

Assessment of DHA in self-tanning creams applied in spray booths

Lena Höglund MSc.
DTC (Danish Toxicology Centre):

Betty Bügel Mogensen
Rossana Bossi
Marianne Glasius
National Environmental Research Institute of Denmark

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Summary and conclusions

Dihydroxyacetone (DHA) containing self-tanning products has existed for many years. Self-tanning spray booths were introduced on the market in 1999. These booths are more or less automatic. The health risks connected to exposure to DHA via inhalation and contact via eye and mucous membranes have not yet been subject to any investigations and are not documented.

According to an agreement with the Danish Environmental Protection Agency, the Danish Toxicology Centre (DTC, a unit of DHI – Institute for Water and Environment) has gone through a number of representative self-tanning preparations and techniques on the Danish market in order to create an initial overview of actual exposure and potential health risks caused by inhalation and exposure to eyes and mucous membranes to DHA.

The National Environmental Research Institute (NERI) was responsible for the monitoring programmes, and DTC conducted customer exposure assessments in connection with three booth techniques:

1. Manual turbine spray technique: The operator sprays the customer manually using a handheld spray pistol.
2. 3rd generation booth is totally closed and fully automatic.
3. 4th generation booth is open and fully automatic; the self-tanning lotion is electrically charged before being sprayed on the customer.

All exposure assessments are based on realistic worst-case calculations. As can be seen from the results, the operators in the salons are more exposed to DHA and the self-tanning preparations than the customers are. Furthermore, exposure of the eyes and mucous membranes to DHA and the self-tanning preparations constitute a greater quantitative exposure than inhalation. Due to the lack of data, such as NOAEL, LOAEL, etc. it is not possible at this level to conclude further on the extent of the exposure.

Due to the findings in this study and the limited data available on the toxic effects of DHA, the following risk reduction measures are recommended until further documentation is made available:

- DHA and the self-tanning preparations should not be inhaled or in contact with mucous membranes.
- Persons with asthma, sensitive skin or wounds should seek medical consultation before exposure to DHA and to self-tanning preparations.
- Keep the mouth closed and protect the lips with lip balm during treatment.
- Pregnant and breast-feeding women should avoid the use of DHA and self-tanning preparations in self-tanning booths
- Continuous weekly treatment over a long time cannot be recommended as long as there is insufficient knowledge of the effects of DHA.

- Self-tanning preparations do not protect sufficiently against the sun. The general recommendations should be followed and sun tan lotion should be used, when exposed to the sun.
- Booths should be equipped with exhaust fans in order to avoid unnecessary exposure of customers as well as operators.

1 Introduction

Self-tanning products have existed for many years as lotions to be rubbed onto the skin. In recent years a new development has taken place in that since 1999 (1) there have been booths on the market in which self-tanning products are more or less automatically sprayed onto the customer's body. The purpose of the booths is to achieve a more even covering of the self-tanning product than through manual application. The customer is usually in the booth for automatic application for between 6 and 60 seconds. Time spent in booths for manual application using a turbine spray is longer, typically from 2-3 minutes.

There are many different types of self-tanning product on the market. The products typically contain dihydroxyacetone as the tanning agent. Dihydroxyacetone (DHA) is the only self-tanning agent the FDA (US Food and Drug Administration) has authorised in self-tanning products (1). DHA reacts with amino acids in the outer layer of the skin and gives the skin a brownish hue. The effect appears after 2-6 hours. Isolated cases of allergies have been described, but in most cases, these are believed to be due to other contents than DHA. Formation of aerosols cannot be avoided during application in booths. Exposure to the substances in self-tanning liquids used in booths occurs through skin contact, inhalation, and through contact with the eyes and the mucus membranes. There is also a risk that aerosols could be spread outside the booth.

Cosmetic products must not pose a threat to the safety or health of the user when they are used. The Danish Consumer Council therefore contacted the Danish Environmental Protection Agency (Danish EPA) to have any risks from the products assessed. The Danish Toxicology Centre (DTC, a unit of DHI Water & Environment) agreed with the Danish EPA to review a number of representative self-tanning products and techniques on the Danish market in order to achieve an overview of exposure and any health risks from inhaling the products, or contact with the eyes or mucus membranes. DTC has also carried out a comprehensive review of the literature, searched the Internet, and contacted manufacturers and suppliers of self-tanning products and booths, in order to obtain data on DHA.

The National Environmental Research Institute of Denmark (NERI) has conducted measurements during use of three different types of spray booth: manual turbine application with a spray gun (air brush), a closed 3rd generation booth, and an open 4th generation booth. Furthermore, background measurements were taken in the room outside the booths in order to assess any exposures for salon staff.

The study has revealed that many different application techniques are used when spraying on self-tanner in booths and salons, and these techniques are regularly being improved. The study has focussed on the three latest techniques on the market. These techniques are today not the most commonly used, but they are expected to win increasing market shares over the coming years. The three techniques included in the study are estimated by the manufacturers to account for 20 per cent of the market, while the traditional manual air-brush technique accounts for the remaining 80 per cent. Today

there is not sufficient knowledge of the health effects of DHA and the project focused on the techniques expected to be used in the future, so that future health assessments in the area can reflect relevant and actual exposure scenarios as far as possible.

Two self-tanning salons have contributed with technical information, demonstrations and meetings, and they have made booths available for the NERI measurements.

2 Objectives

Objective of the project - *A health assessment of dihydroxyacetone (DHA) in self-tanning products used as sprays in booths.*

1. to obtain toxicological data on DHA in order to assess the health effects of inhalation and contact with the eyes and mucous membranes.
2. to find examples of products and types of booth available on the market.
3. to carry out an exposure assessment on the basis of measurements conducted by NERI.

The project does not include a study of products, but it should be viewed as a preliminary assessment of selected products and techniques on the market.

3 Techniques

This study covers three different techniques for applying self-tanning products.

- *Manual turbine spray*, a technique where an operator applies self-tanning products using a hand-held spray gun.

The other two techniques mentioned in the project are automatic in special booths, where the self-tanning product is applied from either moveable or fixed nozzles.

- *Third-generation booths* are completely enclosed.
- *Fourth-generation booths* are open and the liquid is electrically charged before being sprayed on the customer.

3.1 Description of techniques

3.1.1 Manual turbine spray

3.1.1.1 Description of the technique

Figure 3.1 Manual turbine spray



Using the manual turbine spray principle, the customer goes into an open booth and then the liquid containing DHA is sprayed onto the customer by an operator. The system used for NERI's measurements for this project uses the turbine principle; the same as that used in the most modern manual spray systems on the market. The advantages of the turbine principle are described by the supplier:

- It is based on HVLP technology, i.e. High Volume air but under Low Pressure.
- It is faster to apply - a total of 2-3 minutes compared with up to 30 minutes for traditional air-brush systems - and the liquid dries faster.
- Spray width is 13 cm compared with 2 cm for traditional air-brush systems.
- Waste (overspray) is minimal.
- Very low lotion consumption - about 25 ml. per treatment compared with 100 ml for traditional air-brush systems.
- The aerosol/DHA cloud is minimal so that the customer and the operator are not exposed to unnecessary inhalation of the self-tanning product/DHA.

3.1.1.2 Preparation

The customer removes clothes and jewellery as well as any make-up. Paper briefs/panties can be worn. A cap is pulled over the hair so that all the hair is covered, and then it is drawn back so that the hairline and ears are uncovered with a hairline of about 1 cm. The customer then steps onto self-adhesive sandals which protect the customer's feet against over exposure when the customer moves around in the booth. Next, a barrier cream is applied to all areas of dry skin.

3.1.1.3 The spray treatment

The operator turns on the machine and holds the pistol horizontally. The operator must move her whole arm and not just her wrist so that the nozzle is always straight (at right angles to the customer either vertically or horizontally) in order to achieve uniform covering. This also means that the operator must squat or go down on her knees when spraying a customer's legs, and the operator must stand up when spraying the upper body.

The operator must always remember to squeeze the trigger fully before the spray hits the customer's body, and to release the trigger only after the spray is away from the body. This is called "fanning" and it avoids smudging on the customer. For larger areas "full strokes" must be used where the pistol is moved backwards and forwards on an area so that the area is hit twice (once backwards, once forwards). The pistol should always be held at a distance of about 15 cm from the customer's body.

Spraying itself is in the following order:

1. Legs from the front
2. Upper body and neck
3. Arms
4. Legs from behind
5. Back and neck
6. Arms
7. Side of upper body
8. Face, when the customer is asked to close her eyes, purse her lips, and hold her breath while the operator counts to three.

Any wet areas on the customer are air dried by pointing the pistol towards the areas without squeezing the trigger. Treatment has now been completed and the customer can put on loose clothes or a kimono.

3.1.1.4 Manufacturer's safety instructions

The manufacturer's safety instructions recommend that the operator wears a dust mask while using a manual spray system or during automatic application (2). During treatment of face and neck, the customer is asked to close her eyes, purse her lips, and breathe through small filters inserted in both nostrils.

3.1.2 Third-generation booths (closed booths)

3.1.2.1 Description of the technique

Figure 3.2 Third-generation spray booth



Third-generation booths have two compartments. The customer hangs her kimono in the outer compartment and activates the spray program. The inner compartment has three rows of nozzles, located so that the customer is sprayed over her entire body without having to turn around. Treatment takes about six seconds using 60 ml of lotion.

3.1.2.2 Preparation

The customer removes clothes and jewellery as well as any make-up. A bathing cap is pulled over the hair and adjusted so that it just reveals the hairline. Protective cream can be applied to nails, hands, and feet to avoid discolouring.

3.1.2.3 Spray treatment

The customer hangs her kimono in the outer compartment and activates the program. The customer enters the inner compartment. After a 15-second countdown, treatment commences with the self-tanning product being sprayed out of nine nozzles. Treatment lasts about six seconds during which the customer stands with raised arms and palms facing each other, and lifts her right, then her left leg alternately. The customer then returns to the outer compartment and closes the door behind her. At the moment treatment ceases, there is a thick aerosol mist in the inner compartment. The quicker the door is closed, the quicker the aerosol mist is isolated. Extraction starts in the inner compartment as soon as the treatment is over. The customer spreads any excess product over her body, puts the kimono on again and exits the booth. After treatment the inner compartment is cleaned automatically so that the surplus spray mist is removed from the air, and the self-tanning product is cleaned from the walls and floor of the booth.

3.1.2.4 Manufacturer's safety instructions

The customer is asked to keep her eyes and mouth shut during treatment.

3.1.3 Fourth-generation booths (open booths)

3.1.3.1 Description of the technique

Figure 3.3 Fourth-generation spray booths



Fourth-generation spray booths are fully automatic. In contrast to third-generation booths, fourth-generation booths are open.

The self-tanning product is charged to 40,000 V and sprayed out through two vertical rows of nozzles. The spray nozzles are located in a tower on one side of the booth so that the customer has to turn around to be sprayed on the front and the back. Charging means that the aerosol is more accurate and less lotion is used for each treatment. According to the Danish agent, 99 per cent of the aerosol hits the customer and only 15 ml of lotion are used per treatment. The aerosol drops are ten-times smaller than in third-generation booths.

The customer stands on two earthed metal plates so that the lotion hits the customer very accurately using electrostatic energy.

3.1.3.2 Preparation

The customer removes clothes and jewellery as well as any make-up. A cap or paper briefs/panties may be put on. The cap is pulled down over the hair so that all the hair is covered, and then pulled back to leave the ears and a hairline of about 1 cm free.

3.1.3.3 Spray treatment

The customer stands in front of the spray tower with her feet placed on the two earthed metal plates and presses the start button. The nozzles in the tower spray lotion on the customer for 2, 2½ or 3 seconds. The customer turns her back towards the spray tower and is again sprayed for 2, 2½ or 3 seconds. The customer receives instructions throughout the process from loud speakers in the open booth. The treatment takes a total of 4½ to 6½ seconds, including the time it takes for the customer to turn around.

