kg bw/d in 4 (2 m, 2 f) rats. Maize (corn) oils proved suitable as a vehicle for oral administration.

Reliability: (2) valid with restrictions
As a pretest for a subchronic OECD 407 study, this 7-day test was not performed under GLP, however, it was performed at a contract laboratory of high standing under similar conditions as a GLP study.

06-NOV-2002 (98)

5.5 Genetic Toxicity 'in Vitro'

Type: Bacterial reverse mutation assay
System of testing: Salmonella typhimurium, TA97, TA98, TA100, TA102, TA104, TA 1535
Concentration: 0 (solvent control), 100, 333, 1000, 3333 and 10,000 µg test article/plate plus positive control
Cytotoxic Concentration: according to the protocol, the highest test article dose is "limited by toxicity", hence 10,000 µg/plate is considered to be close to the cytotoxic concentration
Metabolic activation: with and without
Result: negative

Method: other: after Haworth et al (1983): Environ Mutagen 5(suppl 1): 3-142.
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: Chemicals (including isophytol) were tested according to a reverse mutation assay as described by Haworth et al. [1983: Environ Mutagen 5(suppl 1): 3-142]. Briefly, a preincubation of the Salmonella test was used; the test chemical is incubated with the tester strain either in buffer or in S9 plus cofactor mix for 20 min at 37 °C prior to the addition of soft agar and plating on minimal agar plates. All chemicals are tested both in the absence of metabolic activation and with exogenous metabolic activation (S9) from Aroclor-1254-induced Sprague-Dawley rats and Syrian hamsters, in Salmonella typhimurium strains TA98, TA100, TA1535 and/or TA97. Each test consists of triplicate plating of concurrent positive and solvent controls [for aliquot A37965 95% ethanol was used as a solvent, for aliquot A23522 dimethyl sulfoxide (DMSO) was used a a solvent] and of at least 5 doses of test chemical. The high dose is limited by toxicity or solubility in pretests, but does not exceed 10 mg/plate. For isophytol, the high dose for both aliquot tests was 10,000 µg/plate.
A positive response is defined as a reproducible, dose-related increase in revertant (histidine-independent) colonies. A chemical is judged positive if a reproducible positive response is observed in any strain/activation combination. An equivocal or ambiguous response is defined as either a non-dose-related increase or a response that is not reproducible.
Remark: In an US National Toxicology Program Results Report dated February 11, 2002, isophytol CAS 505-32-8 is listed on page 106 as "Salmonella test type, negative response, inconclusive". The original, but unpublished, data were received on request from the US National Institute of Environmental Health Sciences.
No year is given for the test proper
Result: In an US National Toxicology Program Results Report dated February 11, 2002, isophytol CAS 505-32-8 is listed on page 106 as "Salmonella test type, negative response, inconclusive".
Based on the original data received from NIEHS (see Remarks),
1) there was one weakly positive and one ambiguous result in the two test runs with 30% rat-liver-induced S9 activation in strain TA97, with aliquot A37965 using 95% ethanol as a solvent;
2) further, there was an ambiguous result in one test run

each with 30% rat-liver-induced S9 activation in strains TA97, TA102 and TA104 and one ambiguous result in a test run without S9 activation in TA97, with aliquot A23522 using DMSO as a solvent.

Source: The original, but unpublished, data were graciously made available on request by the US National Institute of Environmental Health Sciences. Please note that there is a proviso on publishing these data in a formal report: "Data for aliquots A23522 and A37965 [both Isophytol] have not been published. It is requested that you not publish or include the data in any formal report. However, you may reference the conclusions for these tests as 'NTP unpublished results'."

Conclusion: In two test series with different aliquots of isophytol, comprising a total of 87 series of 7 plates, with and wihtout metabolic S9 activation, with DMSO or 95% ethanol as the solvent, there were 5 ambiguous series and one that was considered weakly positive, while 81 series were negative. Based on these results, isophytol is regarded as negative, with some ambiguous results, in a large series of Salmonella reverse mutation assays.
The NTP Results Report states in the published overview "Isophytol, CAS 505-32-8, SA, -,? [Salmonella assay, negative, ambiguous]".

Reliability: (2) valid with restrictions
Detailed methods and complete data for all single test plates available, hence reliability assigned 2.

Flag: Critical study for SIDS endpoint

21-FEB-2003 (107)

Type: Ames test
System of testing: Salmonella typhimurium; TA1535 TA100 TA1537 TA98
Concentration: 20-5000 µg/plate
Cytotoxic Concentration: no toxic effect was observed
Metabolic activation: with and without
Result: negative

Method: OECD Guide-line 471
Year: 1983
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: Isophytol was tested according to OECD 471 in an Ames test, in both a standard plate test and a pre-incubation test, both with and without S-9 mix, in Salmonella typhimurium. The S-9 mix was prepared from livers of Aroclor-1254-dose male rats, 5 days after administration. Histidine-negative (His-) Salmonella strains TA1535, TA1537, TA98 and TA100 were selected for the test. 0.1 ml bacterial suspension was mixed with soft agar, minimal amino acid siolution (0.5 mM histidine and 0.5 mM biotin), 0.1 ml test solution, 0.5 ml S-9 mix in case of metabolic activation experiments or 0.5 ml phosphate buffer in tests without metabolic activation, then the mixed samples were poured onto Vogel-Bronner (minimal glucose) agar plates within 30 seconds for the standard plate tests. For the pre-incubation tests, 0.1 ml test solution, 0.1 ml bacterial suspension and 0.5 ml S-9 mix are incubated at 37 °C for 20 minutes, then soft agar is added and after mixing the samples are poured onto Vogel-Bronner agar plates as above within 30 seconds. After

incubation in the dark at 37 °C for 48 hours, bacterial colonies (his+ revertants) were counted. A negative control was run in parallel as well as positive controls with and without S-9 mix (details in report). Test substance doses were 0 (controls), 20, 100, 500, 2500 and 5000 µg/plate, test concentrations were run in triplicate in parallel.

Result: There was no increase in his+ revertant colonies in comparison with control, both with and without S-9 mix and both with and without pre-incubation, in any of the four tested strains. Further, no bacteriotoxic effect, as evidenced through reduced his- background growth, was noted.

Test substance: Isophytol, batch no. 88/600, produced by BASF, of 97.6% purity.

Conclusion: Isophytol did not cause an increase in his+ revertant colonies in this Ames test, with and without metabolic activation using S-9 mix.

Reliability: (2) valid with restrictions
Full details given in a test report from a professional industry toxicology laboratory, hence validity 2.

