Scientific Committee on Consumer Safety

SCCS

OPINION ON

Dihydroxyacetone

The SCCS adopted this opinion at its 9th plenary meeting on 14 December 2010

About the Scientific Committees
Three independent non-food Scientific Committees provide the Commission with the scientific advice it needs when preparing policy and proposals relating to consumer safety, public health and the environment. The Committees also draw the Commission's attention to the new or emerging problems which may pose an actual or potential threat.
They are: the Scientific Committee on Consumer Safety (SCCS), the Scientific Committee on Health and Environmental Risks (SCHER) and the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) and are made up of external experts.

In addition, the Commission relies upon the work of the European Food Safety Authority (EFSA), the European Medicines Agency (EMA), the European Centre for Disease prevention and Control (ECDC) and the European Chemicals Agency (ECHA).

SCCS
The Committee shall provide opinions on questions concerning all types of health and safety risks (notably chemical, biological, mechanical and other physical risks) of non-food consumer products (for example: cosmetic products and their ingredients, toys, textiles, clothing, personal care and household products such as detergents, etc.) and services (for example: tattooing, artificial sun tanning, etc.).

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This opinion has been subject to a commenting period of four weeks after its initial publication. All comments received during this time have been considered by the SCCS and discussed in the subsequent plenary meeting. Where appropriate, the text of the relevant sections of the opinion has been modified or explanations have been added. In the cases where the SCCS after consideration and discussion of the comments, has decided to maintain its initial views, the opinion (or the section concerned) has remained unchanged.

Keywords: SCCS, scientific opinion, dihydroxyacetone, self-tanning agent, directive 76/768/ECC, CAS 96-26-4, EC 202-4945

Opinion to be cited as: SCCS (Scientific Committee on Consumer Safety), Opinion on dihydroxyacetone, 14 December 2010

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1. BACKGROUND

The substance dihydroxyacetone (DHA, CAS nr. 96-26-4) is used as an ingredient in self-tanning cosmetic products on the EU market since the 1960's. A rapid and indicative survey by the Dutch Food and Consumer Product Safety Authority (VWA) revealed that there is a widespread use of DHA in self-tanning cosmetic products on the Dutch market.

Besides the use in cosmetic products a recent development is the use of DHA solutions in spray cabins with the purpose of obtaining tanned skin without exposure to sunlight or UV radiation. The VWA has raised concerns about the safety of DHA in spray applications, because of the possibility of consumer exposure by inhalation. This exposure is quite different from DHA as an ingredient in self tanning creams, which occurs via the skin only. In a risk assessment on DHA the Dutch National Institute for Public Health and the Environment (RIVM) concluded that neither the risk of inhalatory, nor dermal exposure to DHA could be assessed, due to the lack of data.

In 2006, the Danish Toxicology Centre, on behalf of the Danish Ministry of the Environment, performed an assessment of DHA in self-tanning creams applied in spray booths, including exposure assessment from different kind of spray cabins. This report also states a lack of toxicological data with regard to the use of the substance in self-tanning products.
In 2008, Colipa in 2008 submitted a dossier on the safety profile of DHA in cosmetic products.

2. TERMS OF REFERENCE

1. *Does SCCS consider the use of Dihydroxyacetone (DHA) in cosmetic products safe for the consumers when used in a maximum concentration up to 10.0%, taking into account the data provided?*

2. *DHA may also be used in "spray cabins" in aqueous solutions in concentrations between 8 and 14%. Does the SCCS consider this use and exposure safe for the consumers?*

3. *Does the SCCS have any further scientific concerns regarding the use of DHA in a spray solution as a tanning agent without UV?*

3. OPINION

3.1 CHEMICAL AND PHYSICAL SPECIFICATIONS

3.1.1 Chemical identity

3.1.1.1 Primary name and/or INCI name

Dihydroxyacetone (INCI name)

Ref.: 1

3.1.1.2 Chemical names

1,3-Dihydroxy-2-propanone

Ref.: 1

3.1.1.3 Trade names and abbreviations

1,3-Dihydroxydimethyl ketone
Propane-1,3-diol-2-one
DHA

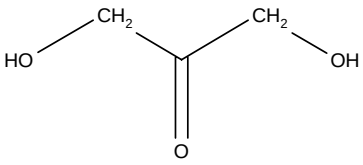
Ref.: 1

3.1.1.4 CAS / EC number

CAS No: 96-26-4
EC No: 202-494-5

Ref.: 1

3.1.1.5 Structural formula



Ref.: 1

3.1.1.6 Empirical formula

$C_3H_6O_3$

Ref.: 1

3.1.2 Physical form

White to almost white fine crystalline free-flowing powder, possibly with granular parts

Ref.: 2, 3, 4, 5

3.1.3 Molecular weight

90.08 g/mol

Ref.: 1

3.1.4 Purity, composition and substance codes

Certificates of analysis are available for 4 different batches of 'Dihydroxyacetone extra pure for cosmetics'. The results are summarized below:

Purity according to specifications	Batch number	Measured	Ref.
98.0 – 102.0%	VL720150	100.3%	2
	VP182150	99.5%	3
	VP182250	99.8%	4
	VP173050	100.0%	5

Batch number VL720150 is described as a typical commercial batch of Dihydroxyacetone (DHA) and has been used for toxicological evaluation in the following studies:

- Local Lymph Node Assay in Mice
- Embryo-foetal developmental toxicity study by the oral route in rats
- 14-day and 13-week oral toxicity (gavage) studies in rats

This batch VL720150 was further characterised as follows:
Chemical identification through ^{1}H NMR and ^{13}C NMR (spectra available)
Loss on drying: 0.05%
Residue on ignition: < 0.01 %

Ref.: 1, 6

3.1.5 Impurities / accompanying contaminants

Impurities:	Heavy metals (as Pb)	≤ 0.001%
	Arsenic	≤ 0.0003%
	Iron	≤ 0.002%
	Protein	≤ 0.1%
	Glycerol	≤ 0.5%
	Water	≤ 0.2%
	Formic Acid	≤ 30 ppm
	Formaldehyde	≤ 50 ppm
Microbiology:	Total viable aerobic count	≤ 100 CFU/g
	E. Coli	absent in 1g
	Pseudomonas aeruginosa	absent in 1g
	Staphylococcus aureus	absent in 1g
	Candida albicans	absent in 1g
	Salmonella species	absent in 10g

Ref.: 2,3,4,5

3.1.6 Solubility

General specifications:
Water: > 930 g/l (at 20°C) according to EC A.6.
Ethanol: soluble

Ref.: 1, 7

3.1.7 Partition coefficient (Log Pow)

Log P_{ow} = -1.822 (not stated whether this is measured or calculated)

Ref.: 1

Measured according to EC A.8 (OECD 107) with DHA Batch number VP983050:
Log P_{ow} = -1.95

Ref.: 8

3.1.8 Additional physical and chemical specifications

pH value (5% in water), according to analytical report:

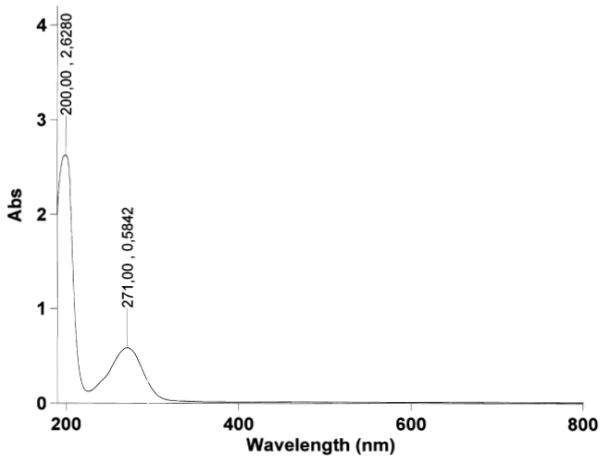
pH according to specifications	Batch number	Measured	Ref.
4.0 - 6.0	VL720150	4.6	2
	VP182150	5.0	3
	VP182250	4.7	4
	VP173050	5.0	5

Additional parameters measured with dihydroxyacetone Batch number VP983050:

Parameter	Method	Result
Melting point	EC A.1 (OECD 102)	96.5°C
Boiling point	EC A.2 (OECD 103)	188°C (decomposition)
Relative density	EC A.3 (OECD 109)	1523 kg/m³ (4°C)
Vapour pressure	EC A.4 (OECD 104)	$2.4*10^{-5}$ hPa (20°C) $5.8*10^{-5}$ hPa (25°C) $3.3*10^{-3}$ hPa (50°C)
Surface tension	EC A.5 (OECD 115)	0.0689 N/m (20°C)
Flammability (solids)	EC A.10	Not a highly flammable solid

Ref.: 9

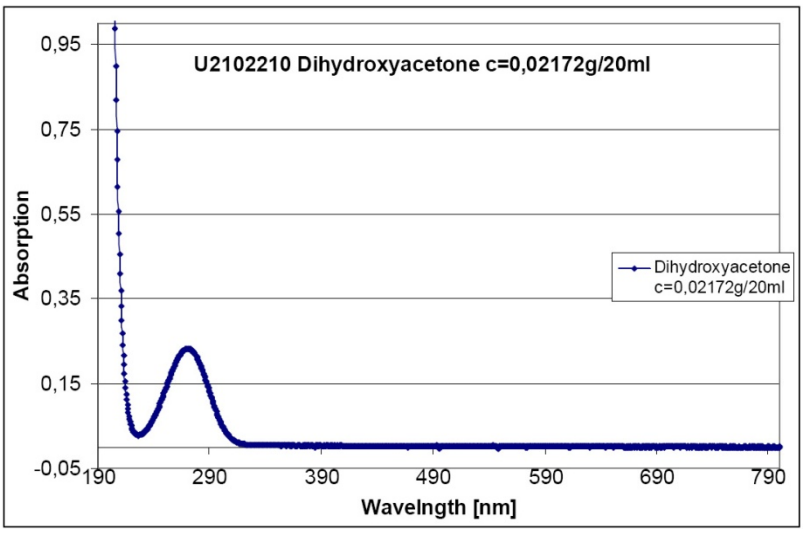
UV / visible light absorption spectrum [0.25% DHA in Aqua dest.]