3.1.3.4 Manufacturer's safety instructions

In material for operators, the manufacturer recommends that customers use disposable briefs/panties, nose filters and eye protection.

3.2 Safety instructions

3.2.1 General remarks on enterprises' safety instructions

Instructions from operators to customers are more or less the same, irrespective of the salon. Most places recommend that customers:

- avoid inhaling the spray mist
- shut their eyes and mouth during spraying.

A manufacturer provides the following instructions for operators for manual application:

"Separate extractors are not necessary, but if you spray more than five customers per day, an external extractor should be used or an extractor booth with built-in filters. The operator should wear a dust mask if a manual spray system is used or if the operator is present during autospray" (2).

Another manufacturer recommends for expectant mothers: "Tanning spray is a non-toxic, food-approved product. Although [the manufacturer] has no information from any health authority that indicates harmful effects on pregnant women, we recommend that you consult your doctor before receiving treatment with self-tanning products in booths" (3).

The same manufacturer's recommendations for diabetics: "[The manufacturer] has no information from any health authority that indicates risks for diabetics. However, [the manufacturer] recommends that anyone with a medical condition should contact their doctor before receiving treatment with self-tanning products in booths" (3).

3.2.2 Safety instructions from the authorities

As a result of the increasing popularity of self-tanning booths, the US Food and Drug Administration (FDA) recommends that customers ask for special protection in order to avoid exposure of the eyes, lips and mucous membranes and in order to prevent inhalation/ingestion, for example by asking for nose filters.

3.2.3 Advice for customers from personnel

Most manufacturers of self-tanning booths have their own website on which they publish instructions and recommendations by replying to FAQs. Operators are trained in the same safety instructions and in how to reply to questions. On the first visit, the operator will usually ask the customer the following health-related questions (1):

Do you know how self-tanning works?

This enables the operator to explain how DHA works.

What is your normal skin colouring (light, medium, dark)?

The darker the skin colour the greater the effect of DHA.

What type of skin do you have (dry, normal, greasy)?

The drier the skin, the more effectively the DHA will penetrate the skin. This also means that areas of dry skin such as feet, knees, elbows, hands and

fingertips get dark smudges. It is suggested that dry areas are protected with a barrier cream before spraying commences.

Do you want to be brown for a special occasion?

If the customer is to go on vacation, the salon personnel should mention that the self-tanning product will not protect against the sun.

Do you use a solarium?

If the customer wants to combine solarium with self-tanning, the salon personnel/manufacturers will recommend that the customer go to the solarium before treatment with self-tanning products. This is because heating the body will open the pores and make the skin more receptive to DHA, and the customer should avoid sweating immediately after application of DHA.

Do you suffer from asthma?

Some asthmatics can also be allergic to DHA. Although customers are asked not to inhale DHA, the salon personnel/manufacturers recommend that the customer bring an inhaler in case of accidents. Some suppliers of self-tanning products also ask asthmatic customers to seek advice from their doctor before treatment.

Are you pregnant?

Expectant mothers are recommended not to use self-tanning products during the first 12 weeks of pregnancy.

Are you breast-feeding?

Nursing mothers are asked to cover their breasts during application to ensure that the child does not ingest DHA residues.

Do you have very sensitive skin?

If the answer is yes, and the customer has not previously had DHA treatment, it may be advisable to make a small test (usually behind the ear) to see whether the skin reacts to the DHA.

Do you have a skin condition?

If the customer has a skin condition such as psoriasis or eczema, DHA will penetrate the dry areas with this condition more intensively. The areas can be protected with barrier cream, but if the condition is widespread, the customer should seek advice before using self-tanner.

Do you have any cuts or grazes?

Open cuts should be protected with plasters and healing cuts with barrier cream.

Would you like to buy a nose filter?

The customer should be made aware that DHA should not be inhaled. The operator can therefore offer the customer a nose filter through which she can breathe during treatment.

Figure 3.4 Nose filters



In the US, the National Tanning Training Institute offers courses leading to certification. These are popular as some US states require personnel training before issuing licences to beauty salons (1).

4 Substances contained in products

The number of self-tanning products on the market is growing, and more manufacturers of self-tanning booths are making their own products. The products assessed in this project are water-based, with a water content of 75-85 per cent. A review of the information on packaging, websites, patents (4) etc. revealed the following information about 15 self-tanning products for spray application on the market. DHA, bronzers and moisturisers are the most common ingredients in this type of commercially available self-tanning product. The remaining ingredients mentioned in 4-11 below are common in many self-tanning products.

1. *Dihydroxyacetone (DHA)*. Ordinary self-tanning agents typically contain 3-14 per cent DHA. Self-tanners sold over the counter contain lower concentrations of DHA (typically 3-5 per cent) than self-tanning products used in professional spraying booths (typically 8-14 per cent). The highest concentrations are in the agents used in modern spraying booths where the consumption of lotion is reduced and the concentration of DHA is increased in order to achieve a better tanning effect (1).
2. *Bronzers*. Many self-tanning products contain an ordinary dyestuff (bronzer) which colours the skin immediately after application. This means that it is easy to see where the self-tanning product has been applied and therefore avoid missed patches without DHA. Many customers also seem to like seeing an immediate effect. It is important to stress that the bronzer does not influence the final effect of the self-tanning product. The bronzer washes off in the shower. The most common bronzers, which are also described in the patents, are caramel, Carmine (CI 75470) and various nut oils from, for example, chestnuts and walnuts.
3. *Moisturisers* are also used by most manufacturers. The most common are glycerine and various plant extracts, e.g. aloe.
4. *Erythrulose* is also contained in some self-tanning products as an active self-tanning substance, either on its own or in combination with DHA.
5. *Perfumes*, e.g. cinnamyl alcohol, citral, citronellol, dipentene, geraniol, hexyl cinnamaldehyde, hydroxycitronellal, linal, linalool.
6. *UV filters* (4,5).
 - a. Without nitrogen: e.g. dicaprylyl ether (CETIOL OE).
 - b. UV B filters: Ethylhexyl Methoxycinnamate, Methylbenzyliden Camphor.
 - c. UV A filters: Benzophenon-3, Butyl Methoxydibenzoylmethane (AVOBENZONE).
7. *Preservatives*. Parabens (methyl-, ethyl- and propylparaben are contained in many products) and phenoxyethanol (4).

8. *Thickening agents.* Cellulose ethers and xanthane gel are often used (4).
9. *Emulgators.* Silicones, sesquioleates, sorbitan esters, alkoxyated sorbitan and fatty-acid esters, alkoxyated mono-, di- and triglycerides, alkoxyated polymers, alkoxyated fatty alcohols, fatty acids, esters and ethers of natural oil derivatives are often used.
10. *Vitamins:* A, E and C.

Chapter 5 includes a toxicological profile of DHA as well as brief profiles of erythulose, ethoxydiglycol, phenoxyethanol, glycerine, polysorbates/sorbitan esters, parabens and PCA/Sodium PCA. These substances have been selected on the basis of their occurrence in many self-tanning products.

5 Health assessment

The health assessment focuses on dihydroxyacetone (DHA) as the active ingredient in self-tanning agents. The other ingredients in self-tanning products vary from manufacturer to manufacturer. However, some ingredients are prevalent in many products, for example ethoxydiglycol, phenoxyethanol, glycerine, polysorbates/sorbitan esters, parabens and PCA/Sodium PCA. The following is a brief health profile of these. Only limited data is available for several of the substances/substance groups.

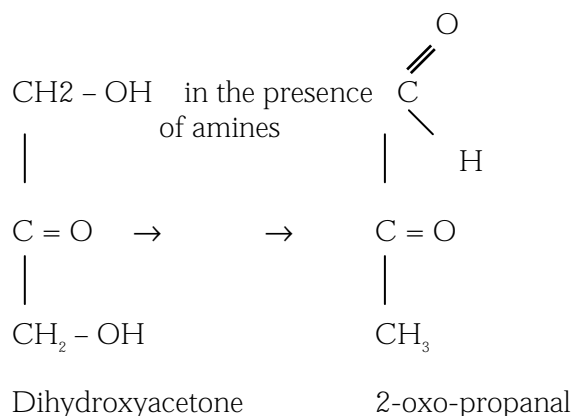
Meetings and other contact with representatives from some of the large manufacturers of booths and products demonstrated that none of them was able to document that DHA does not pose a health risk from exposure of the eyes and mucous membranes, or through inhalation. The US manufacturer Magic Tan has completed studies of the effects of self-tanning products from exposure via inhalation, the eyes and the mucous membranes, but they would not provide information on the results when requested (5).

5.1 TOXICOLOGICAL PROFILE OF DIHYDROXYACETONE (DHA) (CAS NO. 96-26-4)

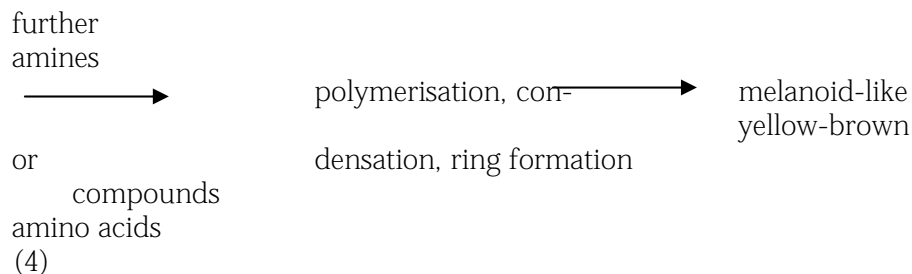
Occurrence and use

DHA is a molecule with three carbon atoms and it is the most commonly used active ingredient in self-tanning preparations. DHA was first used to treat diabetics, as some patients were more tolerant to DHA than to glucose. In 1957 the skin-colouring properties of DHA were discovered at a children's hospital. DHA was administered orally to treat childhood glycogen storage disease. A doctor noticed that when the children spat out some of the DHA preparation, they developed brown marks where the DHA had hit their skin. The first scientific article on DHA was published in 1960. Since then, the physical-chemical and tanning properties of DHA, as well as its reaction on the skin have been studied (1).

The skin-colouring properties of DHA come through a reaction with the amino acids and amino groups found on the outer layer of the skin (stratum corneum) during the formation of high-molecular pigments (melanoids). This occurs in a Maillard-like reaction, where pyruvate and other hydroxycarbonyl compounds are formed from dihydroxyacetone.



The subsequent reaction steps have not yet been fully explained as they seem extremely complicated. We know, however, that keto or aldo compounds react with an amine (the amino acids in the skin) during the formation of a ketoimine or an aldoimine. Furthermore, we know that the resulting compounds are cyclical and linear polymers with yellow-brown colouring and they are assumed to follow the course below:



DHA does not colour other surfaces than the stratum corneum, i.e. not the mucous membranes. On the other hand, there is stronger colouring of skin areas where the stratum corneum is thicker, for example on the palms, soles of feet, knees and ankles. (6).