Flag: Critical study for SIDS endpoint

21-FEB-2003 (10)

Type: other: Liquid Suspension Assay, modified Ames test
System of testing: Salmonella typhimurium; TA 100, TA98
Concentration: 20-5000 µg/plate
Cytotoxic Concentration: no toxicity observed
Metabolic activation: with and without
Result: negative

Method: OECD Guide-line 471
Year: 1983
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: Isophytol was tested in a modified Ames pre-incubation test, both with and without S-9 mix, in Salmonella typhimurium. The S-9 mix was prepared from livers of Aroclor-1254-dose male rats, 5 days after administration. Histidine-negative (His-) Salmonella strains and TA100 were selected for the test. 1.5 ml bacterial suspension was pre-incubated with 0.1 ml test solution or solvent (DMSO) and 0.5 ml S-9 mix in case of metabolic activation experiments or 0.5 ml phosphate buffer in tests without metabolic activation in tightly closed tubes in a shaking water bath at 37 °C for 90 minutes. Subsequently, the bacterial cultures were centrifugated at 5000 rpm for about 10 minutes. The supernatant was removed, the bolus re-suspended in 0.5 ml phosphate buffer (details in report) and 2 ml soft agar, then the mixed samples were poured onto Vogel-Bronner (minimal glucose) agar plates within 30 seconds for the plate tests. After incubation in the dark at 37 °C for 48 hours, bacterial colonies (his+ revertants) were counted. A negative control was run in parallel as well as positive controls with and without S-9 mix (details in report). Test substance doses were 0 (DMSO controls), 20, 100, 500, 2500 and 5000 µg/plate, test concentrations were run in triplicate in parallel.

Result: There was no increase in his+ revertant colonies in comparison with control, both with and without S-9 mix subsequent to liquid suspension pre-incubation, in any of

the two tested strains. Further, no bacteriotoxic effect, as evidenced through reduced his- background growth, was noted.
Test substance: Isophytol, batch no. 90/604, produced by BASF, of 97.5% purity.
Conclusion: Isophytol did not cause an increase in his+ revertant colonies in this Ames test with prior liquid suspension pre-incubation, with and without metabolic activation using S-9 mix.
Reliability: (2) valid with restrictions
Full details given in a test report from a professional industry toxicology laboratory, hence validity 2.
Flag: Critical study for SIDS endpoint
21-FEB-2003 (9)

Type: Ames test
System of testing: TA98, TA100, in part also TA97 and TA1537 plus other, undefined strains
Concentration: no data
Cytotoxic Concentration: no data
Metabolic activation: with and without
Result: ambiguous

Method: other: as described by Zeiger et al. (1992): Environ Mol Mutagen 19(suppl. 21): 1-141
Year: 1984
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Result: In an overview on proportion of mutagens among chemicals in commerce, isophytol CAS 505-32-8 is listed with "?" in an undefined Salmonella mutagenicity assay (defined as "equivocal response" in the footer of the table), based on a 1984 NAS publication.
Reliability: (4) not assignable
Flag: Critical study for SIDS endpoint
06-JAN-2006 (111)

5.6 Genetic Toxicity 'in Vivo'

Type: Micronucleus assay
Species: mouse **Sex:** male
Strain: other: NMRI BR mice (SPF)
Route of admin.: gavage
Exposure period: 24 and 48 hours
Doses: 2000 mg/kg
Result: negative

Method: OECD Guide-line 474 "Genetic Toxicology: Micronucleus Test"
Year: 2002
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Method: Animals
NMRI BR (SPF) mice from Charles River, Sulzfeld, Germany were used. Animals were young adults (6-8 weeks old), females were nulliparous and non-pregnant.
The animals were housed in an air-conditioned room with approximately 15 air changes per hour, a temperature of 21±3 °C and a relative humidity between 30 and over 70%; inspite

of the relative humidity exceeding 70% for part of the test period, no abnormalities were noted in the animals and it was concluded that this deviation did not affect the integrity of the study. The animal room was illuminated for 12 hours per day with artificial fluorescent lighting and was dark for 12 hours.
The animals were housed in randomised groups of 5 each per sex per cage in labelled polycarbonate cages containing purified sawdust (Sawi, Jelu-Werk, Rosenberg, Germany) as bedding material. Paper bedding (BMI Helmond, The Netherlands) was provided for nest material. There was free access to standard pelleted diet (Altromin (code VRF 1), Lage, Germany) and to tap water. Certificates of analysis for all substrates, feed and water are retained in the NOTOX archives. For all animals there was an acclimatisation period of at least 5 days before start of treatment under laboratory conditions.
Treatment groups
3 males and 3 females were used for the dose range-finding test. 5 males per test group respectively as negative and positive controls were used as there were no obvious differences between sexes in the range-finding test. All animals were identifed by a unique number on the tail.
In the main test there were 4 groups, labelled A through D. A was a negative control (vehicle only, 10 ml maize/corn oil/kg bw) group, B and C were treatment groups (2000 mg isophytol/kg bw in maize/corn oil, dose adjusted to a volume of 10 ml/kg bw; group B to be sampled at 24 hours post-dosing, group C at 48 hours post-dosing) and D was a positive control group (50 mg cyclophosphamide/kg bw, dissolved in physiological saline; cyclophosphamide from Asta-Werke, Germany). Feed was withheld 3-4 hours prior to dosing. Administration was by oral gastric intubation.
Observations
The animals were observed at least once a day for signs of toxicity. Prior to dosing the animals were weighed.
Preparation of erythroblasts and erythrocytes
The test animals were killed by cervical dislocation 24 hours (groups A and B) respectively 48 hours (groups C and D) after dosing. In every instance, both femurs were removed and freed of blood and muscles. Then, both ends of the boe were shortened until a small opening to the marrow canal became visible. The prepared bones were flushed with foetal calf serum (FCS), the cell suspension was collected and centrifuged at 1000 rpm for 5 minutes. The supernatant was discarded and the pellets re-suspended in FCS. A drop of the suspension was placed on the end of a previously cleaned and marked (NOTOX study number, animal number) microscopic slide, spread using a clean slide and air-dired, fixed with 100% methanl and automatically stained in a HEMA-tek Slide Stainer (Miles, Bayer Nederland, The Netherlands) and covered with a glass coverslip.
Before analysis, the unique marks of each slide were randomised by covering with an adhesive label bearing the NOTOX study number and a code. Slides were first screened at a magnification of x100 for suitable regions, then scored at x1000. The number of micronucleated polychromatic erythrocytes was counted in a total of 2000 polychromatic erythrocytes per slide. The ratio of polychromatic to normochromatic erythrocytes was determined in the first 1000

erythrocytes scanned. Micronuclei were only counted in polychromatic erythrocytes.
Statistics
After counting, the randomisation was unveiled and averages and standard deviations for the four groups were calculated. A test substance and/or dose would be considered positive if it induced a statistically significant (Wilcoxon Rank Sum test, two-sided test at P < 0.05) increase in the frequency of micronucleated polychromatic erythrocytes, at any dose or sampling time. Conversely, a test substance is considered negative if there is no such statistically siginifcant difference at any dose or sampling time.
Acceptability criteria
A micronucleus test is considered acceptable if it meets the following criteria: 1) the positive control substance, cyclophosphamide, induces a significant increase in micronucleated polychromatic erythrocytes and the incidence of micronucleated polychromatic erythrocytes in the copntrol animals is reasonably within the laboratory historical controls range (mean ± 3 SD).