As shown in the DHA absorption spectrum there is a peak at 271 nm, but no absorption in the UVA/VIS wavelength range (320 – 800 nm).

Ref.: 1

Additional UV / vis absorption spectrum [1.086 g/l DHA (Batch nr. VP195350) in water]



Ref.: 10

3.1.9 Stability

Analytical procedures (HPLC) were developed for analysis of DHA in bi-distilled water. These procedures were shown to be appropriate for measurement of the concentration of DHA in dosing solutions in the concentration range 25 - 100 mg/ml in bi-distilled water.
The test item is stable in dosing solutions when kept for 4 hours at room temperature; recovery rates at the end of this period were within ± 10% of initial values.
According to the authors, the results of this study indicate the appropriate development and validation of the analytical method for the measurement of the DHA concentration in bi-distilled water, as well as the appropriate stability of the dosing solutions used in the different toxicity studies.

Stability tests according to ICH-Q1A Guideline showed that DHA **Batch number VL720150** stored at 2 – 8°C is stable for at least 18 months.
As a general statement, the storage stability of DHA at 4 – 8 °C is declared to be at least 18 months.

Ref.: 1

The summary sheets of some additional stability tests indicate DHA Batch numbers VL438450, VL438550 and VL438650 to be stable for 48 months at 5 ± 3°C.

Ref.: 11

3.2 FUNCTION AND USES

DHA is a self-tanning agent. More specifically, the application of a DHA-containing self-tanning formulation to the skin produces a reaction between DHA and skin contents. DHA reacts with the amino acids and amino groups of proteins present in sweat, keratin and skin, eventually leading to the production of coloured melanoidins. The applicant explains that there is much evidence to suggest that the process is similar to the "Maillard reaction", also known as non-enzymatic browning. 2 - 6 hours after application, a brown coloration appears that has a similar hue to that of natural sun tan. As the DHA molecules are bound to free amino acids and proteins of the skin, the colour obtained is substantive and cannot easily be washed off. It only fades in the course of mechanical abrasion or natural exfoliation of the skin.

DHA has been used worldwide in cosmetic products, i.e. skin care products for face and body, instant tan formulations and 'flash bronzers' in combination with colorants.
The applicant (Colipa, representing 8 cosmetic companies) wishes to support the use of DHA in face creams and body lotions at a concentration of up to 10%. The recommended use levels depend on the skin type and tanning status of the user:

Ca. 3 - 5 % in formulations for persons with light skin (skin type I - II),
Ca. 5 - 10 % in formulations for persons with dark skin (skin type III - IV / V),
Ca. 1 - 2 % in formulations for skin care products (creams and lotions).

The use of DHA solutions in spray cabins, however, is not supported by Colipa, as none of its member companies manufacture products used in spray cabins.

Ref.: 1

A report of the Danish Toxicology Centre (DTC) summarizes the major spraying systems used to treat customers with DHA self-tanning creams (containing 8-14% of DHA):

1) The **manual turbine spray** or spraying 'pistol', spraying a high air volume under low pressure onto the skin. The formulation is sprayed from about 15 cm distance from the customer's body.
2) The **third-generation spray booth**, consisting of a closed compartment in which, after a short countdown, 3 rows of nozzles spray lotion on the entire body. Turning around is not necessary, as the whole body is treated in one spray action. Once the spraying stops, the customer immediately leaves the spraying compartment, in which a thick aerosol mist has meanwhile been formed.
3) The **fourth-generation spray booth**, i.e. an open booth, in which the formulation is charged to 40,000 V and sprayed through 2 vertical rows of nozzles. The customer stands on two earthed metal plates so that the lotion hits her/his body very accurately through electrostatic energy. After 2-3 seconds, the customer turns around and the other side of the body is treated.

In addition, the DTC document reports on some important exposure parameters related to each of the spraying systems, and on the DHA levels measured in the air around the mouth/nose during treatment in a number of operational booths. They are summarized in the following table:

Parameter	Manual turbine spray	Third-generation (closed) spray booth	Fourth-generation (open) spray booth
Amount of formulation used	± 25 ml	± 60 ml	± 15 ml
Application time	2-3 minutes	6 seconds	4-6 seconds
Estimated inhalation exposure	Aerosol cloud is minimal, thus minimal inhalation exposure.	A thick aerosol mist is present in the spraying compartment, thus inhalation is likely.	Aerosol drops are 10 x smaller than in third generation booths; nevertheless, inhalation is expected to be low due to 'electrostatic precision'.
Protective measures to be taken	No specific measures	Customer is advised to keep eyes and mouth shut during treatment.	Customer is advised to use nose filters and eye protection.
Measured DHA concentration (droplets < 12µm)	0.8 mg/m³ air	Up to 238 mg/m³ air	Up to 17 mg/m³ air
Calculated exposure level per treatment[1]	(0.05h x 1.5m³/hour x 0.8 mg/m³) = **0.06 mg DHA**	(0.0017h x 1.5m³/hour x 238 mg/m³) = **0.61 mg DHA**	(0.0017h x 1.5m³/hour x 17 mg/m³) = **0.04 mg DHA**

Ref.: 12

[1] General inhalation rate of 1.5 m³/hour is assumed for adult man (low activity) [Technical Guidance Document on Risk Assessment, Part I, European Commission, ECB, 2003].

3.3 TOXICOLOGICAL EVALUATION

3.3.1 Acute toxicity

3.3.1.1 Acute oral toxicity

Guideline:	Not stated, but mostly corresponding to EC.B1: Acute oral toxicity test.
Date of test:	June 1970
Species/strain:	Wistar rat
Group size:	5 rats/sex/dose
Test substance:	Dihydroxyacetone for cosmetic purposes
Batch:	Batch 446
Purity:	Not stated
Dosages:	800-1,600-3,200-6,400-12,500-16,000 mg/kg bw
Observation period:	14 days
GLP/QAU:	n.a. (before GLP implementation)

Results
In all dose groups the rats showed dazing, staggering and dose-dependent dyspnoea 1 hour after administration. At the highest dose levels convulsive paroxysms (assuming predominantly the abdominal position) were observed. All clinical signs were completely reversible.

Conclusion
The study authors conclude that the LD_{50}-value for Dihydroxyacetone is > 16,000 mg/kg bw.

Ref.: 13

3.3.1.2 Acute dermal toxicity

No data submitted

3.3.1.3 Acute inhalation toxicity

Guideline:	OECD TG 403, EC B.2: Acute toxicity (inhalation)
Date of test:	Dec 2003 - Jan 2004
Species/strain:	Wistar rat
Group size:	5 rats/sex
Test substance:	Dihydroxyacetone
Batch:	VL 157850
Purity:	99.7%
Dosage level:	Limit test at 5 mg/l/4h
Exposure time:	4 hours
Observation period:	14 days
GLP/QAU:	In compliance

Dihydroxyacetone was administered to 10 rats by nose-only, flow-past inhalation exposure for a single 4-hour period, with observation of the animals for a period of 15 days. The nominal concentration in the air was measured to be 5.114 mg/l. Gravimetric measurements of particle size distribution during the exposure produced mass median aerodynamic diameters of 2.92 µm and 2.96 µm.
All animals were observed for clinical signs and mortality during and following the inhalation exposure, i.e. over a 15-day observation period. Body weights were recorded prior to exposure on test day 1, and during the observation period on test days 4, 8 and 15.
On day 15, all animals were sacrificed and necropsied.

Results
There were no deaths, no clinical signs, no relevant adverse effects on body weight development, and no macroscopic pathology findings.

Conclusion
The LC_{50} of DHA in rats obtained in this study was estimated to be > 5.114 mg/l/4h.

Ref.: 14

3.3.1.4 Acute intraperitoneal toxicity

In a study of 1970, Dihydroxyacetone was administered to Wistar rats through the intraperitoneal route at dose levels of 400 - 800 - 1,600 - 3,200 - 6,400 mg/kg bw. At the highest doses, rats showed dazing, staggering, dyspnoea and temporary dragging of the hind paw immediately after injection. All effects were fully reversible.
The LD_{50}-value for Dihydroxyacetone was considered > 6,400 mg/kg bw

Ref.: 13

3.3.2 Irritation and corrosivity

3.3.2.1 Skin irritation

Guideline:	Not stated, but mostly in accordance with EC B.4: Acute Dermal Irritation/Corrosion
Date of test:	June 1970
Species/strain:	New Zealand albino white rabbit
Group size:	6 animals
Test substance:	Dihydroxyacetone for cosmetic purposes
Batch:	Batch 446
Purity:	Not stated
Dose:	0.5 ml of test substance on 6 cm²
Exposure time:	24 hours
Observation period:	1 week
GLP/QAU:	n.a. (before GLP implementation)

A patch with 0.5 g of test material moistened with water, was placed on a ± 4 cm² area of the shaved skin of three female rabbits as well as on the shaved and abraded skin of three male animals. Test solutions were covered with occlusive dressing for 24 hours, after which the patches were removed and the residual substance was rinsed off with water.