Identification

Chemical name	2-propanon, 1,3-dihydroxy-acetone
Synonyms	Dihydroxyacetone (DHA)
CAS No.	96-26-4
EINECS No.	202-494-5
Molecule formula	C ₃ H ₆ O ₃
Molecule structure	$\text{HO C} - \underset{\begin{array}{c} \\ \text{O} \end{array}}{\text{C}} - \text{C OH}$
Legislation Classification in accordance with the list of hazardous substances (Statutory Order no. 439 of 3 June 2002) Statutory Order on Cosmetic Products (Statutory Order, no. 422 of 4 May 2006)	Not classified Unlimited

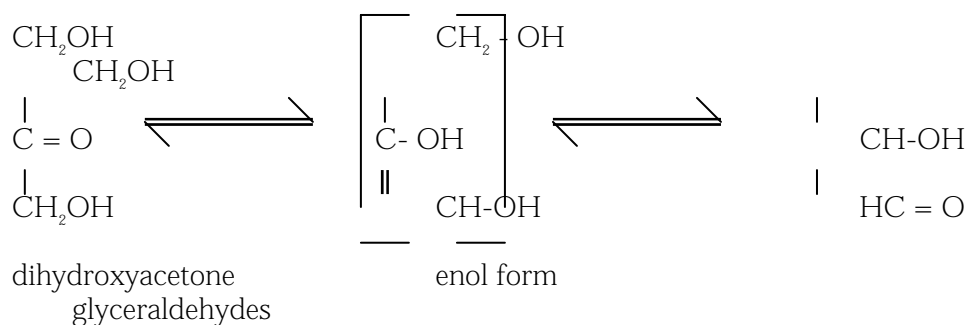
Physical-chemical properties (7,8)

Physical state	Crystalline powder
Molecular weight (g/mol)	90.08
Boiling point, °C	90 °C (68-71 °C) (9)
Vapour pressure	0.021 mmHg (25 °C)
Henry's law constant	1.21x10 ⁻³ atm m ³ /mol (25 °C)
Water solubility (mg/l)	5.03x10 ⁵ mg/l (25 °C)

It has been demonstrated that pure DHA acts as a mixture of monomers and dimers where the dimers are dominant. Heating or melting converts the DHA

to monomer form. The monomer is also formed after about 30 minutes in an aqueous solution. Only the monomer is active in colouring the skin (4).

In aqueous solution the DHA monomer can be gradually automerised to glyceraldehyde. As the equilibrium is shifted towards glyceraldehyde at higher pH values, the equilibrium depends on the pH of the solution.



In alkaline conditions, starting in glyceraldehyde, different isomerisation and condensation reactions take place which lead to formation of brown-coloured oligomers (4).

The stability of DHA depends on the concentration of DHA, pH, and the presence of by-products. The pH value of DHA solutions falls over time to about pH 3 because of the formation of organic acids. DHA is slightly acidic in itself. Glyceraldehyde is an isomer of DHA (4).

DHA phosphate occurs naturally in the human body and is part of the Krebs cycle (citric acid cycle) (4). The pH of the stratum corneum is 4.2-5.6, and for the epidermic is 7.3-7.4. (10)

Acute toxicity

Table 5.1. Summary of toxicological data.

Toxicological data (animals)	
LD ₅₀ , (mg/kg BW), intraperitoneal, rat (United States Patent Document. Vol. #4049795)	8750
LD ₅₀ , (mg/kg BW), intraperitoneal, rabbit (United States Patent Document. Vol. #4049795)	8000
LD ₅₀ , (mg/kg BW), oral, rat (8)	>16000
LD ₅₀ , (mg/kg BW), intraperitoneal, rat (8)	6400

A skin and mucous membrane test on rabbits exposed to DHA revealed no irritation (4,11).

A study by Goldman in the 1960s showed that ingestion of 18 g DHA, three times per day over 2-3 weeks did not produce any harmful effects on adult humans (12). No similar studies have been carried out more recently.

Ingestion of DHA has reduced amounts of body fat in rats (13). DHA is used as a food supplement together with pyruvate as a performance enhancer (14,15,16).

According to a more recent study by Kurz in 1994, DHA reacts with the skin's stratum corneum but it does not penetrate the skin (4). This conflicts with Goldman et al. who, in an older study from 1962, could demonstrate DHA in the blood shortly after applying DHA to the skin (17).

Long-term, repeated impacts

There is conflicting information about the health effects of DHA. Older studies by Akin et al. and Goldman et al. show that DHA has not demonstrated any toxic or carcinogenic effects. In the Akin study, 0.1 ml of aqueous 5 per cent and 40 per cent solutions of 97 per cent DHA were evenly applied to shaven Swiss-Webster mice once a week. The mice were divided into three groups, each with 50 males and 50 females. Treatment continued over 80 weeks. No significant carcinogenic effect could be demonstrated in any of the groups. Goldman refers to experience with the low toxicity of DHA without giving further information about channels of exposure and concentrations of DHA (18,19). According to Draelos et al., DHA has low toxicity, both for ingestion and for application to the skin, with only few reported cases of allergic contact dermatitis. The low toxic effect is not further documented in the article (20). Other *in vitro* studies show that DHA is suspected of causing damage to the DNA (21,22,23). DHA induced a significantly increased number of type 3 foci in 3T3 fibroblasts, indicating *in vitro* neoplastic cell transformation (24). DHA has been found in the blood shortly after application to the skin (17). According to Petersen et al. (23) it is therefore possible that DHA may penetrate down through the stratum corneum and affect the DNA and proteins in other cells in the body.

Because of an *in vitro* study, in which DHA demonstrated genotoxic and mutagenic properties, the substance is suspected of having these properties. These properties have not been confirmed in other test systems. Therefore there is some doubt about the use of DHA in skin treatment over longer periods (23).

The information available about the genotoxicity of DHA can seem contradictory as DHA has demonstrated genotoxic properties *in vitro*, but it is also an intermediary in the carbohydrate metabolism of higher plants and animals, and therefore a natural phenomenon in the body (7).

Local irritation

Frequent use of self-tanning products containing DHA may make skin more prone to irritation (20).

Laboratory tests of a self-tanning product showed no skin irritation, but moderate eye irritation. However, there is no evidence that the irritation is due to the content of DHA (25).

Allergies

Patients have been tested for allergic reactions to DHA using patch tests, but with negative results. The author has a theory that previously described cases of allergies may be due to exposure to other substances at the same time as DHA (26,27).

Morren et al. report on two cases of allergic reactions to DHA as a result of using self-tanning products on the skin (28). The US FDA mentions that it is possible that other ingredients in self-tanning products such as oils or Juglans regia (walnut) extract may cause discomfort for those with nut allergies (29).

5.1.1.1 Critical effect

The critical effect of DMA is considered to be irritation of the eyes. Allergic reactions rarely occur. Because of an *in vitro* study, DHA is suspected of being a genotoxic substance with mutagenic properties.

There is no data on inhalation of DHA. The US Magic Tan (5) have completed studies of the effects of exposure to self-tanning agents through inhalation, and on the eyes and mucous membranes, but they do not want to reveal the results.

There is no data on NOAEL, LOAEL, NOEL or LOEL in the literature.

The attitude of the US FDA to DHA

DHA is an ingredient in food and it has been approved for ingestion by the US Food and Drug Administration (FDA). In fact the health industry is the world's largest user of DHA. As DHA is a pyruvite or "fat-burner", it is used in many slimming products.

DHA has also been approved by the FDA for use as a cream and lotion. The FDA assesses that DHA has no known harmful effects on the body when it is applied to the skin, and DHA is the only self-tanner approved for use in self-tanning products. Approval of DHA as a self-tanner for use in cosmetics is limited to external use. The FDA defines external use as "applied only to external parts of the body and not to the lips or any body surface covered by mucous membrane". Furthermore, no colouring additive may be used in cosmetics intended for use around the eyes unless it has special approval (30).

According to the latest document from the FDA on sunscreens, self-tanning products must be labelled with a warning that they do not protect against UV radiation or sunburn (1).

Recommendations from the FDA

- DHA should not be inhaled as in rare cases it can cause an allergic reaction in allergy sufferers, and the long-term effects of the carbohydrate from repeated inhalation have never been examined.
- The eyes should be kept shut for the reasons mentioned above.
- It may be advisable to protect the lips with a lip-salve and the nose with nose filters prior to treatment.
- The mouth should be kept shut during treatment.

5.2 BRIEF HEALTH ASSESSMENT OF ETHOXYDIGLYCOL (CAS NO. 111-90-0)

Ethoxydiglycol is on the INCI list and there are no restrictions on use in cosmetic products under the Statutory Order on Cosmetic Products (Statutory Order no. 422 of 4 May 2006).

Ethoxydiglycol generally has low toxicity. This applies for injection, inhalation, and acute and sub-chronic skin toxicity tests (31).

Ethoxydiglycol provokes minimal to mild skin irritation in rabbits and mild to serious ocular (eye) irritation (31,32).

Ethoxydiglycol does not provoke an allergic reaction on contact with the skin (31).

Use of ethoxydiglycol is deemed to be low risk when used as an ingredient in cosmetics under current practices for use and concentration. (31).

5.3 BRIEF HEALTH ASSESSMENT OF PHENOXYETHANOL (CAS NO.122-99-6)

Phenoxyethanol is on the INCI list and it is an aromatic ether permitted for use as a preservative in cosmetic products at concentrations of up to 1 per cent. According to the Cosmetic Ingredient Review Expert Panel, phenoxyethanol has low toxicity in rats from ingestion and application to the skin. There is no information on the concentrations tested. On the other hand a subchronic test with phenoxyethanol showed reduced body weight in rats in a 90-day study (33).

It was not possible to demonstrate harmful effects on rats exposed to single exposures of saturated vapours of phenoxyethanol (saturated at 100 degrees C and then cooled to room temperature) for a period of seven hours (Hazardous Substance Databank, HSDB, Phenoxyethanol).

The concentrated phenoxyethanol was extremely irritant on the eyes. This irritation disappeared at concentrations of < 2.2 per cent. Exposure to phenoxyethanol in a concentration of 2 per cent was slightly irritant on the skin in a test on rabbits. The same test on guinea pigs provoked neither irritation nor allergic reactions. There was no sign of teratogenicity, embryotoxicity or foetotoxicity at doses which were toxic for the mother animal in tests on mice where phenoxyethanol was applied to the skin. Phenoxyethanol was not mutagenic in Ames' test, with or without metabolic activation or in micronucleus tests (mice). In clinical studies phenoxyethanol provoked no primary irritation or allergic reaction. Phenoxyethanol did not demonstrate phototoxicity in clinical studies (33).

5.4 BRIEF HEALTH ASSESSMENT OF GLYCERINE (CAS NO. 56-81-5)

Glycerine is on the INCI list and there are no restrictions on use in cosmetic products under the Statutory Order on Cosmetic Products.

When administered on the skin and in the rectum, glycerine can cause irritation. Application of glycerine to the eyes can damage the cornea (34,35).

5.5 BRIEF HEALTH ASSESSMENT OF POLYSORBATES AND SORBITAN ESTERS

Polysorbates and sorbitan esters are on the INCI and there are no restrictions on use in cosmetic products under the Statutory Order on Cosmetic Products. They are used as emulsification agents/surface-active substances in self-tanners.

There is an LD₅₀, oral at >15g/kg (36). The FAO/WHO Expert Committee on Food Additives states the acceptable daily intake of polysorbate esters at 25 mg/kg body weight (34,36).

Polysorbates can increase the absorption of fat-soluble substances. There are instances of allergic reactions after skin contact (34).

Undiluted polysorbates provoked no or slight skin irritation in tests on rabbits. Dilution of 15 per cent gave no skin irritation. Draize' test of undiluted polysorbates in the eyes of rabbits provoked minimal eye irritation. Dilution to 75 or 30 per cent in water or 10 per cent in mineral oil provoked no irritation (36).

Tests on the mucous membranes in the mouth on hamsters with Polysorbate 20 (unspecified volume and concentration) and 10 per cent Polysorbate 40 showed no inflammatory reaction (36).

A lotion containing 4 per cent Polysorbate 40 and a cream formulation containing 1 per cent Polysorbate 85 gave no irritation after application to penile and vaginal mucous membranes on rabbits. Three beagle dogs exposed in the vaginal area to a foam-bath product containing 6 per cent Polysorbate 20 once a day, five days a week for three weeks suffered serious irritation. Exposure to the same foam-bath product diluted to 5 per cent in water provoked no visible effects (36).

Polysorbate 60 and 80 are not deemed to have any harmful effects from ingestion of amounts of up to 25 mg/kg BW (ADI total polysorbate tests) (36).

Polysorbate 20 provoked no harmful effects on the eyes at concentrations of up to 40 per cent. Undiluted Polysorbate 20 applied to the eyes of rabbits gave slight irritation. There is no documentation regarding skin irritation (36).