Result: Dose range-finding study
3 males and 3 females were dosed with 2000 mg isophytol in maize/corn oil per kg bw. All treated animals showed no abnormalities during an observation period of 3 days. Therefore, 2000 mg/kg bw was chosen as the only dose for testing. Moreover, as there were no obvious differences between the sexes, it was decided to use only males in the main test.
Micronucleus test
The mean bodyweights of all four groups, recorded just before dosing, were not statistically different (data available).
All animals treated with 2000 mg/kg bw showed no abnormalities; this was also true for both the negative and positive controls.
Average numbers of micronucleated polychromatic erythrocytes and ratios of polychromatic to normochromatic erythrocytes:

Group	Dose, mg/kg bw	Sampling time, h	Number, mean±SD	Ratio, mean±SD
A, vehicle control	0	24	0.6±0.9	1.15±0.20
B, Isophytol	2000	24	0.2±0.4	1.09±0.11
C, Isophytol	2000	48	0.4±0.9	1.17±0.10
D, cyclophosphamide	50	24	25.8±5.5**	0.39±0.06

** Significantly different from negative (vehicle) control group, P <= 0.01.
All single data are available in the report.

Test substance: Isophytol from Teranol AG, Lalden, Switzerland, lot no. UU02013601, purity 97.5% (weight, GC) respectively 98.0% (area, GC), complying with specification. Certificate of analysis no. 1E2, dated January 23, 2002, Quality Control Department, Teranol, Lalden.

Conclusion: Isophytol at an oral dose of 2000 mg/kg bw did not induce any increase in the incidence of micronucleated polychromatic erythrocytes in this in vivo mouse test. Therefore, isophytol is regarded as negative regarding genotoxic effects in this model.
Further, the test groups treated with isophytol did not show any decrease in the ratio of normochromatic to polychromatic erythrocytes, which reflects a lack of toxic effects of

isophytol on erythropoiesis.
Reliability: (1) valid without restriction
Flag: Critical study for SIDS endpoint
24-JUN-2003 (73)

5.7 Carcinogenicity

Species: Drosophila melanogaster **Sex:**
Strain: other: "tu bw; +su-tu"
Route of administration: other: in feed medium, with possibility of direct epidermal contact of larvae in addition to feeding uptake
Doses: 0 (controls), 2.5, 5 and 10 mM
Control Group: yes, concurrent vehicle

Year: 1968
GLP: no
Test substance: no data

Method: Drosophila flies of strain "tu bw; +su-tu", approximately 90% of which normally develop melanotic tumours, were fed defined, sterilised feed medium containing terpenoid juvenile hormone mimics or structurally similar substances, such as isophytol. The development and occurrence of melanotic tumours was determined subsequent to metamorphosis.
Remark: Approximately 90% of Drosophila flies of strain "tu bw; +su-tu" develop genetically determined melanotic tumours around the time of metamorphosis from larvae to flies, which suggests that the tumourigenic transformation is under hormonal control. Several materials with known insect juvenile hormone effects and structurally related substances (such as isophytol, which has no discernible hormonal effect) were fed via feed medium to larvae of this fly strain in order to determine a tumour-promoting or -suppressing effect.
Result: The incidence of melanotic tumours, given as a tumour expression (TE) index in the results graph, steadily declined with isophytol dose in feed medium. While controls (no additional substances in the medium) had a TE of approx. 1.2, the TE dropped to approx. 1.0 at 2.5 mM isophytol, to approx. 0.6 at 5 mM isophytol and to approx. 0.5 at 10 mM isophytol, the highest concentration tested.
Conclusion: Isophytol suppressed the incidence of melanotic tumours in Drosophila flies. This was unexpected as isophytol had been shown previously not to have juvenile hormone mimic acitivity, in contrast to other terpenoids including the isomer phytol.
Reliability: (4) not assignable
09-APR-2002 (26)

5.8.1 Toxicity to Fertility

Type: One generation study
Species: rat
Sex: male/female
Strain: other: Wistar Crl: (WI) BR (outbred, SPF)
Route of administration: gavage

Exposure Period: males: average 98 (range 91-134) days, females: average 64 (range 52-108) days
Frequency of treatment: once daily
Premating Exposure Period
male: 10 weeks
female: 2 weeks
Duration of test: maximally 134 days
No. of generation studies: 1
Doses: 0 (vehicle control), 250, 500 and 1000 mg/kg bw/d
Control Group: yes, concurrent vehicle
NOAEL Parental: = 500 mg/kg bw
NOAEL F1 Offspring: = 500 mg/kg bw

Method: OECD Guide-line 415 "One-generation Reproduction Toxicity Study"
Year: 2002
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Method: Animals
Male and female Wistar rats Crl: (WI) BR (outbred, SPF-quality) were acquired from Charles River Deutschland, Sulzfeld, Germany. Of the animals assigned to the four original groups, the 96 males were 5-6 weeks old and the 96 females were 13-14 weeks old. During the course of the study, animals that had not successfully mated were subsequently paired with additional, non-treated animals acquired later; among these, both the 11 males and the 14 females were 12 weeks old. All animals were given a health check to ensure good state of health at the beginning of the study. All animals were acclimatised for at least 5 days before assignment by computer-generated randomisation according to body weight, with all animals within ±20% of the sex mean to treatment groups and start of the study. All animals were uniquely identified by tattoo on the tail.
Animal husbandry
All animals were housed in suspeded stainless-steel cages in climate-controlled rooms at 21±3 °C, a relative humidity of 30-70% and a 12-hour-light/12-hour-dark cycle. Animals had free access to standard pelleted rat rat diet (Altromin, code CRF1, Lage, Germany) and tap water. Analyses for all batches of feed and quater-yearly analyses of tap water are retained at NOTOX archives. On arrival, all animals were housed in groups of 4 animals per sex per cage, with males and females being kept in separate rooms. During mating, parental females were caged with parental males on a 1-to-1 basis in suspended stainless steel cages with wire mesh floors. Mated females and males were housed individually in labelled polycarbonate cages containing awdust (SAWI bedding, Jelu-Werk, Rosenberg, Germany) as bedding material. During the final stage of the pregnancy period, from day 16 post coitum, and during lactation, paper (Enviro-dri, BMI, Helmond, The Netherlands) was supplied to the dams for incorporation into the nest. The paper was replaced when soiled.
Treatment
Isophytol was formulated daily using maize/corn oil as the vehicle. Formulations were analytically confirmed to be stable for at least 4 hours at room temperature and to correspond to targeted concentrations. Dosing was by oral gavage using a stainlees steel stomach tube, dose volume was 5 ml/kg bw,

actual volumes were calculated according to the latest individual body weights. Dose levels were 0 (vehicle controls), 250, 500 and 1000 mg/kg bw/d for the four groups; these dose elvels were based on a GLP 28-day subchronic toxicity study with the same dose levels that resulted in a NOEL of 250 mg/kg bw/d and a LOEL of 500 mg/kg bw/d with reversible effects. The main males were exposed for 10 weeks prior to mating up to termination; the mean exposure was 98 days, with a range from 91 to 134 days. The main females were exposed for 2 weeks prior to mating up to termination; the mean duration of treatment was 64 days, with a range of 52 to 108 days. The offspring was not treated.