Results
Slight reddening of the skin was observed at the site of application in all rabbits (intact and scarified skin). This reddening was observed until the 2nd study day in 5 rabbits and until the 4th day in one rabbit. After this the rabbits were clinically normal again.

Ref.: 13

Comment
This study is old and the description does not allow to check whether it is completely according to the current EC B.4 Guideline. A conclusion is not stated.
Nevertheless the substance does not appear to cause any severe irritation after 24h contact under occlusion, even on abraded skin.

3.3.2.2 Mucous membrane irritation

Guideline:	Not stated, but mostly in accordance with EC B.5: Acute Eye Irritation/Corrosion
Date of test:	June 1970
Species/strain:	New Zealand albino white rabbit
Group size:	3 animals
Test substance:	Dihydroxyacetone for cosmetic purposes
Batch:	Batch 446
Purity:	Not stated
Dose:	100 mg
Exposure time:	24 hours
Observation period:	1 week
GLP/QAU:	n.a. (before GLP implementation)

Before the study, the eyes of all rabbits were examined by means of a split lamp and were found to be free from pathologic alterations. Subsequently, an amount of 100 mg of test substance was placed into the conjunctival sac of the left eye of each of 3 female rabbits, whereas the right eye served as control.

Results
There was slight conjunctival reddening in one rabbit after 24 hours. This reddening was no longer detectable after 3 days. Apart from this, no symptoms occurred during the 1-week observation period.

Ref.: 13

Comment
This study is old and the description does not allow to check whether it is completely according to the current EC B.5 Guideline.
Nevertheless the substance does not appear to cause eye irritation in the rabbit.

3.3.3 Skin sensitization: Local Lymph Node Assay

Guideline:	OECD TG 429, EC B.42: Skin sensitisation: Local Lymph Node Assay
Date of test:	March 2007
Species/strain:	CBA/CaOlaHsd mice
Group size:	5 nulliparous non-pregnant females/dose group
Test substance:	Dihydroxyacetone
Batch:	VL 720150
Purity:	100.3%
Dose levels:	0 - 12.5 - 25 - 50 % DHA in ethanol/water (30/70, v/w)
Exposure time:	24 hours
GLP/QAU:	In compliance

Three groups each of 5 mice were treated with 12.5, 25 or 50% Dihydroxyacetone in ethanol/water (30/70, v/w) by topical application at the dorsum of each ear lobe (left and right) on three consecutive days. The control group was treated with the vehicle alone.
Five days after the first application, the mice were injected (i.v.) with radiolabelled ^{3}H-methyl thymidine. Approximately 5 hours after injection, the mice were killed and the draining auricular lymph nodes excised and pooled per animal. Single cell suspensions of lymph node cells were prepared from pooled lymph nodes, subsequently washed and incubated with trichloroacetic acid overnight. The proliferative capacity of pooled lymph node cells was determined by the incorporation of ^{3}H-methyl thymidine measured in a β-scintillation counter.

Results
Up to the highest test item concentration, the treated mice did not show any signs of irritation or any clinical signs. No mortality was observed. Stimulation indices (S.I.) of 1.04, 1.16, and 0.77 were determined with DHA at concentrations of 12.5, 25, and 50% in ethanol/water (30/70, v/v), respectively. The EC_3 value could not be calculated as none of the test concentrations induced a S.I. greater than 3.

Conclusion
Based on the results of this study, DHA is evaluated as not sensitizing to the skin.
Another solvent than normally used in the LLNA was applied here, probably because of the high water solubility. The solvent or solvent mixture, however, might have an effect on the test outcome.

Ref.: 15

3.3.4 Dermal / percutaneous absorption

3.3.4.1 *In vitro* dermal absorption (human skin samples)

Study 1

Guideline:	OECD TG 428: Percutaneous Absorption: *In vitro* Method (2004), SCCP/0970/06
Date of test:	Jul - Sep 2007
Test system:	Excised, dermatomed (400µm) human skin on a flow-through diffusion cell
N° of samples:	12 samples (4 donors, 3 skin samples/donor) per tested concentration
Test substance:	DHA as o/w formulation at 2.5, 5, 7 and 10% (full composition stated in the study report)
Batch:	VL 759550
Purity:	99.5 %
Applied amount:	2.2 - 2.5 mg formulation/cm², rinsed off after 24 hours
Exposure period:	24 hours
GLP/QAU:	In compliance

The *in vitro* percutaneous absorption of [^{14}C]-DHA was determined in human dermatomed skin by using typical oil-in-water self-tanning formulations containing DHA at concentrations of 2.5%, 5%, 7% or 10 %.
Human dermatomed skin samples were cut at approximately 400 µm in thickness (0.340 to 0.473 mm, n=24). Skin integrity was measured with tritiated water (Kp ≤ 2.5 10^{-3} cm/h). For the main assay, skin samples were mounted on 9mm flow-through diffusion cells, using Phosphate Buffered Saline (PBS) as the receptor fluid. About 2 mg/cm^2 of the different DHA-containing self-tanning formulations were applied to skin surface, with actual application rates ranging from 2.2 to 2.5 mg/cm². The dosing formulations were left on the skin for 24 hours after which the skin surface was rinsed off. Receptor fluid samples were collected after 1, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22 and 24 hours. At the end of the study, radioactivity was measured in various skin compartments (skin wash, stratum corneum (SC, removed by ten consecutive tape strips), epidermis, dermis and receptor fluid) using Liquid Scintillation Counting (LSC).

Results
Originally 3 skin membranes per donor from 4 donors were used per test concentration. In three test groups, however, one or two replicates needed to be excluded due to either inadequate recoveries, a clearly deviating absorption profile or an inadequate Kp value in the ^{3}H water skin integrity test.

The following table provides the amounts of DHA as measured in the different compartments. Results are given in µg/cm² and as a percentage of the applied dose.

Amount of DHA in:	**2.56% DHA n= 11**		**5.07% DHA n = 12**		**7.09% DHA n = 10**		**10.04% DHA n = 11**	
	µg/cm²	%	µg/cm²	%	µg/cm²	%	µg/cm²	%
Applied formulation	64.7 ± 11.7	100.0 ± 18.1	122.1 ± 30.8	100.0 ± 25.2	159.4 ± 46.4	100.0 ± 29.1	225.4 ± 38.6	100.0 ± 17.1
Receptor fluid (24h)	0.77 ± 0.87	1.09 ± 1.13	1.07 ± 1.31	0.89 ± 0.99	2.22 ± 3.15	1.46 ± 1.90	2.99 ± 3.91	1.33 ± 1.61
Stratum corneum	12.43 ± 5.13	18.93 ± 5.90	19.95 ± 8.33	15.98 ± 4.17	28.48 ± 10.78	17.86 ± 5.72	37.08 ± 12.58	16.77 ± 5.68
Epidermis	15.80 ± 7.91	24.38 ± 10.37	30.79 ± 12.22	25.13 ± 7.94	42.92 ± 14.52	26.98 ± 6.37	74.46 ± 28.35	32.73 ± 11.16
Dermis	1.30 ± 0.97	1.88 ± 1.15	1.98 ± 1.22	1.60 ± 0.80	4.15 ± 5.99	2.44 ± 2.96	6.68 ± 4.96	3.01 ± 2.10
Skin wash	27.46 ± 14.02	41.52 ± 17.66	53.69 ± 14.31	44.91 ± 9.77	63.51 ± 23.42	40.66 ± 12.79	81.13 ± 17.41	36.80 ± 9.30
Total recovery	63.74 ± 16.95	97.37 ± 11.75	115.77 ± 29.44	94.95 ± 9.32	152.12 ± 41.63	96.60 ± 13.25	214.73 ± 29.35	96.43 ± 11.25
Total absorption	17.97 ± 8.58	27.49 ± 10.65	33.97 ± 13.23	27.73 ± 8.17	49.47 ± 16.63	31.00 ± 6.58	84.41 ± 28.2	37.19 ± 10.84

Conclusion
Total absorption, defined as the sum of the amounts found in the receptor fluid, the receptor compartment wash and skin membrane (epidermis and dermis, excluding tape strips) slightly increased with DHA concentration, *i.e.* values were 27.5%, 27.7%, 31.0% and 37.2% of the applied dose at DHA concentrations of 2.5, 5, 7 and 10% respectively. Total absolute absorption values were 18.0, 34.0, 49.5 and 84.4 µg/cm² at DHA concentrations of 2.5, 5, 7 and 10% respectively.
Most of the DHA considered to be absorbed (*i.e.* 87 to 91% of the total amounts in the receptor fluid, the receptor compartment wash and skin membranes) was found within the epidermis. According to the study authors, this suggests that considerable amounts of DHA were actually bound to residual parts of the stratum corneum that could not be removed by the ten consecutive tape strips performed[2].