Polysorbates are not regulated by the Statutory Order on Cosmetic Products, but use in cosmetic products has been assessed by a panel of experts. On the basis of available toxicological data, the panel of experts assessed that Polysorbates (20, 21, 40, 60, 65, 80 and 85) do not pose a risk for the consumer when they are used in cosmetics under the current practice for use and concentration (36).

5.6 BRIEF HEALTH ASSESSMENT OF ERYTHRULOSE (CAS NO. 40031-31-0)

Erythrulose is not on the INCI list. Erythrulose is a sugar molecule formed through aerobic fermentation of *Gluconobacter*. Erythrulose reacts with free primary and secondary amino groups (Maillard reaction) in the outer layer of the skin (stratum corneum). Acute toxicity tests have revealed LD₅₀ >2000 mg/kg (rats, oral) for 16 per cent erythrulose (43).

Erythrulose is neither irritating nor sensitising on contact with the skin. Erythrulose was not mutagenic in Ames' test (43).

5.7 BRIEF HEALTH ASSESSMENT OF PARABENS (METHYLPARABEN CAS NO. 99-76-3, ETHYLPARABEN, CAS NO. 120-47-8 AND PROPYLPARABEN CAS NO. 94-13-3)

Methyl, ethyl and propylparabens are present in several self-tanning products.

Parabens are used as a preservative and are regulated by the Statutory Order on Cosmetic Products (Statutory Order no. 422 of 4 May 2006). This Statutory Order sets limits on the content of parabens in cosmetics at 0.4 per cent (as acid) for an individual ester, and 0.8 per cent for ester mixtures.

Parabens are effective preservatives in the pH interval 4-8, but they are best at lower pHs. Effectiveness increases and water solubility decreases for longer alkyl chains. The effectiveness of parabens can be influenced by other additives or other ingredients, such as polysorbates (34,35)

Methyl, ethyl and propyl parabens can be absorbed through the skin. Parabens are hydrolysed and conjugated and released through the urine. Data from life-time studies indicate that parabens do not accumulate in the body. Studies with different administration routes on animals indicate that parabens have low acute toxicity and that toxicity seems to fall with increasing lengths of alkyl chain (37)

Parabens can provoke allergic reactions on contact with the skin. The sensitising effect is more limited on normal skin, however. Hypersensitivity to methylparaben will often imply hypersensitivity to ethylparaben and propylparaben. Ingesting food and drugs containing parabens does not seem to lead to rashes on people allergic to parabens, if the mucous membrane is intact (37).

A large number of mutagenic tests, including Ames' test, have been carried out, and none of these indicate the parabens have a mutagenic effect (37).

A teratogenic effect has been demonstrated in rats from administration of a 10 per cent solution of ethylparaben (corresponding to 9,000 mg/kg BW/day). There were no clear teratogenic effects at lower concentrations. Methyl, ethyl and propylparabens have demonstrated potent spermatocidal effects. In rats there were clear oestrogen-like effects at oral doses of 600 mg/kg BW/day (37).

No carcinogenic effects have been demonstrated in studies of propylparaben and methylparaben (37).

Trials lasting 96 weeks with 2 per cent and 8 per cent methyl and propylester showed no significant pathological results. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) has estimated that 2 per cent parabens in food corresponds to 1,000 mg/kg BW (37).

The estimated acceptable daily intake is < 10 mg/kg BW. (34).

5.8 BRIEF HEALTH ASSESSMENT OF PCA (CAS NO. 98-79-3 and 149-87-1) AND SODIUM PCA (CAS NO. 28874-51-3 AND 54571-67-4)

Use of PCA is not restricted in cosmetic products and it is used as a moisturiser.

PCA is polyglutamic acid. Sodium PCA is the sodium salt of polyglutamic acid. It is used in both skin and hair lotions. Recommended concentrations in these substances are 0.2-4 per cent. Sodium PCA has been tested as non-irritant for the eyes and skin at concentrations of up to 50 per cent. There are no indications that Sodium PCA and PCA are photo-toxic, allergenic, or genotoxic (38).

On the basis of available data, it can be concluded that Sodium PCA and PCA are safe ingredients in cosmetic formulas as they are used today (38).

6 User exposure

Exposure to DHA in the self-tanning products used in spray booths has been assessed on the basis of information in the literature, contacting manufacturers and suppliers, and from the results of measurements carried out by NERI.

6.1 Assessment of exposure

The assessment only covers exposure for the mucous membranes, eyes, and through inhalation. The assessment assumes that DHA is taken up 100 per cent via inhalation, the eyes, or mucous membranes.

The exposure scenarios are all realistic worst-case scenarios. NERI has carried out air measurements in the booths during use by customers, representing the impact on the customer. Moreover, measurements have been conducted in the room outside the booths while the customer is using the booths. This represents background concentrations in the room and the impact on salon staff/operators. The background concentrations are also part of the exposure for customers in the time they are in the salon, i.e. before and after treatment in the booths. Measurement data and figures were supplied by NERI and will be described in detail in the NERI report.

6.1.1 Exposure via inhalation

A general inhalation rate of 1.5 m³/hour has been assumed (adult man, low activity) for both customer and operator (39).

Measurements were taken in three types of spray booth in which DHA-containing self-tanning products were used. The three types of booth and the different techniques are described in more detail in 3.1. The following is a description of the exposures in the booths from using the different techniques, as well as background exposures outside the booths. Exposure assessments are all worst-case scenarios, so they do not take into account use of nose filters, for example (as shown in section 3.2.3).

Treatment lasts for 5-7 days. Two different worst-case scenarios have been described. In the first scenario the customer is treated once a week during the winter months, i.e. 1 October to 31 March inclusive (6 months = 26 weeks, i.e. a total of 26 times a year, corresponding to an average daily exposure of $(26/365=)$ 0.07 times a day. The second worst-case scenario describes special cases for, e.g. TV presenters, actors and models, where exposure could be weekly throughout the year (12 months = 52 weeks, i.e. 52 times a year, corresponding to an average daily exposure of $(52/365=)$ 0.14 times per day.

6.1.1.1 Exposure from manual turbine spray

NERI has carried out measurements during treatment of a customer with a manual turbine spray. The customer was sprayed for about 167 seconds and the air was collected from the customer's inhalation zone for 210 seconds. A period of 180 seconds was chosen to measure customer exposure, as this is

the normal treatment time. 25 ml lotion was used. The booth was open and equipped with an extractor fan in the ceiling. For further technical details of the measurement procedure, please refer to the NERI report.

The results from the completed measurements are summarised in the table below.

Table 6.1. Concentration of DHA in droplets < 12 µm in the air around the mouth/nose during treatment with self-tanner using manual turbine spray

	Spraying time sec.	Collection time. sec.	DHA in sample mg/m ³ air	Particle separation µm
Measurement	167	210	0.8	12

Three scenarios were established:

1. Weekly exposure in the winter months
2. Weekly exposure all year
3. Exposure per treatment (acute exposure)

1. Weekly exposure in the winter months

The following calculation models were set up as worst-case scenarios at weekly exposure in the winter months with intake via inhalation:

Weight of subject: 60 kg
 Inhalation rate: 1.5 m³/hour
 Number of treatments: 0.07/day
 Exposure time per treatment (180/60/60) 0.05 hours
 Daily exposure time (0.07 x 0.05): 0.0035 hours

Average daily exposure via airways is calculated as:

Daily exposure x inhalation rate x concentration of DHA in air =
 kg body-weight

$$\frac{0.0035 \text{ hours/day} \times 1.5 \text{ m}^3/\text{hour} \times 0.8 \text{ mg/m}^3 \text{ air}}{60 \text{ kg}} = 7 \times 10^{-5} \text{ mg/kg/day}$$

2. Weekly exposure all year

The worst-case scenario below has been set up for particularly exposed groups such as TV presenters, actors and models who are exposed weekly throughout the year.

Weight of subject: 60 kg
 Inhalation rate: 1.5 m³/hour
 Number of treatments: 0.14/day
 Exposure time per treatment (180/60/60) 0.05 hours
 Daily exposure (0.14 x 0.05): 0.007 hours

Average daily exposure via airways is calculated as:

Daily exposure x inhalation rate x concentration of DHA in air =
 kg body-weight

$$\frac{0.007 \text{ hours/day} \times 1.5 \text{ m}^3/\text{hour} \times 0.8 \text{ mg/m}^3 \text{ air}}{60 \text{ kg}} = 14 \times 10^{-5} \text{ mg/kg/day}$$

3. Exposure per treatment

A person with an inhalation rate of 1.5 m³/hour, treated for 0.05 hours at a concentration of inhalable DHA in the air of 0.8 mg DHA/m³ air is exposed to (0.05 hours x 1.5 m³/hour x 0.8 mg/m³ air =) **0.06 mg** DHA via inhalation per treatment.

6.1.1.2 Exposure using third-generation booth (closed booth)

During treatment in a third-generation booth, the customer is exposed to concentrated aerosol for 6 seconds and 60 ml lotion is used per treatment. The customer leaves the booth after spraying has stopped. Immediately after this, extraction fans start and the booth is rinsed so that excess aerosol is removed from the booth.

Table 6.2. Concentration of DHA in droplets < 12 µm in the air around the mouth/nose during treatment with self-tanning spray in third-generation booth

	Spraying time sec.	Collection time sec.	DHA in sample mg/m ³ air	Particle separation µm
Measurement Customer 1	6	14	238	12
Measurement Customer 2	6	16	115	12

Three scenarios were established:

1. Weekly exposure in the winter months
2. Weekly exposure all year
3. Exposure per treatment

1. Weekly exposure in the winter months

The following methods of calculation are set up as worst-case scenarios for uptake via inhalation and weekly exposure in the winter months.

Weight of subject: 60 kg
Inhalation rate: 1.5 m³/hour
Number of treatments: 0.07/day
Exposure time per treatment (6/60/60) 0.0017 hours
Daily exposure (0.07 x 0.002): 0.0001 hours

Average daily exposure via airways is calculated as:

$$\frac{\text{Daily exposure} \times \text{inhalation rate} \times \text{concentration of DHA in air}}{\text{kg body-weight}}$$

Measurement 1.

$$\frac{0.0001 \text{ hours/day} \times 1.5 \text{ m}^3/\text{hour} \times 238 \text{ mg/m}^3 \text{ air}}{60 \text{ kg}} = 59.5 \times 10^{-5} \text{ mg/kg/day}$$

Measurement 2.

$$\frac{0.0001 \text{ hours/day} \times 1.5 \text{ m}^3/\text{hour} \times 115 \text{ mg/m}^3 \text{ air}}{60 \text{ kg}} = 28.8 \times 10^{-5} \text{ mg/kg/day}$$

2. Weekly exposure all year

The worst-case scenario below has been set up for particularly exposed groups such as TV presenters, actors and models who are exposed weekly throughout the year.

Weight of subject: 60 kg
 Inhalation rate: 1.5 m³/hour
 Number of treatments: 0.14/day
 Exposure time per treatment (6/60/60) 0.0017 hours
 Daily exposure (0.14 x 0.002): 0.0003 hours

Daily exposure x inhalation rate x concentration of DHA in air =
 kg body-weight

Measurement 1.

$$\frac{0.0003 \text{ hours/day} \times 1.5 \text{ m}^3/\text{hour} \times 238 \text{ mg/m}^3 \text{ air}}{60 \text{ kg}} = 0.0018 \text{ mg/kg/day}$$

Measurement 2.

$$\frac{0.0003 \text{ hours/day} \times 1.5 \text{ m}^3/\text{hour} \times 115 \text{ mg/m}^3 \text{ air}}{60 \text{ kg}} = 0.0009 \text{ mg/kg/day}$$

3. Exposure per treatment.

A person treated for 0.0017 hours at concentrations of DHA in air of 238 and 115 mg DHA/m³ air respectively is exposed as a worst-case to (0.0017 hours x 1.5 m³/hour x 238 or 115 mg/m³ air =) **0.61** or **0.29 mg** DHA respectively via inhalation per treatment.