Mating procedures

Main pairing. Females were paired on a one-to-one basis with males from the same treatment group. Each morning the trays under the mating cages were inspected for ejected copulation plugs. The day on which a copulation plug was found was designated as day 0 of gestation. Once mating had occurred, the males and females were separated. In case no copulation plug was detected within 3 weeks of pairing, the male and female were separated.

Additional pairing. According to the guideline, all animals that had not successfully mated during the main study or in which other effects on reproduction were observed, were later mated with non-treated additional animals to check for normal or reduced fertility.

Parturition

The pregnant females were allowed to litter normally. Day 1 of lactation was defined as the day when a litter was found completed (ie, membranes, placentas cleaned up, nest built up and/or feeding of pups started). Females that were in the process of littering were left undisturbed.

Culling offspring

On day 4 after birth the size of each litter was adjusted at random by eliminating extra pups to yield, as closely as possible, four male and four female pups per litter. Elimination of runts only was not appropriate. Whenever the number of pups per sex did not allow four plus four, partial adjustement was made to come as close as possible to that ratio, eg, three males plus five females. No adjustement was made for litters of eight pups or less.

Identification of offspring

Pups were identified individually by means of intracutaneous injection of Indian ink or by tattoo on the feet.

Termination

All survivors were killed by exsanguination after iso-flurane anaesthesia. The main males were killed after confirmation of the pregancy of the female they had been mated with or after successful delivery of the respective dam. The main females were killed at day 21 post partum or shortly thereafter. Additional males were killed as soon as mating with a treated dam that had not mated successfully before had been confirmed. Additional females were killed shortly after delivery of their litter or, in case mating was unsuccessful, after two weeks pairing. Pups were killed either at adjusting litters on day 4 post partum or at the end of the study at day 21 post partum.

Observations

Parental animals were observed twice daily for behavioural and clinical signs, the latter were recorded according to fixed scales. Cage debris of pregnant females were examined to

detect abortion or premature birth. Signs of difficult or prolonged parturition were recorded. Males and females were weighed on the first day of exposure and weekly thereafter. Mated females were weighed on days 0, 7, 14 and 21 of gestation and during lactation on days 1, 4, 7, 14 and 21. Food consumption was recorded weekly for males and females, with exception of the mating period. Food consumption of mated females was recorded on gestation days 0, 7, 14 and 21 and during lactation on days 1, 4, 7, 14 and 21. Regarding water consumption, subjective appraisal was maintained during the study as there were no suspicions of any effect of treatment. Reproductive basic data such as numbers of animals mated, mating date, confirmation of pregnancy and day of delivery were recorded.
For the main offspring, the numbers of live and dead pups at first litter check (= day 1 of lactation) and daily thereafter was recorded as well as the individual weight of all live pups on days 1, 4, 7, 14 and 21 of lactation, the sex of the pups by assessment of the ano-genital distance, the number of pups with physical or behavioural abnormalities daily. Litters of additional offspring were not examined as these only served to confirm the basic reproductive competence of parental animals that had not mated in the main pairing round.
Pathology
After killing or natural death all parental main animals were subjected to external examination and to macroscopic examination during dissection, specifically the cranial, thoracic and abdominal organs and tissues, with special attention to the reproductive organs. All macroscopic abnormalities were recorded. The additional animals were not subjected to macroscopic examination.
The terminal body weight and the following organ weights were recorded from the main parental animals on the day of death: cervix plus uterus, epididymides (both together), kidney, liver, ovaries, pituitary (weighed after 24 h fixation), prostate (weighed after 24 h fixation), seminal vesicles together with coagulating gland and fluids, spleen and testes.
During dissection, samples of the following organs and tissues were collected from all main parental animals and fixed in neutral, phosphate-buffered 4% formaldehyde solution: all gross lesions, cervix, coagulation gland, epididymides (fixed in Bouin's, transferred to formalin after 24 h), kidney, liver, ovaries, pituitary, prostate, seminal vesicles, spleen, testes (fixed in Bouin's, transferred to formalin after 24 h), uterus and vagina. In case a female was not pregnant, the whole uterus was stained after Salewski in order to determine any early post-implantation losses through evidencing implantation site scars.
Histopathology. All organ and tissue samples as listed below were processed, embedded, microtomed at 2-4 µm and stained with haematoxylin and eosin: kidneys from all animals of all treatment groups; liver and prostate from 10 randomly selected animals per sex from all treatment groups; epididymides, ovaries, prostate, seimnal vesicles, testis and uterus from 10 randomly selected animals per sex of both the vehicle control and highest-dose groups; slides from all animals which died spontaneously or were killed in extremis and all gross lesions found from all groups; the reproductive organs from all main animals suspected of infertility. All slides were examined by a professional histopathologist, abnormalities were described

and included in the histopathology report. The histopathologist was asked to add an interpretation of the findings.
Pups. Main offspring found dead or killed before day 14 of lactation were sexed and externally examined if practically possible. The stomach was examined for the presence of milk.
Main offspring found dead or killed on or after day 14 of lactation were sexed and subjected to external examination of the thoracic and abdominal tissues and organs; all abnormalities were recorded. If possible, defects or cause of death were evaluated. The pups of additional females were not subjected to macroscpic examination. No pups were preserved.
Statistical evaluation
For variables assumed to follow a normal distribution, the Dunnett test was applied; for other assumed distributions the Steel test was used. In those cases where variables could be dichotomised without loss of information, the exact Fisher test was applied. All tests were two-sided, significance was accepted at $p < 0.05$.