In summary, the dermal absorption of DHA contained in typical self-tanning formulations was estimated to be 27.5 % (18.0 µg/cm^2), 27.7 % (34.0 µg/cm^2), 31.0 % (49.5 µg/cm^2) and 37.2 % (84.4 µg/cm^2) of the applied dose at DHA concentrations of 2.5, 5, 7 and 10%, respectively.

Ref.: 16, 17, 18

Study 2

Guideline:	Bronaugh et al., Methods for *in vitro* percutaneous absorption studies VII: Use of excised human skin. *J. Pharm. Sci.* 75:1094-7, 1986
Date of test:	1999
Test system:	Excised, dermatomed (200-300µm) human skin on a flow-through diffusion cell

[2] The applicant adds that such an inefficiency of standard tape stripping procedures was confirmed by other investigators who reported that at least 50 tape strips are needed to remove 95% of the SC (Ref. 17), or that a total of 92 subsequent tape strips is not efficient at removing the entire SC from human skin samples (Ref. 18).

N° of samples:	3 samples (n° of donors not stated) per tested formulation & concentration
Test substance:	[^{14}C]-DHA at 2.5% and 5.0% in water [^{14}C]-DHA at 5.0% in o/w emulsion (composition stated in report)
Batch:	980709
Purity:	99 %
Applied amount:	15 mg of aqueous solution/cm², 3 mg of o/w emulsion/cm² rinsed off after 24 hours
Measurements:	every 6 hours receptor fluid samples were taken
Exposure period:	24 hours
GLP/QAU:	No documents available

Disc samples of dermatomed skin (200-300 µm in thickness) were mounted on diffusion cells using HEPES-buffered Hanks' balanced salt solution (HHBSS) as the receptor fluid. The skin surface temperature was maintained at 32°C. The barrier integrity of the skin was verified by using the 20 min ^{3}H-water test.
Either a 2.5% or a 5% DHA aqueous solution, or an oil-in-water emulsion containing 5% DHA was applied on skin samples at 15 mg/cm² and 3 mg/cm², respectively.
Samples of receptor fluid were collected at 6-hour intervals over a period of 24 hours. After 24 hours of application, skin samples were washed, and the dermal absorption of DHA was determined by measuring the absorbed radioactivity by liquid scintillation counting in skin washes, stratum corneum (isolated by ten tape strips), viable epidermis + dermis and receptor fluid.
In order to study the potential covalent binding of DHA to skin proteins, additional studies were performed on the skin homogenates from skin samples that had been subject to 10 tape strippings.
Finally, any possible metabolism of DHA in the skin was investigated using HPLC analysis of skin homogenates.

Results

% of applied DHA in:	2.5% DHA in water	5.0% DHA in water	5.0% DHA in o/w emulsion
Receptor fluid (24h)	0.4 ± 0.2	0.5 ± 0.2	0.5 ± 0.1
Stratum corneum 1 (Tape strips 1-5)	6.5 ± 1.3	5.5 ± 1.3	3.9 ± 0.9
Stratum corneum 2 (Tape strips 6-10)	4.5 ± 1.6	4.8 ± 1.8	5.2 ± 0.7
Epidermis + dermis	12.4 ± 8.1	9.7 ± 6.0	12.8 ± 7.0
Skin wash	60.5 ± 8.9	66.4 ± 8.2	65.9 ± 2.1
Total recovery	84.3 ± 2.4	88.1 ± 2.8	88.3 ± 4.3
Total absorption*	23.9 ± 10.6	21.7 ± 9.9	22.3 ± 5.7

* The amount retrieved in the stratum corneum is considered dermally absorbed by the authors of this study

Mean relative absorption of DHA into the receptor fluid over 24 hours was 0.4%, 0.5% and 0.5% of the applied dose for 2.5% DHA in water, 5% DHA in water and 5% DHA in o/w emulsion, respectively. DHA contents in the epidermis/dermis amounted to 10% (5% DHA in water) and 13% of the applied dose (5% DHA in o/w emulsion).
Total relative absorption, defined as the summed amounts of DHA in the receptor fluid and skin membranes (epidermis and dermis, excluding tape strips), amounted to 10.2% (5% DHA in water) and 13.3% of the applied dose (5% DHA in o/w emulsion).
Most of the amounts of DHA considered to be absorbed were actually found within the epidermis/dermis (95% to 97%). This suggests that considerable amounts of the test material might actually be bound to residual parts of the stratum corneum that could not be removed by the 10 tape strips performed. However, covalent binding analysis of the residual skin homogenates (after tape stripping) showed that the majority of radioactivity

was washed out with the two first washes, indicating that the material was not covalently bound.
Finally, no metabolism of DHA into the skin was evidenced as indicated by the presence of a single HPLC chromatogram peak for DHA in skin homogenates.

Conclusion
Total absorption, defined as the sum of the amounts found in the receptor fluid, the receptor compartment wash and skin membrane (epidermis and dermis, excluding tape strips) showed to amount to 10 to 13% of the applied dose. The type of formulation does not appear to significantly influence the dermal absorption.
Most of the absorbed DHA was found within the dermis/epidermis compartment. The fact that DHA does not appear to covalently bind to skin proteins is quite surprising, as the brown staining of the skin does not disappear for hours after application. Therefore the study authors conclude that DHA probably binds to and/or interacts with skin constituents in a manner that consists of a combination of covalent and other intermediate chemical bond forms.

Ref.: 19

3.3.4.2 *In vivo* dermal absorption (human volunteers)

Guideline:	Internal protocol 'Pharmacokinetics: transdermal penetration, excretion in urine and faeces'
Date of test:	22 Jan - 12 Feb 1993
Group size:	6 male human volunteers, age 52-61
Test substance:	1g of 'gel formulation' (also referred to as 'o/w creme for dermal application') containing 50 mg of [^{14}C]-Dihydroxyacetone (42.97 µCi) (full composition stated in the report)
Batch:	not stated
Purity:	not stated
Dosage levels:	topical application: 1g of gel formulation on a skin surface area of 333 cm² on the upper arm
Contact period:	6 hours under non-occlusive cover
Sampling period:	72 hours after application
Quality control:	Ethical approval and Informed Consent documents are available

Approximately 1g of a [^{14}C]-Dihydroxyacetone gel was applied to 333 cm² of the upper arm of 6 healthy male volunteers. The skin was protected by a non-occlusive cover, which was not allowed to come into contact with the gel layer. 6 hours after the single application, the radiolabelled gel was removed by five consecutive skin washings with cotton wool plugs soaked with ether. Thereafter the remaining gel was removed by tape stripping.
Blood samples were drawn up to 72 hours after application and urine and faeces were collected until discharge.

Results
All subjects completed the study and only one adverse effect (headache) was noted. The tolerability of the test substance was judged to be good.

The following measurements were recorded:

Parameter	Mean radioactivity (%) ± SD (n=6)
Cotton wool plugs	14.6 ± 7.1
Skin strips (6h post-application)	29.3 ± 5.1
Skin strips (at discharge)	3.3 ± 1.0
Renal excretion	0.065 ± 0.044
Fecal excretion	0.008 ± 0.009
Total recovery	47.3 ± 3.3

The plasma levels of radioactivity were at all times below two times the radioactivity background value; therefore pharmacokinetic parameters could not be calculated.
Recovery from skin washings after the 6 hour application varied between 6 and 26%, whereas recovery from tape stripping ranged from 20 to 34%. Excretion occurred mainly by the renal route with maximal 0.13% in contrast to excretion via the faecal route, which did not exceed 0.025%. The total recovery of test substances ranged from 42 to 51% of [^{14}C]-Dihydroxyacetone.

Conclusion
The low amount of radioactivity measured in urine and faeces, together with the low plasma levels of [^{14}C]-Dihydroxyacetone, indicate that no noticeable dermal absorption took place in the setting of this study.
The low recovery rate is explained by the galenic formulation (part of it evaporates and is shed off the skin immediately) and by the mechanism of reaction of Dihydroxyacetone within the skin (binds covalently with proteins/lysine residues in the upper layers). The fact that the volunteers' treated skin area was still coloured (pale brown) on the day of discharge supports this theory.

Ref.: 20

3.3.5 Repeated dose toxicity

3.3.5.1 Repeated dose (28 days) oral / dermal / inhalation toxicity

Guideline:	EC Method B.7: Repeated dose (28 days) toxicity (oral); OECD TG 407.
Date of test:	27 Dec 2006 - 21 Mar 2007
Species/strain:	HanRcc:WIST (SPF) Wistar rat
Group size:	5 animals/sex/dosage group
Test substance:	Dihydroxyacetone (Art. 110150)
Batch:	VL720150
Purity:	100.3%
Dosage levels:	0 - 250 - 500 - 1000 mg/kg bw/day
Observation period:	14 days
GLP/QAU:	In compliance

Groups of 5 male and 5 female rats received 250, 500 or 1000 mg/kg bw/day of Dihydroxyacetone by daily oral gavage for 14 days (7 days per week). Control animals received 10 ml/kg bw bi-distilled water only.
Clinical signs, body weights and food consumption were recorded periodically during acclimatization and treatment. At the end of the dosing period, blood samples were withdrawn for haematology and plasma chemistry analyses. All animals were killed, necropsied and examined post mortem. Histological examinations were performed on organs and tissues from all control and high dose animals, and all gross lesions from all animals.