6.1.1.3 Exposure using fourth-generation booth (open booth)

Fourth-generation booths are open. The customer is sprayed twice for 2, 2½, or 3 seconds each time. For NERI's measurements the booth was set to spray for 2 x 3 seconds. A total of 15 ml lotion is used per treatment, and the spray mist is almost invisible immediately after treatment.

Table 6.3. Concentration of DHA in droplets < 12 µm in the air around the mouth/nose during treatment with a self-tanning spray in fourth-generation booth

	Spraying time sec.	Collection time sec.	DHA in sample mg/m ³ air	Particle separation µm
Measurement Customer 1	6	35	3.3	12
Measurement Customer 2	6	35	17	12

Three scenarios were established:

1. Weekly exposure in the winter months
2. Weekly exposure all year
3. Exposure per treatment

1. Weekly exposure in the winter months

The following methods of calculation are set up as worst-case scenarios for uptake via inhalation and weekly exposure in the winter months:

Weight of subject: 60 kg

Inhalation rate:	1.5 m ³ /hour
Number of treatments:	0.07/day
Exposure time per treatment (6/60/60)	0.002 hours
Daily exposure (0.07 x 0.002):	0.0001 hours

Daily exposure x inhalation rate x concentration of DHA in air =
kg body-weight

Measurement 1.

$$\frac{0.0001 \text{ hours/day} \times 1.5 \text{ m}^3/\text{hour} \times 3.3 \text{ mg/m}^3 \text{ air}}{60 \text{ kg}} = 0.8 \times 10^{-5} \text{ mg/kg/day}$$

Measurement 2.

$$\frac{0.0001 \text{ hours/day} \times 1.5 \text{ m}^3/\text{time} \times 17 \text{ mg/m}^3 \text{ air}}{60 \text{ kg}} = 4.3 \times 10^{-5} \text{ mg/kg/day}$$

2. Weekly exposure all year

The worst-case scenario below has been set up for particularly exposed groups such as TV presenters, actors and models who are exposed weekly throughout the year.

Weight of subject:	60 kg
Inhalation rate:	1.5 m ³ /hour
Number of treatments:	0.14/day
Exposure time per treatment (6/60/60)	0.002 hours
Daily exposure (0.14 x 0.002):	0.0003 hours

Daily exposure x inhalation rate x concentration of DHA in air =
kg body-weight

Measurement 1.

$$\frac{0.0003 \text{ hours/day} \times 1.5 \text{ m}^3/\text{hour} \times 3.3 \text{ mg/m}^3 \text{ air}}{60 \text{ kg}} = 2.5 \times 10^{-5} \text{ mg/kg/day}$$

Measurement 2.

$$\frac{0.0003 \text{ hours/day} \times 1.5 \text{ m}^3/\text{hour} \times 17 \text{ mg/m}^3 \text{ air}}{60 \text{ kg}} = 12.8 \times 10^{-5} \text{ mg/kg/day}$$

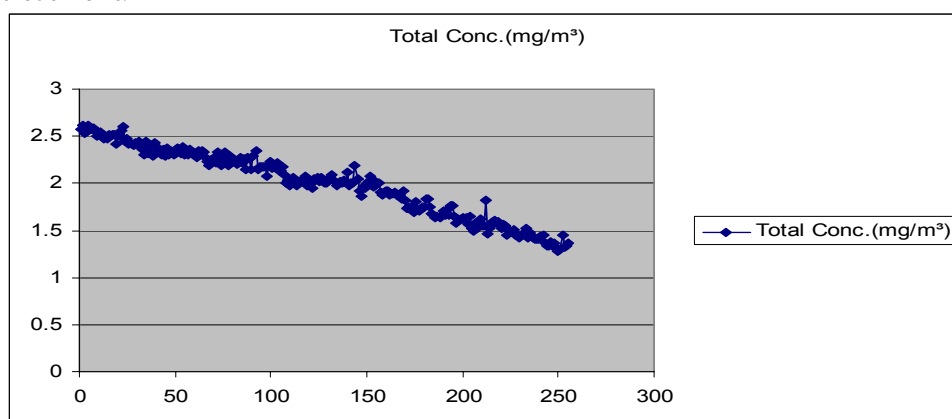
3. Exposure per treatment

A person treated for 0.002 hours at concentrations of DHA in air of 3.3×10^{-5} and 12.8×10^{-5} mg DHA/m³ air respectively is exposed as a worst-case to $(0.002 \text{ hours} \times 1.5 \text{ m}^3/\text{hour} \times 3.3 \times 10^{-5} \text{ or } 12.8 \times 10^{-5} \text{ mg/m}^3 \text{ air}) = 1 \times 10^{-7}$ or **3.8×10^{-7} mg** DHA respectively via inhalation per treatment.

6.1.1.4 Background exposure

Besides measuring the aerosol mist in the booth, measurements were also carried out of the background concentrations outside the booth, typically where the operator and waiting customers are.

Figure 6.1 shows how the concentration in air of self-tanning product falls with time in the room outside the booth. The x axis shows secs. after treatment.



Extrapolating the curve to zero shows that it takes 9 minutes for the concentration of self-tanning product in the air to fall to zero. The results in table 6.4 have been used to calculate exposure to DHA.

Table 6.4. Concentration of DHA in droplets < 12 µm in the air around the mouth/nose beside a spray booth after three treatments with self-tanning spray

	Collection time minutes	DHA in sample mg/m ³ air	Particle separation µm
Manual and open booth	30.5	0.29	12
Closed booth	30	0.5	12

The worst-case scenario used is an operator in the room outside the booths where three customers are treated per day for 227 working days per year. The values measured are a worst-case scenario as the three treatments are completed within a short period of time. These are usually spread over the working day. NERI's background measurements during treatment of customers were carried out for open booths and manual turbine spraying together, i.e. it is not possible to separate the air impacts from the two types of treatment.

The following method of calculation has been set up as a background scenario for an operator exposed via inhalation.

Weight of subject:	60 kg
Inhalation rate:	1.5 m ³ /hour
Number of exposures (3 x 227/365 =)	1.86/day
Exposure each time (9/60 =)	0.15 hours
Daily exposure time (1.86 x 0.15 =)	0.28 hours

Daily exposure x inhalation rate x concentration of DHA in air =
kg body-weight

Open booth:

$$\frac{0.28 \text{ hours/day} \times 1.5 \text{ m}^3/\text{hour} \times 0.29 \text{ mg/m}^3 \text{ air}}{60 \text{ kg}} = 0.0020 \text{ mg/kg/day}$$

Closed booth

$$\frac{0.28 \text{ hours/day} \times 1.5 \text{ m}^3/\text{hour} \times 0.5 \text{ mg/m}^3 \text{ air}}{60 \text{ kg}} = 0.0035 \text{ mg/kg/day}$$

The worst-case scenario for the customer is that the customer waits/gets ready for five minutes while another customer is being treated. When the customer has finished treatment, she gets dressed for 10 minutes while a new customer is being treated. This means the customer is exposed for 15 minutes.

Weekly exposure, winter months:

Weight of subject:	60 kg
Inhalation rate:	1.5 m ³ /hour
Number of treatments:	0.07/day
Exposure time per treatment (15/60)	0.25 hours
Daily exposure time (0.07 x 0.25 =):	0.018 hours

$$\frac{\text{Daily exposure} \times \text{inhalation rate} \times \text{concentration of DHA in air}}{\text{kg body-weight}}$$

Open booth:

$$\frac{0.018 \text{ hours/day} \times 1.5 \text{ m}^3/\text{hour} \times 0.29 \text{ mg/m}^3 \text{ air}}{60 \text{ kg}} = 0.00013 \text{ mg/kg/day}$$

Closed booth

$$\frac{0.018 \text{ hours/day} \times 1.5 \text{ m}^3/\text{hour} \times 0.5 \text{ mg/m}^3 \text{ air}}{60 \text{ kg}} = 0.00023 \text{ mg/kg/day}$$

Weekly exposure, all year:

Weight of subject:	60 kg
Inhalation rate:	1.5 m ³ /hour
Number of treatments:	0.14/day
Exposure time per treatment (15/60=)	0.25 hours
Daily exposure time (0.14 x 0.25 =)	0.035 hours

$$\frac{\text{Daily exposure} \times \text{inhalation rate} \times \text{concentration of DHA in air}}{\text{kg body-weight}}$$

Open booth:

$$\frac{0.035 \text{ hours/day} \times 1.5 \text{ m}^3/\text{hour} \times 0.29 \text{ mg/m}^3 \text{ air}}{60 \text{ kg}} = 0.00025 \text{ mg/kg/day}$$

Closed booth:

$$\frac{0.035 \text{ hours/day} \times 1.5 \text{ m}^3/\text{hour} \times 0.5 \text{ mg/m}^3 \text{ air}}{60 \text{ kg}} = 0.00043 \text{ mg/kg/day}$$

In addition to this is exposure during the treatment itself. The total exposure has been calculated in section 6.1.3.

6.1.1.5 Size of particles and deposition

When using spray booths a spray mist is formed composed of small droplets of lotion (aerosols). The physical and chemical properties of aerosols determine where they are deposited in the airways and the possibilities for the lungs to clear themselves (clearance).

Large aerosols/particles are relatively independent of the surrounding movement in the air, whereas small aerosols follow the movements in the air. Large aerosols (30 μm - 60 μm) are filtered out via nasal hair. Aerosols/particles which get through the nose/mouth are called inspirable or the inhalable fraction (< 25 μm). Many of these will be deposited in the upper airways and on the larynx. The upper airways go from the larynx up to the surrounding/outer air. This includes the mouth (40).

The fraction of aerosols which is not deposited in the upper airway or the larynx passes the larynx and makes up the lung fraction (thoracic fraction (< 10 μm)), and it comprises the aerosols deposited in the trachea, the large bronchus and the small bronchus. This is called the tracheobronchial part (41).

The aerosols passing here and reaching right down to the respiratory bronchials and alveolar ducts are called the respirable part (<1-5 μm). Not all respirable particles are deposited in the alveolar ducts, some are exhaled again. This fraction will primarily be composed of aerosols < 1 μm (41).

The distribution of the droplet size was only registered at one measurement. The techniques in the various booths are different and therefore the droplet size also differs. For the preliminary calculations it was assumed that 100 per cent of the measured inhalable aerosols (particle size < 12 μm) were taken up.

Figure 6.2 Result of NERI measurements during treatment in a third-generation booth

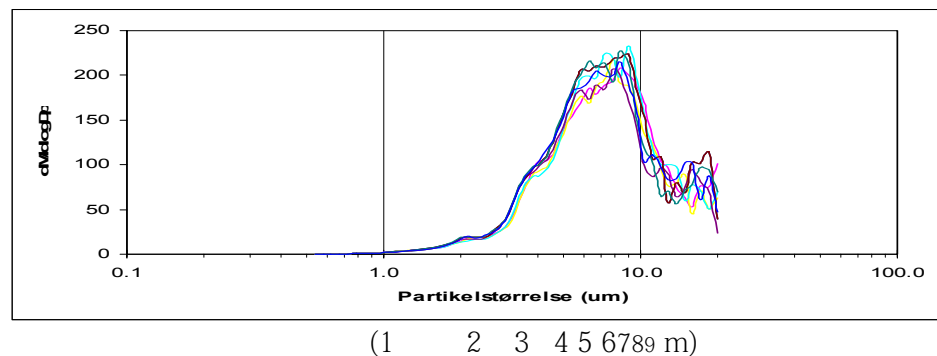


Figure text
Particle size

The vertical axis is the amount and the horizontal the particle size. Note that the axis for particle size is logarithmic. Particles were collected <12 μm . The figure above shows that most particles are in the range 5-10 μm , i.e. a thoracic fraction. The respirable (<1-5 μm) and inhalable fractions (<25 μm) make up the next largest fractions.

The technical description of fourth-generation booths states that the aerosols are 10-times smaller than in the third-generation booths, and this means that the size of the droplets will primarily be < 1 μm . Much of this fraction will be exhaled again (41).