Result:

Protocol deviations
14 protocol deviations are listed in the report. All 14 were evaluated and considered not to have affected the integrity of the study or of the results.
Dose preparations
Analyses of formulations showed values for accuracy within the range of 100±8% and values for homogeneity within the range of 100±5%. A stability analysis showed a decrease over 4 hours of1.3% and 0.1% for groups 250 and 1000 mg/kg bd/d.
Mortalities
There were 8 unscheduled deaths out of a total of 96 main parental animals; all 8 animals were females. Two were spontaneous deaths, one each in the 0 and the 500 mg/kg bw/d groups after 45 respectively 33 days of treatment; no evident cause of death was noted. The other six animals were all killed in extremis for humane reasons, one from the 0 (control) group, three from the 250 group and two from the 1000 group. The three from the 250 group were killed because of severe delivery difficulties, the other three for extreme signs of bad health. Dissection showed no consistent picture for the eight animals, with exception of the three with delivery problems, and there was no dose-response relationship present. Therefore, these deaths were considered not to be related to the treatment with the test substance.
Clinical signs
Females of the 1000 group showed an increased incidence in lethargy, hunched posture and piloerection. Incidental findings consisted of aggressive behavious, alopecia, scabs, thickened area of the tail, necrosis fo the tail apex, absent tail apex, rales, wounds, brown fur staining, lethargy, hunched posture, psresis of a leg, piloerection, emaciation, ptosis of one eye, dark tail base, laboured respiration, pale appearance, nodule at the tail, salivation, abnormal posture, uncoordinated movements, abrnomal gait, flat gait, hypotonia, slow breathing and broken upper incisors. No relationship with the treamtnet could be established for these signs. Moreover, based on experience in the test lab, these signs were considered to be within the normal biological variation for rats of this strain and age.
Body weight
Body weights were affected by treatment at 1000 mg/kg bw/d:

Body weights and body weight gain of males of the 1000 group were slightly decreased during the whole treatment period. Females of the 1000 group showed a slight body weight loss during the lactation period, which recovered at the end of the lactation period. Body weight gain of males of the 500 group was significantly increased on day 15 of the pre-mating period; this was an isolated, incidental finding that was not considered to be toxicologically relevant. See table further below for body and organ weights.
Food consumption
Absolute and relative food consumption of females of the 1000 group was significantly decreased during the lactation period. Statistically significant increases in relative food consumption were observed during several days of the tratment period in males and females of the 500 and 1000 groups; no explanation for this effect can be given, however, it was not considered to be an adverse effect. Realtive food consumption was statistically significantly decreased in males of the 250 group on days 29-36 of the pre-mating period; This finding was incidental in nature and not considered to be toxicologically relevant.
Macroscopic examination
No clearly treatment-related macroscopic findings were identified but a number of findings that were considered incidental in nature.
In the control group at planned necropsy, one male showed an accessory liver lobe attached to the diaphragm; one male showed an enlarged liver; one male showed flaccid testes, reddish discolouration of the left testis and epididymides reduced in size; one male showed a nodule at the tip of the tail, two males showed an accentuated lobular pattern of the liver; one non-pregnant female showed a fluid-filled uterus; one female showed an accessory liver lobe; one females showed a red-brown hard nodule at the ovaries; one female a reddish discolouration of the right side of the thymus. In the control group, one female that died spontaneously showed an enlarged liver, both uterus horn containing haemorrhagic fluid, an enlarged spleen and a soft nodule at the thymus; one female that was killed in extremis showed red-brown contents of the uterus and 18 foetuses in autolysis.
In the 250 group at planned necropsy, one male showed a dark red thymus; three males showed pelvic dilation of the right kidney; one male showed an enlarged liver; two males showed a reddish discolouration of the mandibular lymph node; one female showed an accessory lymph node attached to the diaphragm; one female showed a dark red noduel at the right uterus horn. In the 250 group, one female that was killed in extremis showed five foetuses in the birth channel, the uterus containing haemorrhagic fluid and a thickened knee region; one female that was killed in extremis showed two foetuses in the birth channel, of which one in breech presentation, and the uterus containing haemorrhagic fluid; one female that was killed in extremis shwoed two foetuses stuck in the left uterus horn and isolated gray-white foci at the heart.
In the 500 group, at planned necropsy one male showed an enlarged liver; two females showed an accessory liver lobe that caused a diaphragmatic hernia, of which one additionally had a thickened spleen; one female showed watery cysts at the right ovary; one female showed an enlarged liver. In the 500 group, one female that died spontaneouslyshowed an enlarged

liver and spleen, several dark red foci on the thymus and dark red discolouration of the mandibular lymph node.
In the 1000 group at planned necropsy, one male showed an enlarged liver; one male showed an accentuated lobular pattern of the liver; one male showed pelvic dilation of both kidneys; one male showed pelvic dilation of the right kidney; six females showed a fluid-filled uterus; one female showed pale discolouration of the kidneys; one female showed dark red discolouration of the mandibular lymph node; one female showed accentuated lobular pattern of the liver. In the 1000 group, one female that was killed in extremis showed intussusception of the colon and an enlarged spleen. The incidence of fluid in the uterus in females was slightly increased at 1000 mg/kg bw/d, hwoever, these findings were without histological correlates or else were associated with physiological changes (dilation, endometrial hypertrophy), therefore this was considered an incidental finding.
Organ weights
The following changes were considered to be related to treatment. In the 1000 group, the males showed decreased terminal body weight, increased relative kidneys weight, decreased relative and absolute prostate weight, decreased absolute seminal esicles weight. In the 1000 group, the females showed increased absolute and relative kidneys weight and increased absulute and relative uterus weight. In the 500 group, females showed increased absolute and relative kidneys weight.
The following effects were considered to be unrelated to treatment through lack of histopathological or reproductive effects or lack of recognisable dose-response relationship. Males oth the 250 and 500 group showed significantly decreased absolute and relative prostate weights. Males of the 250, 500 and 1000 groups showed significanty increased relative and/or absolute liver weights. Females of the 500 group showed significantly increased terminal body weights, increased absolute and relative liver weights and increased absolute spleen weights.
Body and organ weight table (weights in grams):

Males							
Dose group	bw	kidney		liver		seminal vesicles	
	abs	abs	rel	abs	rel	abs	rel
0	546	3.52	0.65	18.41	3.38	2.851	0.529
250	538	3.46	0.64	20.41	3.78	2.601	0.487
500	529	3.64	0.69	21.29	4.01	2.683	0.508
1000	500	3.62	0.73	20.20	4.04	2.387	0.479
Females							
Dose group	bw	kidney		liver		uterus	
	abs	abs	rel	abs	rel	abs	rel
0	352	2.41	0.69	17.33	4.94	0.537	0.155
250	356	2.65	0.74	17.69	4.94	0.497	0.141
500	378	2.85	0.76	20.41	5.41	0.522	0.148
1000	338	3.17	0.94	16.55	4.87	0.969	0.209

All values are averages for the respective group.
abs = absolute weight, g; rel = relative weight, %.