Results
All animals survived to scheduled necropsy. Neither clinical signs nor effects on food consumption or body weight gain were observed.
No test related changes in clinical laboratory investigations (haematology and clinical biochemistry) were recorded. Organ weights were not affected in any of the dosed animals and there were no macroscopic or microscopic findings of note at pathology.

Conclusion
Daily treatment with Dihydroxyacetone for 14 days at oral dosage levels of 250 to 1000 mg/kg bw/day did not cause adverse effects in the Wistar rat.
Based on the results of this study, dosage levels of 250, 500 and 1000 mg/kg bw/day were proposed for the 90-day oral study in the rat.

Ref.: 21

Comment
The above assay is a dose-range finding study for the 90-day subchronic toxicity test with a shortened administration period (14 days instead of 28 days).

3.3.5.2 Subchronic (90 days) oral / dermal / inhalation toxicity

Guideline:	EC Method B.26: Repeated dose 90-day oral toxicity study in rodents; OECD T G 408
Date of test:	21 Feb - 24 Sep 2007
Species/strain:	HanRcc:WIST (SPF) Wistar rat
Group size:	10 animals/sex/dosage group; additional 5 animals/sex for control and high dosage groups
Test substance:	Dihydroxyacetone (Art. 110150)
Batch:	VL720150
Purity:	100.3%
Dosage levels:	0 - 250 - 500 - 1000 mg/kg bw/day
Observation period:	17 weeks
GLP/QAU:	In compliance

Dihydroxyacetone was administered daily to groups of 20 Wistar rats at dose levels of 250, 500 and 1000 mg/kg bw/day for a period of 13 weeks. A control group was treated similarly with bi-distilled water only. Additional 10 rats were used at 0 and 1000 mg/kg bw/day. These animals were treated for 13 weeks and then allowed a 4-week treatment-free recovery period after which they were sacrificed.
Clinical signs, outside cage observation, food consumption and body weights were recorded periodically during pretest, treatment and recovery period. Ophthalmoscopic examinations were performed at pretest, the end of the treatment and recovery periods. Functional observational battery, locomotor activity and grip strength were performed during week 13 and week 17. At the end of the dosing and the treatment-free recovery period, blood samples were withdrawn for haematology and clinical biochemistry analyses. Urine samples were collected for urinalyses. All animals were killed, necropsied and examined post mortem. Histological examinations were performed on organs and tissues from all control and high dose animals, and all gross lesions from all animals.

Results
All animals survived to scheduled necropsy. Neither clinical signs nor effects on food consumption or body weight gain were observed.
Neither ophthalmoscopic examinations nor functional observational battery revealed test item-related changes.
No test related changes in clinical laboratory investigations (haematology, clinical biochemistry and urinanalysis) were recorded. Organ weights were not affected in any of the dosed animals and there were no macroscopic or microscopic findings of note at pathology.

Conclusion
Treatment for 13 weeks with Dihydroxyacetone at daily oral dosage levels of 250 to 1000 mg/kg bw/day did not cause adverse effects in the Wistar rat.
Based on the results of this study, the dosage level of 1000 mg/kg bw/day was chosen as the NOEL-value.

Ref.: 22

3.3.5.3 Chronic (> 12 months) toxicity

No data submitted

3.3.6 Mutagenicity / genotoxicity

3.3.6.1 Mutagenicity / genotoxicity *in vitro*

A. Bacterial Reverse mutation test 1

Date of study:	6-15 July 1983
Guideline:	OECD TG 471, EC Method B.13/14: Mutagenicity: reverse mutation test using bacteria
Species/strain:	Salmonella typhimurium TA98, TA100, TA102, TA1535, TA1537
Replicates:	4
Test substance:	Dihydroxyacetone
Batch:	V216350
Purity:	99.0%
Concentrations:	50 - 250 – 1,250 – 2,500 – 5,000 - 10,000 µg/plate, with and without S9-activation
GLP/QAU:	Signed document available, although test performed before official GLP implementation

Five strains of Salmonella typhimurium (TA100, TA102, TA1535, TA98 and TA1537) were used to evaluate the mutagenic potential of DHA in two independent experiments in the presence and absence of Aroclor-induced S9. DMSO was used as the solvent. Since DHA was freely soluble and had shown to be non-toxic in a preliminary experiment, doses used were 50 - 250 – 1,250 – 2,500 – 5,000 - 10,000 µg/plate.
Known mutagens were used as positive controls, and cultures treated with DMSO (solvent) were used as negative controls. Four plates per treatment condition were used.

Results
All solvent and positive controls gave counts of revertants within expected ranges, and the experiments were therefore considered to be valid. No noteworthy toxicity was observed in any strain tested, either in the presence or absence of S9. DHA did not show mutagenic activity with the test strains TA98, TA1535 and TA1537 up to a maximum test concentration of 10,000 µg/plate. However, reproducible, dose-related increases in revertant counts were obtained for test strains TA100 and TA102 in the presence and absence of S9. The presence of S9 had no modifying effect and the increase was only seen at relatively high test concentrations (i.e. at ≥ 2,500 µg/plate).

Conclusion
With and without the addition of S9, DHA was mutagenic in two test strains (TA100, TA102) under the experimental conditions described in this study.

Ref.: 23

B. Bacterial Reverse Mutation test 2

Date of study:	6-15 July 1983
Guideline:	OECD TG 471, EC Method B.13/14: Mutagenicity: reverse mutation test using bacteria
Species/strain:	Salmonella typhimurium TA98, TA100, TA102, TA1535, TA1537
Replicates:	4
Test substance:	Dihydroxyacetone
Batch:	V878 (referred to as 'old sample from reserve')
Purity:	100.1%
Concentrations:	50 - 250 - 1250 - 2500 - 5000 - 10000 µg/plate - with and without S9-activation
GLP/QAU:	Signed document available, although test performed before official GLP implementation

Five strains of Salmonella typhimurium (TA100, TA102, TA1535, TA98 and TA1537) were used to evaluate the mutagenic potential of DHA in two independent experiments in the presence and absence of Aroclor-induced S9. DMSO was used as the solvent. Since DHA was freely soluble and non-toxic, doses used were 50 - 250 - 1250 - 2500 – 5000 - 10000 µg/plate.
Known mutagens were used as positive controls, and cultures treated with DMSO (solvent) were used as negative controls. Four plates per treatment condition were used.

Results
All solvent and positive controls gave counts of revertants within expected ranges, and the experiments were therefore considered to be valid. No noteworthy toxicity was observed in any strain tested, either in the presence or absence of S9. DHA did not show mutagenic activity with the test strains TA98, TA1535 and TA1537 up to a maximum test concentration of 10,000 µg/plate. However, reproducible, dose-related increases in revertant counts were obtained for test strains TA100 and TA102 in the presence and absence of S9. The presence of S9 had no modifying effect and the increase was only seen at relatively high test concentrations (i.e. at ≥ 2,500 µg/plate).

Conclusion
With and without the addition of S9, DHA was mutagenic in two test strains (TA100, TA102) under the experimental conditions described in this study.

Ref.: 24

C. Reverse mutation test 3, including influence of protein on mutagenic potential of DHA in S. typhimurium TA100

Date of study:	7 Sep 1983 – 18 Mar 1984
Guideline:	OECD TG 471, EC Method B.13/14:Mutagenicity: reverse mutation test using bacteria
Species/strain:	Salmonella typhimurium TA100
Replicates:	4
Test substance:	Dihydroxyacetone
Batch:	V216350 (I/83)
Purity:	99.0%
Concentrations:	2,500 – 5,000 – 10,000 µg/plate, with different activation systems: 1) S9 mix from Aroclor 1254-induced rats 2) inactivated S9 mix from Aroclor 1254-induced rats 3) bovine serum albumin 4) combination of 1) and 3) 5) combination of 1) and 2)
GLP/QAU:	n.a. (before GLP implementation)

The aim of this additional investigation was to examine whether the mutagenic activity of DHA could be influenced by different kinds of proteins or by different concentrations of these proteins. Therefore the reverse mutation test in S. typhimurium TA100 was repeated making use of the plate incorporation test and of modifications to the *in vitro* metabolising system (i.e. different amounts of Aroclor-induced S9, inactivated Aroclor-induced S9, bovine serum albumin alone and Aroclor-induced S9 supplemented with either bovine serum albumin (BSA) or inactivated Aroclor-induced S9). DMSO was used as the solvent and doses used were 2,500 – 5,000 – 10,000 µg/plate.
Known mutagens were used as positive controls, and cultures treated with DMSO (solvent) were used as negative controls. Four plates per treatment condition were used.

Results
The solvent and positive controls gave counts of revertants within expected ranges. Without the addition of proteins, DHA showed a mutagenic activity in S. typhimurium strain TA100, as expected. The Aroclor-induced S9 caused a decrease in the number of colonies, whereas the inactivated S9 did not show this effect. BSA produced a slight increase in mutagenic activity. When BSA or inactivated S9 was combined with S9, the decrease of mutagenic activity observed with the latter was diminished.

Conclusion
Under the experimental conditions of this report the mutagenic activity of DHA in Salmonella typhimurium TA100 was only slightly affected by the addition of different protein preparations to the test system. Nevertheless, according to the study authors, this study shows that the direct acting mutagen DHA was inactivated by mammalian metabolizing enzymes.