6.1.2 Exposure via the eyes and mucus membranes

Customers are instructed to keep their eyes shut during all treatments. This means that the eyes are only exposed when the customer accidentally blinks.

In a worst-case scenario the exposed part of the eye is deemed to be the same size as the eye lid. The eye's surface was estimated to comprise one-third of the area on which eye-shadow is applied, i.e. $1/3 \times 24 = 8 \text{ cm}^2$ (42).

Several salons recommend using disposable panties. In a worst-case situation the customer will not use these, and this means exposure to the outer parts of the genitals. The worst case will be a female customer. No measurements were found in the literature. An estimate of the exposed part of the female genitals is 25 cm^2 .

The total area of the exposed mucous membranes and eyes is estimated at $(2 \times 8 \text{ cm}^2 + 25 \text{ cm}^2 =) 41 \text{ cm}^2$.

Mucous-membrane and eye exposure is estimated to be the same for treatment using all three of the techniques described. Compared with the background exposure via the air, it is estimated that the background exposure via the mucous membranes is very limited as the operator or customer are both usually dressed outside the booth. Two worst-case scenarios for customers. 1) a customer who repeats treatment each week during the winter months. 2) special cases such as TV presenters, actors and models, where exposure could be weekly throughout the year. The genitals will be exposed for a long time as it is recommended not to take a bath or shower within 24 hours after treatment. Therefore, it is assumed that absorption of DHA via the eyes and mucous membranes is 100 per cent.

Estimates of the amount of product applied which contains DHA are based on information from SCCNFP (42) for skin lotion (8,000 mg lotion per $15,670 \text{ cm}^2$ skin surface is used, i.e. 0.5 mg/cm^2 skin). The stated amount of self-tanning lotion used in the various booths (manual application: 25 ml; closed booth: 60 ml; open booth: 15 ml) only gives consumption and not the exact amount deposited on the body. There will be some waste, although this will probably be least in open booths where the lotion is first charged and where the spray is directed more accurately. The following calculations are therefore based on the SCCNFP estimates. As mentioned above, the exposed area of the mucous membranes and eyes is estimated at 41 cm^2 . The amount applied is about 20.5 mg each time. An employee in the salon sitting outside the booths will only be exposed ($2 \times 8 \text{ cm}^2 = 16 \text{ cm}^2$) in the eyes, i.e. 8 mg. It is assumed that three treatments are performed per day, spread over the day. I.e. the employee is exposed to 24 mg per day. The number of exposures will be on average ($3 \times 227/365 =$) 1.8 per day.

The result of the exposures is calculated in the EU (SED or Systemic Exposure Dose) in mg of substance per kg body-weight per time and/or per day on the basis of the following data:

1/ Customers receiving weekly treatment in the winter months:

Weight of person, adult:	60 kg
Number of daily applications (once/week for 6 months):	0.07 times/day
Estimated amount per application:	20.5 mg product
Highest concentration of DHA self-tanner:	14 weight-%
Absorption via eyes and mucous membranes (worst case):	100%

Daily exposure amount, DHA, adult:

$$\text{SED} = \frac{0.07 \times (14/100\% \times 20.5\text{mg/day})}{60 \text{ kg body weight}} = 0.003 \text{ mg/kg body-weight/day}$$

2/ Customer receiving weekly treatment all year:

Weight of person, adult:	60 kg
Number of daily applications (once/week for 12 months):	0.14 times/day
Estimated amount per application:	20.5 mg product
Highest concentration of DHA self-tanner:	14 weight-%
Absorption via eyes and mucous membranes (worst case):	100%

Daily exposure amount, DHA, adult:

$$\text{SED} = \frac{0.14 \times (14/100\% \times 20.5 \text{ mg/day})}{60 \text{ kg body weight}} = 0.007 \text{ mg/kg body-weight/day}$$

3/ Employee in salon:

Weight of person, adult:	60 kg
Number of daily applications:	1.8 times/day
Estimated amount per application:	8 mg product
Highest concentration of DHA self-tanner:	14 weight-%
Absorption via eyes (worst case):	100%

Daily exposure amount, DHA, adult:

$$\text{SED} = \frac{1.8 \times (14/100\% \times 8 \text{ mg/day})}{60 \text{ kg body weight}} = 0.034 \text{ mg/kg body-weight/day}$$

6.1.3 Total exposure

The following presents the overall results of the exposure calculations.

Table 6.5 Exposure via inhalation using self-tanning booths weekly during the winter months

Place for collection of particles	Daily exposure time, hours	DHA in sample mg/m ³ air	Exposure mg/kg/day
<i>Manual turbine spray</i>	0.0035	0.8	7 x 10 ⁻⁵
<i>Closed booth 1st measurement</i>	0.0001	238	59.5 x 10 ⁻⁵
<i>Closed booth 2nd measurement</i>	0.0001	115	28.8 x 10 ⁻⁵
<i>Open booth 1st measurement</i>	0.0001	3.3	0.8 x 10 ⁻⁵
<i>Open booth 2nd measurement</i>	0.0001	17	4.3 x 10 ⁻⁵

Table 6.6 Exposure via inhalation using self-tanning booths weekly all year

Place for collection of particles	Daily exposure time, hours	DHA in sample mg/m ³ air	Exposure mg/kg/day
<i>Manual turbine spray</i>	0.07	0.8	14 x 10 ⁻⁵
<i>Closed booth 1st measurement</i>	0.0003	238	0.018
<i>Closed booth 2nd measurement</i>	0.0003	115	0.0009
<i>Open booth 1st measurement</i>	0.0003	3.3	2.5 x 10 ⁻⁵
<i>Open booth 2nd measurement</i>	0.0003	17	12.8 x 10 ⁻⁵

Table 6.7 Exposure via inhalation per treatment (acute exposure)

Place for collection of particles	Exposure per treat. hours	DHA in sample mg/m ³ air	Exposure, mg DHA
<i>Manual turbine spray</i>	0.05	0.8	0.06
<i>Closed booth 1st measurement</i>	0.0017	238	0.61
<i>2nd measurement</i>		115	0.29
<i>Open booth 1st measurement</i>	0.002	238	1 x 10 ⁻⁷

<i>2nd measurement</i>		115	3.8×10^{-7}
------------------------	--	-----	----------------------

Table 6.8 Background exposure via inhalation calculated for customer and operator respectively

Place for collection of particles	Exp. time/treat. hours	DHA in sample mg/m ³ air	Exposure mg/kg/day
Background (closed booth) Operator	0.28	0.5	0.0035
Background (closed booth) customer, winter months	0.018	0.5	0.00023
Background (closed booth) customer, all year	0.035	0.5	0.00043
Background (open booth + manual) operator	0.28	0.29	0.0020
Background (open booth + manual) customer, winter months	0.018	0.29	0.00013
Background (open booth + manual) customer, all year	0.035	0.29	0.00025

Exposure of customers to DHA via the eyes and mucous membranes applied as self-tanning products in spray booths:

- 1/ Weekly in the winter months **0.003 mg/kg body-weight/day**
- 2/ Weekly all year **0.007 mg/kg body-weight/day**
- 3/ Exposure of employee to DHA via the eyes **0.034 mg/kg body-weight/day**

Table 6.9 Total exposure to DHA from direct exposure via respiratory system, mucous membranes and background exposure

Place for collection of particles	Total exposure to DHA mg/kg/day
<i>Customer</i>	
Manual turbine spray, winter months	0.0033
Manual turbine spray, all year	0.0087
<i>Customer, winter months</i>	
Closed booth 1st measurement	0.0048
Closed booth 2nd measurement	0.0035
<i>Customer, all year</i>	
Closed booth 1st measurement	0.0092
Closed booth 2nd measurement	0.0083
<i>Customer, winter months</i>	
Open booth 1st measurement.	0.0031
Open booth 2nd measurement	0.0032
<i>Customer, all year</i>	
Open booth 1st measurement	0.0073
Open booth 2nd measurement	0.0074
<i>Operator</i>	
Closed booth	0.0375
<i>Operator</i>	
Open booth: + manual	0.036

Table 6.6 shows that personnel are more exposed than customers to any effects from DHA and self-tanning products by up to a factor of 10, and the various treatment booths make no difference to the exposure of personnel or customers. The scenario with personnel includes three treatments per day for 227 days a year. The average figure for treatment is closer to 1-2 per day for the personnel studied. The above calculations also show that exposure of the eyes and mucous membranes is a greater quantitative load than exposure via inhalation.

Ventilation/extractors and cleaning the booths start immediately after treatment. This was delayed for the NERI measurements. This meant higher air concentrations in the background measurements than for normal operation.

100 per cent absorption has been assumed. This has been assumed as no information was found in the literature.

The operators recommend that customers keep their eyes shut throughout treatment. Customers only rarely open their eyes. Any spray mist which comes into contact with the eyes will possibly be rinsed away by tears and so will not remain on the eyes and be absorbed 100 per cent. The eyes of the operators are exposed to significantly lower concentrations of DHA than the value used in the calculations above.

Operators instruct customers on using nose filters and pursing their lips during treatment in automatic booths so that they avoid inhaling the products. This is harder during manual application as this takes 2-3 minutes. The operator asks the customer to keep her eyes closed, use nose filters, and purse lips when spraying the upper part of the body around the neck and head.

Because of the lack of data such as NOAEL, LOAEL, etc. it is not possible to make further conclusions on the size of exposure.

6.2 Safety assessment of DHA

Because of the possible allergenic effect of DHA, people allergic to the substance should avoid skin contact as there is no lower limit for this health effect.

DHA should not be inhaled, as there is no documentation available on the effects of inhalation.

It is not possible to know for certain whether DHA has any effect on the mucous membranes and eyes, as the only test results are for DHA as an ingredient in a product and not for DHA alone.

Only limited documentation is available on the toxicity of DHA and this mostly builds on poorly documented and out-of-date methods. In laboratory tests, DHA has demonstrated genotoxic potential and mutagenic properties. DHA is also a naturally occurring intermediary in the carbohydrate metabolism of higher plants and animals. There are no results showing that DHA has the same effects on human skin. The health effects from long-term use of DHA in self-tanning products are unknown, and therefore it is uncertain how DHA affects the skin after longer usage.

On the basis of this study and the existing knowledge on the substance there is no reason to assume that the use of self-tanning products in spraying chambers poses a health risk for consumers and professional users. On the basis of the limited knowledge on the health effects of DHA, however, it is recommended that the following precautions be taken until further documentation is available:

- Self-tanning products should not be inhaled or come into contact with mucous membranes.
- People with asthma, sensitive skin and cuts and grazes should consult a doctor before treatment.
- Keep the mouth closed and protect lips with lip salve during treatment.
- Expectant and nursing mothers should not use self-tanning products in spray booths.
- Regular weekly use over a long period is not recommended while knowledge about DHA is so limited.
- Self-tanning products do not adequately protect against the sun's rays and therefore when exposed to the sun it is still important to follow advice and use sun cream.

Furthermore, booths should be equipped with extraction devices so that spray is not spread in the surrounding premises with resulting unnecessary exposure of personnel and customers.

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7 Annex 1 - Analysis report

Exposure to
dihydroxyacetone (DHA)
during self-tanning with
aerosols

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Summary and conclusions

Dihydroxyacetone (DHA) is used for artificial tanning. The Danish Environmental Protection Agency initiated an investigation into the health risks associated with DHA tanning by spray application. The National Environmental Research Institute in Denmark performed a study of inhalation exposure during three different spray scenarios, measuring the concentration of DHA in aerosol droplets < 12 µm in diameter. Further, we studied the exposure to DHA in rooms adjacent to the tanning booths. We developed methods for sampling and analysis of DHA in self-tan lotion aerosols. We used a series of impingers containing a derivatisation agent. The derivatisation product was analysed using LC-MS-MS.