Microscopic examination
The following changes were considered to be related to treatment. Females of the 1000 group showed minimal to

moderate periportal hepatocyte vacuolation. In the kidneys of males and females of 500 and 1000 groups, basophilic aggregates and an increase in the incidence of basophilic tubules were noted. In the kidneys of males and females of the 250, 500 and 1000 groups, dilated tubules and general mineralisation were observed. In kidneys of the males of the 250, 500 and 1000 groups, there was a decrease in the incidence of hyalin droplets.
No histopathological changes were found to correlate with the observed decreases in prostate and seminal vesicles weight.
Reproduction
Reproduction parameters were affected by treatment at 1000 mg/kg bw/d. Females of the 1000 group showed a slightly increased mean pre-coital time, a decreased fertility index, a decreased conception rate and a decreased number of pups at birth. In the 1000 group, seven females were non-pregnant and one female discarded all pups before firts litter check. In the 500 group, two females were non-pregnant. In the 250 group, three females were killed in extremis after showing delivery difficulties, one female was non-pregnant and one female discarded all pups before first itter check. in the 0 (control) group, two females were non-pregnant and three males did not mate within the 10-day mating period.
Breeding data
Breeding parameters were affected by treatment at 1000 mg/kg bw/d. The number of dead pups at first litter check, postnatal loss and breeding loss were increased in litters of the 1000 group. Postnatal loss was also increased in litters of the 250 and 500 groups when compared to the 0 (control) group; however, as these values were within the range of historical control values, this finding was considered to be caused by chance and to be not toxicologically significant.
Additional animals
Among the additional, untreated animals that were later paired with main animals which had not mated successfully during the main study, no unscheduled deaths were observed. One female shwoed alopecia during the study. Body weights, body weight gain, food consumption and relative food consumption were normal. One untreated additional female was non-pregnant; this female was mated with a male from the control group which had not mated sucessfully before.
Among the main, treated or control animals that were later paired with non-treated additional animals, one female of the control group was non-pregnant, two females of the 1000 group were non-pregnant and one female fo the 1000 group did not mate. One female of the 1000 group was killed in extremis on days 4 of lactation. During lactation, all 1000 group females showed body weight decrease and reduced food consumption. All pups of the three litters of the 1000 group died within five days of lactation.

Test substance: Isophytol from Teranol, Lalden, Switzerland, batches UU01113408 (purity by GC 97.0 weight-% respectively 98.0 area-%) and UU02013601 (purity by GC 97.5 weight-% respectively 98.0 area-%).

Conclusion: Reproductive toxicity was assessed by observing mating performance, fertility indices and number of live pups at birth. At 1000 mg/kg bw/d, reproductive toxic effects consisted of slightly increased mean pre-coital time, decreased fertility index, decreased conception rate and decreased number of pups at birth. Comparable effects were

seen in the 1000 group main animals after additional mating. Therefore, regarding fertility, parental and filial reproductive effects parameters, both the parental and the F1-generation NOEL and NOAEL were 500 mg/kg bw/d.

Reliability: (1) valid without restriction
GLP OECD study.

Flag: Critical study for SIDS endpoint

02-OCT-2003 (21)

5.8.2 Developmental Toxicity/Teratogenicity

Species: rat **Sex:** male/female
Strain: other: Wistar Crl: (WI) BR (outbred, SPF)
Route of administration: gavage
Exposure period: 10+ weeks
Frequency of treatment: once daily
Doses: 0 (vehicle control), 250, 500 and 1000 mg/kg bw/d
Control Group: yes, concurrent vehicle
NOAEL Maternal Toxity: < 250 mg/kg bw
other: NOAEL Embryo-/Fetotoxicity :
= 500 mg/kg bw
other: NOAEL Postnatal Developmental Toxicity :
= 500 mg/kg bw

Method: other: OECD 415
Year: 2002
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Method: Please refer to 5.8.1, Toxicity to Fertility, for detailed methods.

Result: In the following results section, only those data that relate to embryo-/fetotoxicity or teratogenicity are listed. For general results please refer to section 5.8.1, Toxicity to fertility.
Breeding data
Breeding parameters were affected by treatment at 1000 mg/kg bw/d. The number of dead pups at first litter check, postnatal loss and breeding loss were increased in litters of the 1000 group. Postnatal loss was also increased in litters of the 250 and 500 groups when compared to the 0 (control) group; however, as these values were within the range of historical control values, this finding was considered to be caused by chance and to be not toxicologically significant.
Pups
The development of pups was affect at 1000 mg/kg bw/d. Several of the pups showed very bad health (eg, very small or cold appearance, little or no milk uptake, dying). Mean body weights were significantly decreasedon days 4-7 of lactation in both male and female pups of the 1000 group when compared to controls.
Incidental findings consisted of small, cold, pale or purple/bluish appearance, little or no milk uptake, cannibalism, wounds at tail or base of leg, red nose, thickened area at abdomen or breast, scales/scabs on several parts of the body, alopecia, swelling of the leg, dying.
Macroscopic examination of the pups revealed pelvic dilation of the right kidney in some cases. No relationship with the treatment was established for these observations, or they were

considered to fall within the normal biological variation for rats of this age and strain. One pup of the 500 group sowed several major abnormalities.

Test substance: Isophytol from Teranol, Lalden, Switzerland, batches UU01113408 (purity by GC 97.0 weight-% respectively 98.0 area-%) and UU02013601 (purity by GC 97.5 weight-% respectively 98.0 area-%).

Conclusion: Embryo- and fetotoxic effects were seen as a decreased number of pups at birth at 1000 mg/kg bw/d. Only one pup (from the 500 mg/kg bw group) in the whole study showed multiple malformations; due to the singular nature this was not assessed as a treatment-related effect. Survival and general fitness of pups was decreased in the 1000 mg/kg bw/d group, as evidenced by increased number of dead pups at first litter check, increased postnatal losses, increased incidence of clinical signs and decreased body weights of pups during the lactation period. Comparable effects were seen in the 1000 group main animals after additional mating. Therefore, regarding developmental and breeding parameters affecting the F1-generation, the NOEL and NOAEL for doses administered to the parental animals was 500 mg/kg bw/d.

Reliability: (1) valid without restriction
GLP OECD study.

Flag: Critical study for SIDS endpoint

27-DEC-2002 (21)

5.8.3 Toxicity to Reproduction, Other Studies

5.9 Specific Investigations

Endpoint: other: physiological activity in skeletal muscles: creatine kinase efflux, muscle cation content, non-enzymic lipid peroxidation, lipoxygenase assay, PGE2 release

Species: rat

Strain: Wistar **Sex:** male

Year: 1989

GLP: no data

Test substance: as prescribed by 1.1 - 1.4

Method: In order to study the release of the muscle cytosolic enzyme, creatine kinase (CK), in physiological or pathological skeletal muscle damage, a rat model was used. Healthy male Wistar rats were killed by cervical dislocation and both soleus (leg) muscles were carefully and rapidly dissected and removed. Muscles were mounted in special holders and pre-incubated in mammalian Ringer solution. Muscles were then treated for 30 min with the Ca(2+) ionophore A23187 to induce CK efflux from the muscle cells, then muscles were incubated in Ringer or test compound solution (see below). From every animal, one soleus was used as an experimental muscle and the contralateral as a control.
CK enzyme efflux determination
Vitamin E (alpha-tocopherol) and related compounds (alpha-tocopherol acetate; Trolox C, corresponding to the chromanol structure of vitamin E without the phytyl chain;