Ref.: 25

D. *In vitro* mammalian cell gene mutation test

Date of study:	Jun 1993
Guideline:	OECD TG 476, EC Method B.17: Mutagenicity: *In vitro* mammalian cell gene mutation test
Species/strain:	V79 Chinese hamster cells [*hprt* locus for 6-thioguanine resistance]
Replicates:	2
Test substance:	Dihydroxyacetone
Batch:	V360350
Purity:	99.7%
Vehicle used:	Distilled water
Concentrations:	+ S9 mix: 31.6 - 100 - 316 – 1,000 µg/ml - S9 mix: 158 - 500 – 1,580 – 5,000 µg/ml
GLP/QAU:	In compliance

DHA was tested in two independent experiments. Duplicate cultures were exposed to DHA at 31.6 - 100 - 316 – 1,000 µg/ml for 24 hours in the absence of S9-mix and at 158 - 500 – 1,580 – 5,000 µg/ml for 2 hours in the presence of S9 (from Aroclor 1254-treated rats). The highest test concentration used in the presence of S9 was the limit dose for this assay, whilst the highest dose used in the absence of S9 was based upon the results of a preliminary toxicity test.
Two parallel cultures were treated with N-Methyl-N'-nitro-N-nitrosoguanidine (MNNG, at 1 µg/ml) or 7,12-dimethylbenz[a]anthracene (DMBA, at 20 µg/ml) in the absence and presence of S9, respectively, as positive controls. Three parallel cultures were established for the negative controls and treated with the solvent (distilled water) alone. Increases in mutant frequency were determined at a single expression time 8 days after treatment.

Results
Mutant frequencies in the solvent negative control remained within normal ranges, and treatment with the positive controls MNNG and DMBA yielded distinct increases in mutant frequencies. Accordingly, the study was considered to be valid.
When tested up to 1,000 or 5,000 µg/ml, DHA did not increase the mutant frequencies in the *hprt* locus of V79 cells either in the absence or the presence of S9-mix, respectively.

Conclusion
DHA was, up to concentrations reaching the solubility limits of the compound, not mutagenic in this mammalian cell gene mutation test in V79 cells (*hprt* locus) either in the absence or presence of metabolic activation under conditions where the positive controls exerted potent mutagenic effects.

Ref.: 26

E. *In vitro* mammalian chromosome aberration test

Date of study:	Feb 1994
Guideline:	OECD TG 473, EC Method B.10: Mutagenicity: *In vitro* mammalian chromosome aberration test
Species/strain:	V79 Chinese hamster cells
Replicates:	2
Test substance:	Dihydroxyacetone
Batch:	V360350
Purity:	99.7%
Vehicle used:	Distilled water
Concentrations:	± S9 mix: 140 - 445 - 1400 – 2,500 µg/ml
GLP/QAU:	In compliance

DHA was tested in two independent experiments in the presence or absence of a metabolic activation system (S9-mix from Aroclor 1254-treated rats). Distilled water was used as a vehicle. Cells were treated for 5 hours with test concentrations of 140 - 445 - 1400 – 2,500 µg/ml (in experiment 2 a high dose of 2,500 µg/ml was used). Ethylmethane sulfonate (EMS, at 500 µg/ml) and Cyclophosphamide (CPA, at 2 µg/ml) were used as positive controls. Medium and solvent controls were included as well. Cells were sampled for metaphase analysis 18 hours after initiation of treatment for the intermediate and low dose groups, and 7, 18 and 28 hours after initiation of treatment for the high dose and vehicle control groups. Four slides (= cultures) were investigated per concentration (two +S9 and two –S9) and per time point by scoring 100 well spread metaphases per slide for chromosomal aberrations, whilst a total of 1000 mitoses per slide were analysed for polyploidy induction. Cytotoxic effects were estimated by suppression of mitotic index based upon a sample size of 2000 cells per dose point.

Results
The positive control substances induced the expected clear increase in the portion of the cells with chromosomal aberrations. The aberration rates of the negative controls were within the usual range of the cell line used in the performing laboratory. DHA decreased the mitotic index at the highest test material concentration tested (2,500 µg/ml) to relative values ranging from 34 to 67% (with and without S9-mix) as compared to the respective solvent control values.
In the absence and in the presence of the S9-mix DHA did not increase the proportion of cells with aberrant chromosomes as compared to the negative controls. Furthermore, DHA did not lead to an increase in the number of polyploid cells.

Conclusion
DHA was not clastogenic in this test system under conditions where the positive controls exerted potent clastogenic effects.

Ref.: 27

3.3.8.2 Mutagenicity / genotoxicity *in vivo*

In vivo micronucleus test

Date of study:	4 Feb – 19 Mar 1983
Guideline:	OECD TG 474, EC Method B.12: Mutagenicity: *In vivo* mammalian erythrocyte micronucleus test
Species:	ddY mice
N° of animals:	1 male and 1 female mouse per dose group in the preliminary studies 6 male mice per dose group in main study
Sacrifice time:	24h post-dose in the main micronucleus test
Test substance:	Dihydroxyacetone
Vehicle used:	Physiological saline
Batch:	00420 CM
Purity:	98%
Doses tested:	Small scale acute toxicity test: 1,000 – 2,000 – 3,000 – 4,000 – 5,000 mg/kg bw Pilot micronucleus test: 10 - 100 - 500 - 1,000 - 5,000 mg/kg bw Micronucleus test (main study): 1,250 - 2,500 - 5,000 mg/kg bw
GLP/QAU:	n.a. (before GLP implementation)

DHA was given intraperitoneally to male ddY mice, at dose levels of 1,250 - 2,500 - 5,000 mg/kg bw. Experimental conditions were based on a dose-range finding acute toxicity test and a pilot micronucleus test, respectively. Mice of the negative control group received the vehicle (physiological saline) only. Mitomycin C was used as a positive control. For the main study, a total of 30 animals were used (6 animals per group). Bone marrow smears were prepared from each animal and stained with Giemsa's solution. Sampling time was 24 hours after administration of the test material based on the results of the pilot micronucleus test. For microscopic investigations, at least two specimens from each animal were prepared and coded. The number of micronucleated polychromatic erythrocytes (MNPCEs) per 1000 polychromatic erythrocytes (PCEs) per animal was determined. The proportion of PCEs to total erythrocytes based on 1000 erythrocytes per animal was also determined to evaluate the bone marrow toxicity of DHA.

Results
Although the substance was shown to reach the target organ, no statistically significant or biologically relevant increase in the number of polychromatic erythrocytes with micronuclei was observed in any of the DHA-treated groups compared to the negative control. The PCE ratio was not changed in the treatment groups.

Conclusion
DHA was not mutagenic in this *in vivo* micronucleus assay in male mice under conditions where the positive controls exerted potent mutagenic effects.

Ref.: 28

Comment
The full study report of this *in vivo* micronucleus test is not available, only a extensive summary.

3.3.7 Carcinogenicity

A short publication of 1984 describes a dermal carcinogenicity study with DHA in Swiss-Webster mice. Animals were treated by topical application with aqueous solutions of DHA (5 or 40%) on the shaved dorsal regions once weekly for 80 weeks. Except for the brown coloration of application sites in DHA-treated mice, no differences in gross physical appearance or clinical signs were observed. Body weight gains were reported to be similar in all groups. Survival rate was not affected by DHA treatment. Histopathologically, there were no changes attributed to treatment with DHA. The tumours observed were typical of the type normally observed in mice of this strain and age, and they were equally distributed among control and treated groups.
The authors conclude that DHA shows to be non-carcinogenic in the presented study.
Ref.: 29

3.3.8 Reproductive toxicity

3.3.8.1 Two generation reproduction toxicity

No data submitted

3.3.8.2 Teratogenicity

Guideline:	Annex V to Dir. 67/548/EEC (2004/73/EC), B.31: Prenatal developmental toxicity study; OECD Technical Guideline N° 414.
Date of study:	22 Jan - 11 Jun 2007
Species/strain:	HanRcc:WIST (SPF) Wistar rat
Group size:	22 animals/dosage group
Test substance:	Dihydroxyacetone (Art. 110150)
Batch:	VL720150
Purity:	100.3%
Dose levels:	0 - 100 - 300 – 1,000 mg/kg bw/day
GLP:	In compliance

Three groups of 22 pregnant rats received Dihydroxyacetone by gavage at doses of 100 - 300 – 1,000 mg/kg bw/day from day 6 through day 20 post coitum. A fourth group of 22 pregnant rats received milli-Q water only.
Animals were checked twice daily for mortality and clinical signs. Food consumption and body weight were recorded at designated intervals during pregnancy. On day 21 post coitum, the animals were killed and examined macroscopically. Foetuses were removed by Caesarean section, examined for gross external abnormalities and subjected to microdissection.

Results
All females survived until scheduled necropsy. No signs of discomfort or clinical symptoms from the treatment with the test item were observed. No macroscopic findings were noted during necropsy of the females.
Mean food consumption, mean body weight and corrected body weight gain (corrected for the gravid uterus weight) were not affected by treatment with the test item in any dose group. Post-implantation losses and the mean number of foetuses per dam were not affected by treatment with the test item at all dose levels.
No test item-related effects on foetal body weights or sex ratios were noted. During the external and visceral examination of the foetuses, no test item-related abnormal findings were noted. Neither were any abnormalities noted during examination of foetal skeleton and cartilages.

Conclusion
Based on the results described above, 1,000 mg/kg bw/day of Dihydroxyacetone is considered to be the No-Observable- Effect-Level (NOEL) for maternal and foetal organisms.