The highest exposure occurred in a closed booth with concentrations of 115 and 238 µg/l air as an average during 16 and 14 seconds of exposure inside the booth. In an open booth working with an electrospray system, the concentration of DHA amounted to 3.3 and 17 µg/l air at an average of 35 seconds of exposure. During manual application using a turbine principle the average concentration was 0.8 µg/l air during 210 seconds of treatment.

The measurements are minimum concentrations as there may be some DHA that passed the sampling equipment without reaction with the derivatisation agent.

During one spray in the closed booth we measured size distribution of droplets between 0.5 and 20 µm in diameter. There was a big proportion of particles less than 10 µm during the application. The apparatus for measuring size distribution was not available during the measurements in open booths and with manual spray.

Introduction

The project aims to:

- determine the amount of DHA in small droplets that a person will potentially inhale during professional treatment with self-tanning lotion.
- measure the amount of DHA in small droplets in rooms adjacent to booths used for treatment with self-tanning lotion.

Dihydroxyacetone (DHA) CAS No: 96-26-4

Dihydroxyacetone or 1,3-dihydroxy-2-propanone is a white, hygroscopic, crystalline powder with a melting point of 75 °C. Formula: $C_3H_6O_3$ Molecular weight 90.08. Vapour pressure is unknown. Water solubility: easily soluble (Merck 1983 p 463)

Materials and methods

Treatment places and methods

At the start of this project, the Danish EPA and Danish Toxicology Centre had already established contact to two providers of self-tanning treatments that had offered to participate in a study to elucidate the health risks of spray treatment.



The different treatment methods are reviewed below.

- a. Open booth for spraying with aerosol according to the electro-spray principle. The customer stands on two metal plates that work as grounders. Self-tanning lotion is sprayed from two vertical rows of nozzles in fine droplets. The droplets are subjected to a voltage of 40,000 Volts and are then sucked onto the customer's skin. Spraying is done in 2 sessions of 2, 2½ or 3 seconds. In this study, spraying was done 2 times 3 seconds. 15 ml of lotion was used per treatment. The customer is instructed to close her eyes and hold her breath during treatment. The skin did not seem wet after treatment. Samples were collected during the period when the test person was in the booth.

Open booth for auto spray treatment uses the electro-spray principle. Equipment for collection of droplets $< 12 \mu\text{m}$ consists of an inlet held up to the nose/mouth, four purifying flasks with derivatisation reagents in a box with crushed ice, and a pump for sucking air through the purifying flasks.

b. Manual turbine spray

The spray used worked according to an HVLP (High Volume Low Pressure) turbine principle that gives a smaller lotion surplus than the turbine spray used in some places. Self-tanning lotion is applied to the entire body whilst the customer turns around. The entire treatment lasted less than three minutes. 25 ml lotion was used for the treatment. The customer is instructed to close her eyes during treatment. A small filter, placed in both nostrils, filters inhalation air. Samples were collected during the period the person was treated standing in the booth.



During manual spraying in booths with extractor fans, breathing is through a nose filter. Eyes are kept closed during treatment.

c. Closed booth

The booth consists of two compartments. The customer hangs her kimono in the outer compartment and activates the spray programme. The customer then steps into the inner compartment, where self-tanning lotion is sprayed through three rows of nozzles. The spray programme lasts six seconds. The customer then steps into the outer compartment and closes the door behind her. The moment the treatment ends, there is a thick aerosol mist in the inner compartment. The sooner the door is closed behind the customer, the sooner the aerosol is shut off. Approx. 60 ml lotion is used per treatment. Samples were collected from the beginning of the treatment until the customer stepped out into the outer compartment. The skin was wet after treatment. Excess lotion was rubbed into the skin until it was dry. After a treatment, the inner compartment is cleaned with an automatic shower system that removes excess lotion from the air and walls in the booth.



Closed booth for auto spray. The outer compartment where the customer hangs her kimono is separated from the spraying booth by a door.

DHA in adjacent rooms.

The concentration of DHA was measured in the area outside the spraying booth at both locations. Air was collected for 30 minutes.

Collection method

We wished to collect inhalable droplets smaller than approx. 10 µm. For this purpose, an inlet was developed to remove larger drops (figure 4). The separation of the two size classes, larger than and smaller than 10 micrometres, builds on a turn/change in the airstream in the inlet through which the smallest size class follows the airstream while the larger particles separate and are collected (not analysed). The principle behind the separation of the two sizes is the same as in an impactor for collection of particles/droplets of different sizes.

The separation of the two size classes is determined by, for example, the flow at the entrance and the distance to the surface across the airstream. The aerodynamic diameter (d_{ae}) where the particles are separated is calculated on the basis of the equation (Mercer and Stafford 1969):

$$d_{ae}^2 = \frac{0.25 \cdot 18 \cdot D(\text{cm}) \cdot 1.8 \cdot 10^{-4} (\text{g/cm} \cdot \text{s})}{v(\text{cm/s}) \cdot 1 (\text{g/cm}^3)}$$

Here D is the diameter of the entrance hole and v is the flow at the entrance hole.

The separation was calculated at 12 µm for the actual flows in the tests. The goal was to separate at 10 µm, but this was not achievable in this study.

Figure text:

Hose for collection of small droplets

Inflow of droplets

Large drops run down here. To be cleaned subsequently – NOT to be analysed.

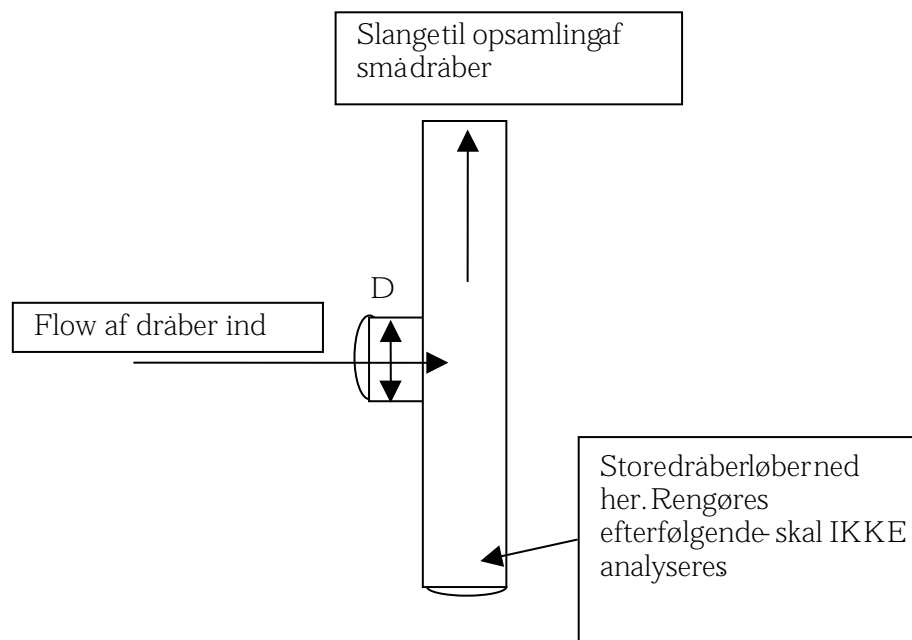


Figure 4. Outline illustrating the principle for the inlet to collect droplets.

We applied a method for collection of DHA described by Spaulding et al. 1999. These authors measured various carbonyls in outdoor air. They are the first to have been able to measure hydroxycarbonyls, such as hydroxyacetone. They collected air with low concentrations of carbonyls in periods of three hours. During collection, the air passes through four purifying flasks placed in series (Figures 1 and 5). The purifying flasks contain 150 ml derivatisation reagent that reacts with carbonyls which thus form a bond with the liquid (see analysis method). We modified the method for short-term collection of samples with a high content of DHA by increasing the concentration of derivatisation reagent in the purifying flasks from 25 mg/l to 250 mg/l. The air is sucked by means of a pump through the inlet described above and on through the purifying flasks. A needle placed in the connection tube between the purifying flasks and the pump regulates the flow rate. The flow rate aimed at was 500 ml/min. The exact flow rate was measured before each sample was taken as the mean value of five measurements measured with a Gilibrator Bubble Flow meter.

Figure text:
 Sample inlet (from booth)
 Flow measured at 150 ml H₂O
 Flow control (needle)
 To pump

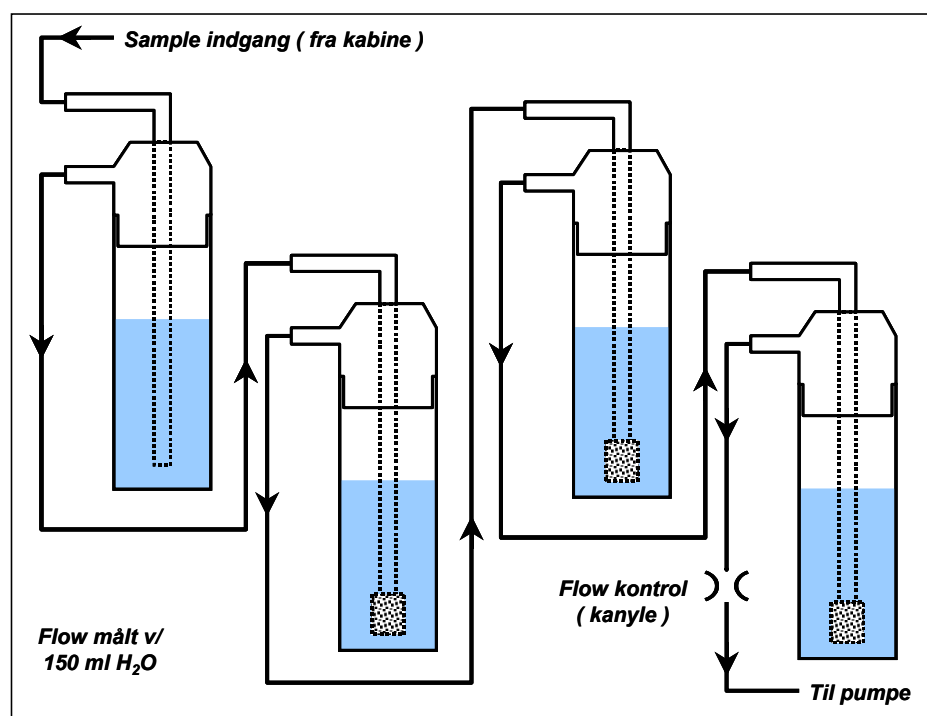


Figure 5. Outline illustrating the principle for collection setup. In the front purifying flask, the airflow passes through an open tube to catch drops. In the other three flasks, the tube ends in a frit to give the best possible contact between air and derivatisation reagent.

In order to minimise evaporation of DHA during sample collection, the derivatisation liquid was kept cold during transport, and the purifying flasks

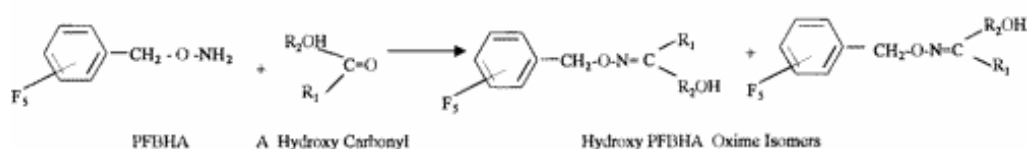
were placed in a box with crushed ice during collection. The intake to the purifying flasks has a glass inlet that is held in front of the mouth and nose during self-tanning treatment. After collection, the samples were poured into Duran bottles. At the manufacturer, this was done on a trolley in the room next to the booths. At another manufacturer it was done behind a closed door in a room next to the salon itself. First, the purifying flasks were rinsed with approx. 25 ml derivatisation reagent which was poured into the sample, and then with two-times 150 ml Millipore water, which was subsequently discarded. Before collection of air from adjacent rooms, the equipment was rinsed with more water.