phytol; isophytol) were tested for their potential to inhibit or reverse A23187-induced CK efflux. Inhibition was tested by co-incubation of A23187 and the test compound while reversal was tested with the test compound only after A23187 incubation. Test compounds were dissolved in 100% ethanol and added to the incubation media; an equal amount (10 µl) of ethanol only was added to the control muscle media. The medium was replaced every 30 min and CK concentrations were measured in the spent media. All test compounds were tested separately for any inhibitory effect on the CK assay.
Analysis of muscle cation content
At the end of the incubation experiment, muscles were freeze-dried and analysed for Ca, Mg, K and Na as previously described [Jackson et al. (1984): Eur J Clin Invest 14: 369-374].
Non-enzymic lipid peroxidation
The effect of all compounds used in the muscle incubation system was investigated in autoxidising mouse muscle homogenates as previously described by Jackson et al. (1983: Biosci Rep 3: 609-619). The absorbance of thiobarbituric-acid-reactive substances (TBARS) is measured spectrophotometrically at 532 nm as an index of lipid peroxidation. Homogenates (2% w/v) of fresh mouse skeletal muscle in K2PO3 buffer, pH 7.4, were incubated with 50 µM ascorbate and 50 µM FeSO4 at 37 °C in the absence or presence of test substances. After 2 h the reaction was stopped and the TBARS measured by addition of an equivalent volume of 0.61 M trichloroacetic acid, 55.5 mM thiobarbituric acid and 1 mM disodium ethylene diamine tetraacetic acid solution. The mixture was heated at 100 °C for 12 min, cooled and the TBARS/TBA chromogen extracted into 1-butanol and the A(532) read against appropriate blanks.
Lipoxygenase assay
Any possible inhibition of lipoxygenase enzyme activity by the test compounds was investigated using purified soya-bean type 1 lipoxygenase (EC 1.13.11.12, Sigma Chemical Co). Lipoxygenase activity was determined spectrophotometrically by monitoring the formation of conjugated dienes as described by Ben Aziz et al. (1970: Anal Biochem 34: 88-100), modified by the use of deoxycholate as a substrate [Nishikimi et al. (1980): Biochem Biophys Acta 627: 101-108]. Compounds to be tested were solubilised in dimethyl sulfoxide and preincubated with the lipoxygenase for 10 min at 37 °C. The reaction was started by addition of the enzyme.
Statistics
Statistical significance of results was assessed by Student's t test, with P values >0.05 considered non-significant.

Result: CK enzyme flux
A23187 treatment for 30 min massively enhanced CK efflux to approx. 160% of baseline (data from graph) within 30 min, with this effect continuing after stopping the A23187 treatment, up to >300% after an additional 120 min.
When added parallel to A23187, the standard alpha-tocopherol prevented respectively lowered CK flux. When added after A23187 treatment alpha-tocopherol lowered CK flux to or beneath 100% within 60 min. Trolox C, representing the

chromanol structure of alpha-tocopherol, did not lower CK flux at all. In contrast, isophytol, representing the phytyl chain of alpha-tocopherol, lowered CK flux similar to alpha-tocopherol. Similarly, both phytol and alpha-tocopherol acetate did lower CK efflux but not as strongly as alpha-tocopherol and isophytol.
Muscle cation content
Treatment with the Ca(2+) ionophore A23187 significantly enhanced Ca content of soleus muscles. Both alpha-tocopherol and isophytol, but not the other test compounds, significantly reduced this enhanced Ca content. No consistent effect of the various compounds on Mg, K or Na muscle content was seen.
Non-enzymic lipid peroxidation
Both alpha-tocopherol and Trolox C, representing the antioxidant chromanol moiety of the former, markedly decreased the amount of TBARS respectively non-enzymic lipid peroxidation. Both phytol and isophytol had some effect on lowering TBARS production while, as expected, alpha-tocopherol acetate had very little antioxidant activity.
Effect on the activity of lipoxygenase enzymes
Both alpha-tocopherol, phytol and isophytol inhibited lipoxygenase by about 50% at a concentration of 230 µM, whereas alpha-tocopherol acetate and Trolox C were essentially ineffective.

Conclusion: Isophytol had protective effects similar to vitamin E (alpha-tocopherol) on rat muscles treated with a calcium ionophore: it lowered the efflux of creatine kinase, it reduced calcium content, it weakly reduced non-enzymic lipid peroxidation and it strongly reduced lipoxygenase activity. In the wording of the authors these results "indicate that vitamin and certain related compounds [specifically isophytol] can inhibit muscle sarcolemmal changes induced by intracellular calcium overload, which leads to intracellular enzyme efflux. The mechanism by which this occurs is at least partially dependent upon the phytyl chain of the tocopherol molecule rather than its antioxidant ability. Some results suggest that this effect may be mediated by an ability of phytyl compounds to inhibit lipoxygenase enzymes."

Reliability: (2) valid with restrictions
probably not GLP but highly detailed study with full methods

17-APR-2002 (83)

Endpoint: other: promotion of percutaneous absorption
Species: rat
Strain: Wistar **Sex:** male
Route of administration: dermal application

Year: 1991
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: Several terpenes including isophytol were tested for their ability to enhance percutaneous absorption of indomethacin from a gel ointment applied to rat skin. The gel ointment consisted of 1.0 g indomethacin, 2.0 g carboxyvinyl polymer, 2.5 g triethanolamine, 50.0 g ethanol, 1.0 g of the respective terpenes and pure water ad 100.0 g.

Male Wistar rats weighing 160-190 g were anaesthetised using urethane saline solution (25%, 3 ml/kg bw i.p.), fixed on their back and the ventral skin was gently shaved using electric clippers. Then, glass cells (16 mm inside diameter, 10 mm deep) containing 1.5 g of the above gel ointment were attached to the shaved skin using cyanoacrylate adhesives at the rim. To measure indomethacin uptake, blood samples (0.5 ml each) were taken from the jugular vein at 2, 4, 6 and 8 h after fixation of the ointment cells.
Indomethacin was determined in the blood samples using an HPLC method described in detail in the paper.

Result: Gel ointment containing 1% isophytol as the only terpene clearly enhanced percutaneous indomethacin absorption in comparison with gel ointment without any terpene, where no indomethacine was found in blood samples. However, the penetration-promoting effect of isophytol was relatively weak, clearly lower than any of the 7 tested monoterpenes, within the range of 4 tested sesquiterpenes; isophytol showed the lowest promoting effect of the 3 diterpenes to which group isophytol itself belongs.
There is no information on percutaneous penetration of isophytol itself in this publication.

Test substance: The terpenes used in this study [including isophytol] were of extra pure reagent grade, purchased from Tokyo Chemical Industries Co. Ltd, Japan.

Conclusion: 1% isophytol in a gel ointment has a relatively weak percutaneous absorption promoting effect for indomethacin. Based on this result it may possibly also enhance the dermal penetration of other substances.