Ref.: 30

3.3.9 Toxicokinetics

See 3.3.4.2: The absorption of radioactivity of ^{14}C-Dihydroxyacetone (DHA) through the skin after single dose dermal application of 50 mg to six healthy volunteers.
Ref.: 20

3.3.10 Photo-induced toxicity

3.3.10.1 Phototoxicity/photoirritation and photosensitisation

No data.

3.3.10.2 Phototoxicity / photomutagenicity / photoclastogenicity

A. Reverse mutation test with UV irradiation 1

Date of study:	July 1983
Guideline:	Based on OECD TG 471, Annex V to Dir. 67/548/EEC, Method B.13/14: Mutagenicity: reverse mutation test using bacteria; adapted by including exposure to UV-light
Species/strain:	Salmonella typhimurium TA98, TA100, TA102, TA1535, TA1537
Replicates:	4
Test substance:	Dihydroxyacetone (exposed to light)
Batch:	V216350
Purity:	99.0%
Concentrations:	50 - 250 – 1,250 – 2,500 – 5,000 – 10,000 µg/plate, with and without S9-activation
GLP/QAU:	n.a. (before GLP implementation)

The mutagenic potential of DHA, exposed to light before testing, was evaluated in two independent experiments in the presence and absence of Aroclor-induced S9 and in five strains of Salmonella typhimurium (TA100, TA102, TA1535, TA98 and TA1537). DMSO was used as the solvent. Since DHA was freely soluble and shown to be non-toxic, doses used were 50 - 250 – 1,250 – 2,500 – 5,000 – 10,000 µg/plate.
Known mutagens were used as positive controls, and cultures treated with DMSO (solvent) were used as negative controls. Four plates per treatment condition were used.

Results
All solvent and positive controls gave counts of revertants within expected ranges, and the experiments were therefore considered to be valid. No noteworthy toxicity was observed in any strain tested, either in the presence or absence of S9. DHA did not show mutagenic activity with the test strains TA98, TA1535 and TA1537 up to a maximum test concentration of 10,000 µg/plate. Reproducible, dose-related increases in revertant counts were obtained for test strains TA100 and TA102 in the presence and absence of S9. Nevertheless, the magnitude of increases in revertant counts of ≥ 2,500 µg/plate were very similar to those obtained in the tests using DHA not exposed to light.

Conclusion
With and without the addition of S9, DHA was mutagenic in two test strains (TA100, TA102) under the experimental conditions described in this study. However, deliberate exposure of DHA to light did not result in a significant enhanced mutagenic response.

Ref.: 31

B. Reverse mutation test with UV irradiation 2

Date of study:	12 Mar - 5 Aug 1993
Guideline:	Based on OECD TG 471, Annex V to Dir. 67/548/EEC, Method B.13/14: Mutagenicity: reverse mutation test using bacteria; adapted by including exposure to UV-light
Species/strain:	Salmonella typhimurium TA100, TA102, TA1537; and Escherichia coli WP2
Replicates:	3
Test substance:	Dihydroxyacetone (exposed to light)
Batch:	not stated
Purity:	not stated
Concentrations:	0 - 5 - 50 - 500 – 1,580 – 5,000 – 7,500 – 10,000 µg/plate, with and without S9-activation
GLP/QAU:	no undersigned documents available

The photomutagenic potential of DHA was evaluated in two independent series in the absence of an exogenous metabolizing system. The UV source was a Heraeus Suntest® lamp (with an appropriate UV filter) and the UVB component corresponded to 1/6 of the UVA component. UVA/UVB doses were measured with an Osram Centra® measuring device and a range of different UV doses were used for each bacterial strain (ranging from 1 to 36 mJ/cm^2). Deionised water was used as the solvent. Concentrations from DHA ranged from 50 to 5,000 µg/plate and two higher concentrations (7,500 and 10,000 µg/plate) were additionally tested in the experiments with TA100 and TA102. 8-Methoxypsoralen was used as a photomutagenic positive control. Cultures treated with DMSO (solvent) were used as negative controls. For each condition three plates were used.

Results
DHA did not increase the number of revertants of TA1537 and WP2 compared to the solvent controls under the conditions of this experiment. At concentrations ≥ 5,000 µg/plate, DHA clearly increased the number of revertants of TA100 and TA102 in the absence of UV. The number of revertants was however not increased by exposure to UV light.

Conclusion
DHA was not photomutagenic under conditions whereas the positive control, 8-methoxypsoralen, induced a clear UV-dependent mutagenic effect.

Ref.: 32

3.3.11 Human data

A 2004 publication describes that the application of a 20% DHA cream applied twice per day on the volar forearm of 10 volunteers with light skin types (types II-III) did not cause any adverse effects. The cream even appeared to generate a sun protection factor ranging from SPF 3.0 on day 1 to SPF 1.7 on day 7 after application.

Ref.: 33

3.3.12 Special investigations

No data submitted

3.3.13 Safety evaluation (including calculation of the MoS)

CALCULATION OF THE MARGIN OF SAFETY

Weekly[3] **topical application** of a self-tanning lotion containing DHA up to 10%

Amount of body lotion applied daily	=	8000 mg/day
Maximum concentration of DHA in formulation	=	10%
Maximum absorption through the skin	=	48.03%[4]
Typical human body weight	=	60kg
Systemic exposure dose (SED)		
(8000mg/day x 10/100 x 48.03/100 x 1/7) / 60kg	=	0.91 mg/kg bw/day
NOEL (90 days-oral-rat) (highest concentration tested)	=	1000 mg/kg bw/day

Margin of Safety = NOEL / SED = **1093**

Worst case daily[5] **topical application** of self-tanning lotion and face cream both containing DHA up to 10%

Amount of body lotion applied daily	=	8000 mg/day
Amount of face cream applied daily	=	1600 mg/day
Maximum concentration of DHA in formulation	=	10%
Maximum absorption through the skin	=	48.03%[4]
Typical human body weight	=	60kg
Systemic exposure dose (SED)		
(9600mg/day x 10/100 x 48.03/100) / 60kg	=	7.68 mg/kg bw/day
NOEL (90 days-oral-rat) (highest concentration tested)	=	1000 mg/kg bw/day

Margin of Safety = NOEL / SED = **130**

Spraying application of self-tanning lotions containing DHA up to 14%

As stated in the DTC report (see 3.2), depending on the type of spraying system used, 15 to 60 ml of DHA-containing formulations are sprayed on and in the vicinity of the customer's body.

In the following calculation of the MoS, the third-generation spraying booths are chosen as the worst case scenario, as they not only involve the largest application volume of 60 ml of formulation, but equally generate the highest DHA air concentration of 238 mg/m³ air. The latter is calculated to correspond to the highest potential systemic exposure level of DHA through inhalation (0.61 mg) among the examined spraying systems.

[3] The Danish EPA report (Ref. 2) takes into account that common application may occur once a month, or in more specific cases (TV-presenters, models), once per week. Therefore, the first calculation of the MoS takes into account a weekly application averaged out over 7 days, leading to the maximal frequency of application of 1/7.

[4] According to the SCCS 'Basic criteria for the *in vitro* assessment of dermal absorption of cosmetic ingredients' (SCCS/1358/10), the mean + 1SD is to be used for the calculation of the MoS when studies comply with the basic requirements of the SCCS, i.e. 37.19% + 10.84% = 48.03% (see 3.3.4.1, Study 1).

[5] Assuming daily application of a 10% DHA-containing tanning preparation is an unrealistic worst-case scenario. Nevertheless, the obtained MoS shows that, even under these extreme exposure conditions, the use of both self-tanning lotion and face cream containing 10%DHA, can be considered as safe.

During the spray application, the peak exposure (238 mg/m³) lasted only for 6 seconds. In studies on acute toxicity by inhalation with Wistar rats, no relevant toxicity or adverse effects were observed after 4 hour exposure to 5000 mg/m³. In addition, consumers are advised to hold their breath during application. Thus, the peak exposure is not expected to be of concern.
As far as the dermal SED determination is concerned, some assumptions need to be made due to the lack of information with regard to the exact composition and the relative density of the formulations used in the booths. Firstly the spray formulation is considered to have a relative density of 1, meaning that 60 ml would correspond to 60g. As the DHA concentration in the formulation is unknown, the maximal concentration of 14% is used for further calculation. This means that exposure to 60 ml of formulation could lead to an absolute maximal dermal exposure to 8.4 g of DHA. Taking into account the amount of substance that unavoidably will be spilled in the spraying booth, this is a clear overestimation of the real exposure.

Supposing the application would be at the maximum application frequency of once/week, the systemic exposure through the dermal route can be calculated as follows:

(8400mg/day x 48.03/100 x 1/7) / 60kg = 9.6060 mg/kg bw/day

The systemic exposure through the inhalation route would subsequently be the following:

(0.61mg/day * 1/7) / 60kg = 0.0014 mg/kg bw/day

Taking into account both exposure routes, the MoS for spraying applications can be calculated as follows:

SED (dermal route)	= 9.6060 mg/kg/day
SED (inhalation route)	= 0.0014 mg/kg/day
Total $SED_{derm+inh}$	= 9.6074 mg/kg/day
NOEL (90 days-oral-rat) (highest concentration tested)	= 1000 mg/kg bw/day

Margin of Safety = NOEL / SED = **104**

3.3.14 Discussion

Dihydroxyacetone (DHA) is used as a self-tanning agent in leave-on cosmetic products up to 10%. In addition, DHA is also reported to be used in spray cabins in aqueous solutions in concentrations between 8 and 14% with the purpose of obtaining tanned skin without exposure to sunlight or UV radiation.

Physico-chemical properties
The submission contains the required information with regard to the identification/purity of several specific batches of DHA, including the batch used in the LLNA and in the most recent long term toxicity studies (14 and 90 day oral toxicity and teratogenicity).
Test reports for important physicochemical parameters (water solubility, Log P_{ow}, pH, melting point, boiling point, relative density, vapour pressure, etc) are available.

Acute systemic toxicity
DHA has a low acute toxicity profile, with an acute oral LD_{50} value of more than 16,000 mg/kg bw and an inhalation LC_{50} above the limit test threshold of 5 mg/l/4h. The intraperitoneal LD_{50} value was determined to be above 6,400 mg/kg bw.

Skin and eye irritation
A relatively old Draize skin irritation study (1970) indicates that DHA does not appear to cause any severe irritation after 24h contact under occlusion, even on abraded skin. An analogous eye irritation study suggests that DHA is non-irritating to the eye.
Although both studies are outdated, the available raw data allow supporting the conclusion that DHA is neither a skin nor an eye irritant.

Skin sensitisation
A Local Lymph Node Assay (carried out in 2007) shows DHA to be non-sensitising to the skin in that *in vivo* model.
However, DHA is known to be a contact allergen in man. (Ref. 34)

Dermal absorption
Three dermal absorption studies are presented, of which 2 *in vitro* studies and one *in vivo* assay in human volunteers.
The most recent (2007) *in vitro* study investigates the dermal absorption values of self-tanning o/w formulations containing DHA at different concentration levels, from 2.5 to 10%. The study is well performed and estimates that, when used at 10% in an o/w formulation, 37.2% of the applied DHA-amount (or 84.4 µg DHA/cm²) may become systemically available. This assay also shows that the total dermal absorption value increases with an increasing DHA concentration in the final formulation.
A second *in vitro* study dates from 1983 and investigates the dermal absorption of DHA when applied at 2.5 and 5.0% in an aqueous solution and at 5.0% in an o/w emulsion. The results show that the percentage of absorbed DHA amounts up to 13% and that the type of formulation does not significantly influence dermal absorption of the compound. The assay also includes some investigations with regard to the alleged covalent binding of DHA with skin proteins. This is supposed to be the basic principle for the self-tanning properties of the molecule.
The compound appears not to be easily washed out of the skin compartments, leading the study authors to conclude that DHA probably binds to and/or interacts with skin constituents in a manner that consists of a combination of covalent and other intermediate chemical bond forms.

In the *in vivo* dermal absorption study with human volunteers, a 5% DHA formulation is applied on the forearm of 6 male volunteers. This assay reveals that DHA only reaches minor concentration levels in plasma, urine and faeces. DHA can only be found in the skin strips withdrawn to estimate the amount of the compound in the stratum corneum of the volunteers. The theory of protein binding by DHA is supported by the fact that the volunteers' treated skin area is still coloured (pale brown) on the day of discharge.
For the calculation of the MoS, the SCCS proposes to use the value of the most recent *in vitro* dermal absorption study as this is well described and performed according to the current guidelines. In addition, it describes the dermal absorption of a representative formulation containing increasing DHA concentrations, including the highest envisaged use concentration of 10% (body lotions and face creams). Finally, the study probably provides an overestimation of the actual dermal absorption value, as DHA may not become systematically available due to protein binding in the human skin in a real-life situation. As prescribed by the SCCS 'Basic criteria for the *in vitro* assessment of dermal absorption of cosmetic ingredients' (SCCS/1358/10), the (**mean + 1SD**) will be used as dermal absorption value for the calculation of the MoS, i.e. (37.19% + 10.84%) or **48.03%.**

Repeated dose systemic toxicity
The submission contains 14 day and 90 day oral tests with the rat with DHA dosage levels up to 1000 mg/kg bw/day, the limit dosage for this type of studies. The results reveal that treatment for 13 weeks with DHA at daily oral dosage levels of 250 to 1000 mg/kg bw/day

did not cause adverse effects in the Wistar rat, wherefore the level of 1000 mg/kg bw/day can be established as the NOEL-value and used for the MoS calculation.

Reproductive toxicity
A teratogenicity study, in which DHA is tested up to 1000 mg/kg bw/day, is available and shows that the compound does not display any embryotoxic properties. The NOEL for maternal and foetal toxicity is 1000 mg/kg bw/day.
No 2-generation reproduction toxicity study is provided.

Carcinogenicity
No fully described carcinogenicity study is available. Instead, a short publication of 1984 describes a dermal carcinogenicity study with DHA in Swiss-Webster mice. No conclusion could be drawn from the study.

Mutagenicity / genotoxicity
The submission contains 3 reverse mutation tests, which all reveal a mutagenic potential of DHA in two particular Salmonella typhimurium strains (TA100 and TA102). One of them includes an investigation on the effect of different kinds of proteins or different concentrations of these proteins on the mutagenic activity of DHA. According to the study authors, the results indicate that direct acting mutagen DHA may be inactivated by mammalian metabolizing enzymes. This means that the mutation effects would not occur in an *in vivo* setting.

An *in vitro* mammalian cell gene mutation test in V79 cells shows DHA to be non-mutagenic up to concentrations reaching the solubility limits of the compound. This appears to be true as well in the absence as in the presence of metabolic activation. DHA also shows to be non-clastogenic in the presented *in vitro* mammalian chromosome aberration assay.

Finally, an *in vivo* micronucleus test in the mouse reveals no statistically significant or biologically relevant increase in the number of polychromatic erythrocytes with micronuclei in any of the DHA-treated groups compared to the negative control.
Although the *in vitro* mutagenicity/genotoxicity testing battery is known to yield a high rate of false positive results and even though the mammalian cell gene mutation as well as the chromosome aberration test gave negative results, the consistent positive results obtained in the reverse mutation assays cannot be denied. Therefore, the SCCS invited external experts to discuss the *in vitro* positive results. In first instance, the presented *in vitro* / *in vivo* testing battery was considered of good scientific quality. Subsequently, a large-scale study of the *in vitro* genotoxicity results obtained for known carcinogens and *in vivo* genotoxic compounds was presented in detail in order to help elucidating the ambiguities related to the obtained positive *in vitro* results for DHA. One of the key findings of this comprehensive study was that all bacterial reverse mutation positive carcinogens and the majority of bacterial reverse mutation positive *in vivo* genotoxins also produced positive results in one of the performed *in vitro* genotoxicity assays with mammalian cells (personal communication). As DHA only showed to be positive in two specific bacterial strains (S. Typhimurium TA 100 & TA 102) and was negative in an *in vitro* mammalian cell gene mutation test, in an *in vitro* mammalian chromosome aberration assay and in an *in vivo* micronucleus test, the experts considered it unlikely that the compound would have any *in vivo* genotoxic potential. Moreover, the use of the S9 mix was questioned, as DHA is expected to be quickly metabolized in the endogenous glycolytic pathway (Ref. 35), without having the opportunity to undergo extensive CYP450 metabolism. One of the experts made the comment that the DHA could have been used by the bacterial cultures as an alternative carbon source, which might then influence the test outcome.

The experts unanimously came to the conclusion that based on the presented raw data and a weight of evidence approach, there is no reason to consider DHA as an *in vivo* mutagenic/genotoxic substance.

Photo-mutagenicity
Two photo-bacterial reverse mutation assays are presented and confirm the mutagenic potential of non-radiated DHA in Salmonella typhimurium strains TA100 and TA102. However, exposure of DHA to light did not result in an enhanced mutagenic response.

4. CONCLUSIONS

1. *Does SCCS consider the use of Dihydroxyacetone (DHA) in cosmetic products safe for the consumers when used in a maximum concentration up to 10.0%, taking into account the data provided?*

   Based upon the available data, the SCCS is of the opinion that the use of Dihydroxyacetone as a self-tanning ingredient in cosmetic formulations up to 10% will not pose a risk to the health of the consumer.

2. *DHA may also be used in "spray cabins" in aqueous solutions in concentrations between 8 and 14%. Does the SCCS consider this use and exposure safe for the consumers?*

   When using DHA in spray cabins in aqueous solutions, exposure via inhalation cannot be excluded. The exposure may be single (frequency of use less than once per month) or 'repeated' (e.g. in extreme cases once per week).
   For the single exposure, reference is made to the presented acute inhalation study in rats, where the animals were exposed to DHA aerosols during 4 hours to the limit dose level of 5000 mg DHA/m³. No effects were observed on the clinical level or on macroscopic findings related to the respiratory tract or other organs.
   As far as repeated exposure to DHA-containing self-tanning formulations is concerned, the potential systemic exposure through inhalation appears to be negligible compared to the calculated worst-case dermal exposure levels. The calculated overall systemic exposure level generates a sufficiently high Margin of Safety.
   Therefore, based upon the available information, the SCCS considers that the use of Dihydroxyacetone as a self-tanning ingredient in spray cabins up to 14% will not pose a risk to the health of the consumer.

3. *Does the SCCS have any further scientific concerns regarding the use of DHA in a spray solution as a tanning agent without UV?*

   In light of the answer to question 2, the SCCS has no further concerns.

5. MINORITY OPINION

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