17-APR-2002 (101)

Endpoint: other: competitive binding to retinol-binding protein

Species: human

Year: 1976

GLP: no

Test substance: as prescribed by 1.1 - 1.4

Method: Retinol-binding protein (RBP) from the urine of patients suffering from "Itai-Itai" disease was purified by ammonium sulfate fractionation, gel filtration on Sephadex G-100, chromatography on DEAE-cellulose, gel filtration on Sephadex G-100 and finally chromatography on DEAE-cellulose. Details are given in the paper.
For the competitive binding experiment with isophytol, 0.1 ml of isophytol and 4.0 ml of standardised RBP solution (0.31 mg protein/ml) in Tris buffer were mixed, gently stirred for 1 minute and left to stand at room temperature for 30 minutes. To this mixture, 0.2 ml of a 0.35% retinol solution in n-heptane was added. Then the mixture was gently stirred for 10 minutes at room temperature and subsequently centrifuged at 3,000 rpm for 5 minutes. The aqueous layer containing the RBP fraction was analysed in a Hitachi EPS-3T spectrophotometer. The molar ratio of retinol to RBP was derived from the A330/A280 absorbance ratio. From this ratio the relative respectively competitive binding was derived. Details are given in the paper.

Remark: Retinol-binding protein (RBP) is a blood protein specific for vitamin A (retinol) transport. RBP is excreted in the

urine of patients of certain diseases. RBP was purified from such urine and the relative binding to RBP of vitamin A derivatives and selected terpenes with structural similarities to parts of retinol, as well as a long-chained (C10) alcohol and a long-chained (C17) fatty acid, was determined.

Result: In comparison with the retinol standard, RBP pre-exposure to isophytol resulted in only 39% retinol binding, respectively 61% retinol-binding inhibition.

Test substance: Isophytol, purity not detailed, obtained from Takasago Perfume Co., Japan.

Conclusion: The high affinity of isophytol to RBP was, in the words of the authors, "surprising". Among terpenoids, competitive binding was only higher in beta-ionone and beta-ionylidene acetic acid on one hand, both of which are characterised by a closed beta-ionone ring identical to the one in retinol, and by citral and pseudoionone, both of which have a terminal respectively subterminal carbonyl group. Competitive binding of phytol, an isophytol isomer with a terminal carboxyl group, was much lower than of isophytol (29% vs 61%). In conclusion, RBP showed a high affinity for isophytol and isophytol is a potential inhibitor of RBP.

07-AUG-2002 (54)

Endpoint: other: neurophysiological modulation/stimulation
Species: mouse
Strain: ICR **Sex:** male
Route of administration: inhalative/olfactory
No. of animals: 32
Control Group: yes, concurrent no treatment

Year: 1992
GLP: no data

Method: Groups of 12 mice of the same age were placed in experimental chambers with odorised air or pure air (controls) during 4 hours' accommodation time. Odorisation of test chamber air was made by evaporating either jasmin oil (Egyptian Jasmin absolute, Argeville, France) or single, synthesised compounds previously identified as components of Jasmin absolute.
All experimental mice were anaesthetised using 55 mg/kg bw sodium pentobarbital administered i.p., returned to the respective test chamber and placed on the back. Pentobarbital-induced sleep time was determined as that time (to the nearest minute) from i.p. administration to regaining the ability to spontaneously right themselves.

Result: Exposure of mice to Jasmin-absolute-enriched air significantly lowered the pentobarbital-induced sleep time to 81% of controls (29 animals). Exposure to single identified components of Jasmine oil showed that pure phytol significantly reduced the pentobarbital-induced sleep time to 65% of controls (33 animals; $p < 0.01$) while isophytol did not significantly decrease the pentobarbital-induced sleep time (94% of controls, 32 animals). Similarly, other identified fractions did not show significant effects.

Test substance: Pure tests substances including isophytol from Kuraray Co., Japan. No data on purity.

Conclusion: Jasmin absolute oil has a stimulating effect on the central nervous system as determined using a pentobarbital-induced

sleep time model in mice. Phytol was identified as being the active substance in bringing about this effect, but isophytol did not show a significant effect.
09-APR-2002 (105)

Endpoint: Endocrine System Modulation
Type: other: insect hormone-mimic activity

Year: 2000
GLP: no

Result: In an overview article on insect hormone systems, phytol was reported to have some (limited) juvenile hormone activity while isophytol had none.
Test substance: commercial isophytol of > 90% purity
09-APR-2002 (53)

5.10 Exposure Experience

Type of experience: Health records from industry

Result: During more that 30 years of isophytol production at the Teranol Lalden plant, Switzerland, no remarkable observations in connection with exposure to isophytol have been registered by the safety and environmental protection department nor by the occupational health service.
Reliability: (2) valid with restrictions
23-JUL-2002 (43)

5.11 Additional Remarks

6.1 Analytical Methods

Method: Gas Chromatography with Flame Ionisation Detector (GC/FID)
Test substance: Isophytol

Reliability: (2) valid with restrictions
27-MAR-2002 (71) (97)

Method: Thin-Layer Chromatography (TLC)
Test substance: Isophytol

Reliability: (2) valid with restrictions
27-MAR-2002 (71)

Method: Paper Chromatography
Test substance: Isophytol

Reliability: (2) valid with restrictions
27-MAR-2002 (71)

6.2 Detection and Identification

7.1 Function

7.2 Effects on Organisms to be Controlled

7.3 Organisms to be Protected

7.4 User

7.5 Resistance

8.1 Methods Handling and Storing

Safe Handling: processing in closed systems, if possible under inert gas
Fire/Exp. Prot.: prevent electrostatic charging
Storage Req.: room temperature; under inert gas for quality (not safety-related) reasons
Container: tightly closing; high-grade stainless steel, coated steel

17-APR-2002 (49)

8.2 Fire Guidance

Ext. Medium: water mist; foam, dry powder; carbon dioxide
Unsuit. Ex. Med.: water spray, water jet (fat explosion hazard)
Add. Information: substance is hazardous for water; contain fire-fighting wastewater

17-APR-2002 (49)

8.3 Emergency Measures

Type: injury to persons (eye)

Remark: rinse immediately with tap water, open eyelids forcibly; consult physician
17-APR-2002 (49)

Type: injury to persons (skin)

Remark: immediately remove contaminated clothes, wash affected skin with water and soap; do not use any solvents
17-APR-2002 (49)

Type: injury to persons (inhalation)

Remark: remove the casualty to fresh air and keep the casualty calm; consult physician
17-APR-2002

Type: accidental spillage

Remark: contain spills; may be ecotoxic at higher concentrations, hence do not allow to enter drains, surface water or groundwater; collect using inert absorbent and dispose of by incineration
17-APR-2002 (49)

8.4 Possib. of Rendering Subst. Harmless

Domain: Industry/skilled trades
Process: Destruction
Type of destruction: Incineration

17-APR-2002 (49)

8.5 Waste Management

Memo: Possibility of destruction: incineration

17-APR-2002 (49)

8.6 Side-effects Detection

8.7 Substance Registered as Dangerous for Ground Water

8.8 Reactivity Towards Container Material

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