Analysis method

No published method for analysis of DHA in air has been found. The method applied was modelled on Spaulding 1999. This method was developed to analyse a number of carbonyls in outdoor air, including hydroxyacetone, but not dihydroxyacetone. The carbonyl group in DHA reacts with *o*-(2,3,4,5,6-pentafluorobenzyl)-hydroxylamine (PFBHA), cf. figure 6. This reaction begins already during sample collection and continues at room temperature for 24 hours in darkness. In the original method, the carbonyls were analysed with GC-MS after further derivatisation with bis(trimethylsilyl)-trifluoroacetamide (BSTFA). However, preliminary lab tests showed poor recovery rates, probably because the dihydroxy compound is not sufficiently derivatised with BSTFA. Instead, a method was developed for analysis of the PFBHA derivative by means of LC-MS-MS.

When the samples were received in the laboratory, they were spiked with ^{13}C acetone which was also derivatised. Calibration standards in concentrations ranging from 0.100 to 500 $\mu\text{g/ml}$ were produced by mixing DHA standard with PFBHA solution and derivatising during the same period as the samples. After 24 hours, the samples were pH adjusted with 1 ml 18N H_2SO_4 and extracted three times with 10 ml dichloromethane. The solvent was evaporated to dryness and the samples were again dissolved in 1 ml water.

Scheme 1: Derivatization of a Hydroxy Carbonyl with *o*-(2,3,4,5,6-pentafluorobenzyl) hydroxylamine (PFBHA)



Scheme 2: Derivatization of a Hydroxy PFBHA Oxime with bis(trimethylsilyl) trifluoroacetamide (BSTFA)

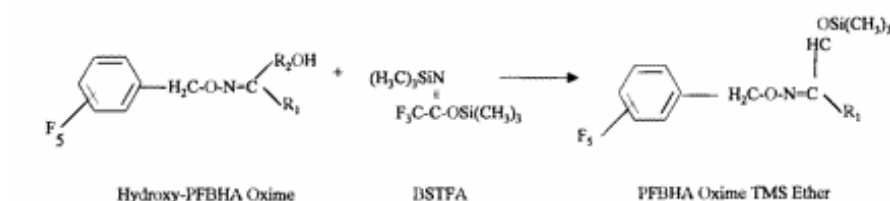


Figure 6. Reaction schemes for derivatisation of hydroxycarbonyls with PFBHA and for further derivatisation of the oxim formed with BSTFA. (Spaulding et al. 1999). The last step did not work for dihydroxyacetone.

The concentrated sample was analysed using reversed phase HPLC on an Agilent 1100 HPLC with a Thermo Hypersil column C18 250x2.1 mm. Eluent A was 1 per cent methanol, 99 per cent 5 mmol ammonium acetate and 0.01 per cent formic acid. Eluent B was 90 per cent methanol and 10 per cent 5 mmol ammonium acetate. The chromatography took place over a 45-

minute period with a linear gradient. Figure 7 shows a chromatogram. Subsequently, the components were detected using a double mass spectrometry (MS-MS) on a Sciex API 2000 mass spectrometer. The substances were ionised with electrospray ionisation (ESI), which forms positive molecular ions $[M+H]^+$. The ionised molecule is isolated in the first MS (quadrupole) and fragments when exposed to electrical energy and a collision gas (nitrogen). A characteristic fragment is isolated in the second MS (quadrupole) whereby only the component meeting these requirements as well as the retention time requirement (same retention time for standard and sample) is positively detected in the sample.

DHA derivatised with PFBHA forms a positive ion at m/z 286 and 2 specific product ions at m/z 268 and 181 after fragmentation.

The DHA content in the samples is calculated using linear regression against calibration standards.

For each sampling session, the concentration was measured in all four purifying flasks.

Distribution of droplet sizes

In connection with the sampling session, the distribution of droplet sizes was measured using an Aerodynamic Particle Sizer Spectrometer (from TSI). The instrument is on loan from the National Institute of Occupational Health (Keld Alstrup Jensen).

The Aerodynamic Particle Sizer (APS) measures the aerodynamic diameter of particles in 51 size classes from 0.5 and up to 20 micrometres. The time resolution in the tests was 1 second. The distribution of drop sizes was measured in four periods of 250 seconds each. In the room next to the booth, measurements were taken before the first treatment, during both treatments with people in the booth, and after all three spraying sessions in the booth. In the booth itself, the distribution of droplet sizes was measured during a spraying session without a person in the booth.

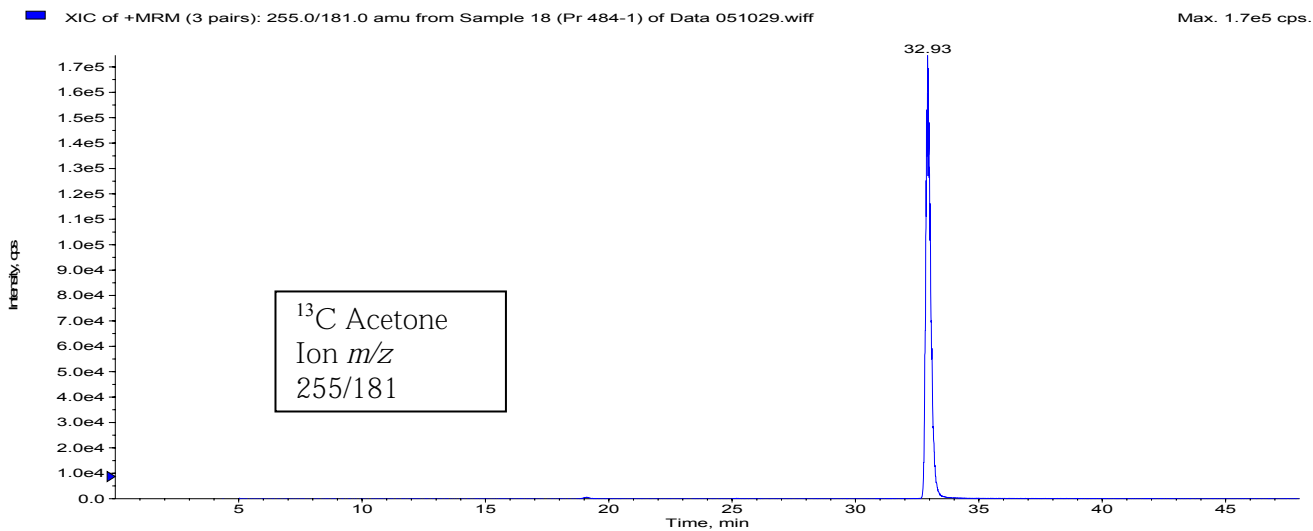
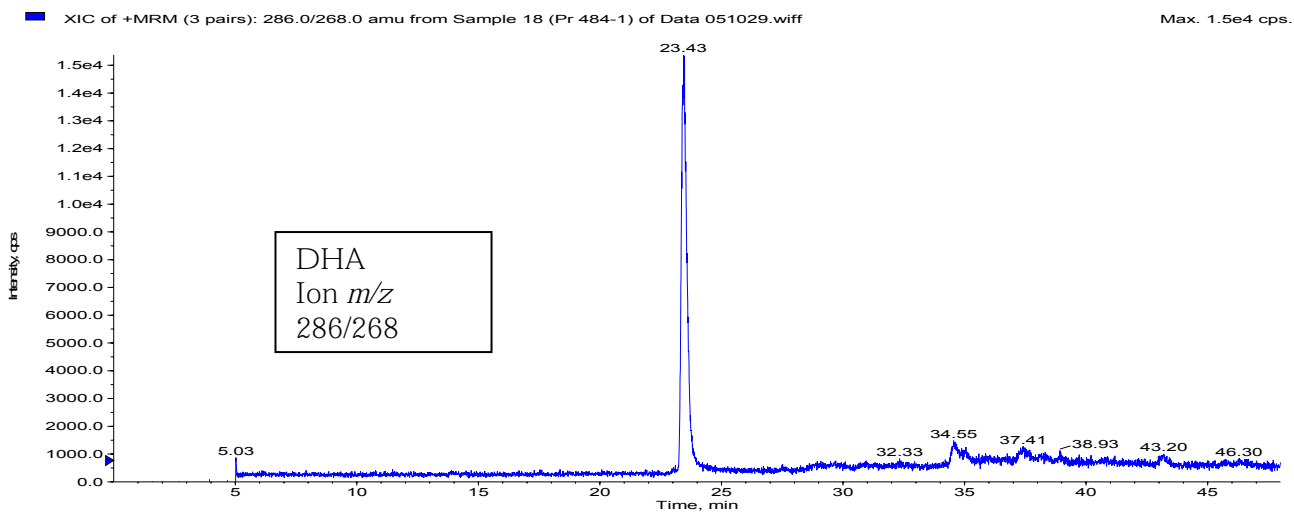
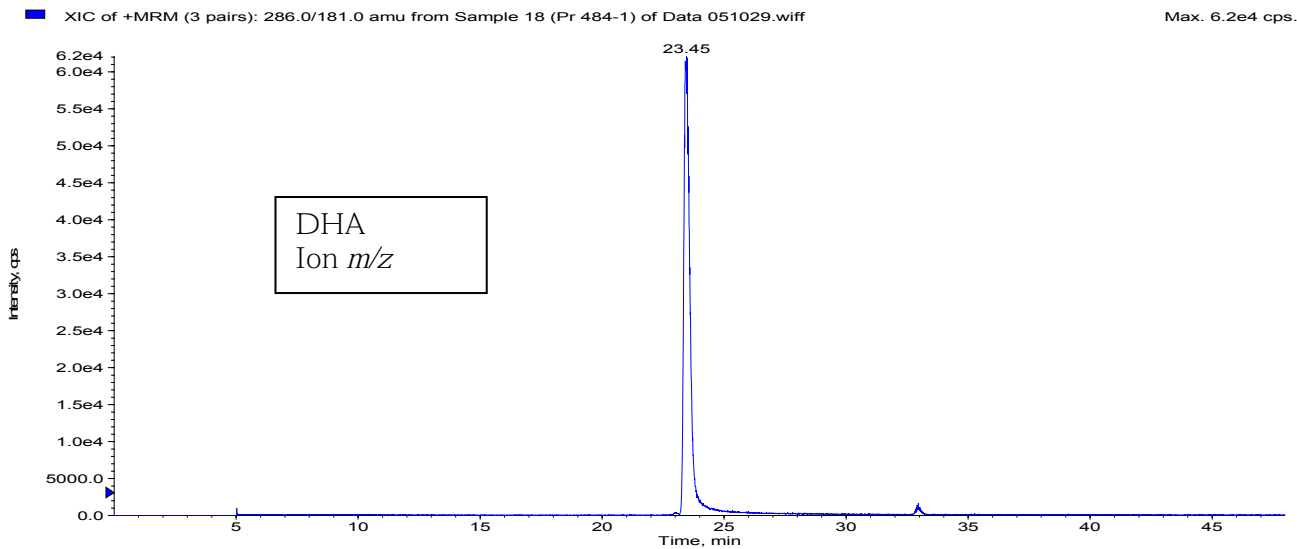


Figure 7. Ion chromatogram of a concentrated sample

Results and discussion

Concentration measurements

Table 1 shows the results of a measurement of DHA in drops < 12 µm in diameter in the treatment booths. The concentration is calculated on the basis of the total amount of DHA in all four purifying flasks. There was also DHA in the last bottle in the series, which seems to indicate that DHA has not had enough time to react quantitatively with the derivatisation reagent. At one sampling location, the purifying flasks were rebottled in the room next to the booths. A field blind sample, which had had the lid screwed off during rebottling, showed that there was a passive uptake of DHA in the derivatisation reagent when it was in contact with air with DHA. The samples from spray collection at this location were thus adjusted for blind value.

Table 1. Concentration of DHA in droplets < 12 µm in the air around the mouth/nose during treatment with self-tanning spray

	Treatment method	Spraying time sec.	Collection time sec.	Flow rate ml/min	DHA in sample µg/l air	Particle separation µm
a	auto spray open booth	2 x 3	35	538	3.3	12
a	auto spray open booth	2 x 3	35	514	17	12
b	manual turbine spray	167	210	529	0.8	12
c	auto spray closed booth	6	14	542	238	12
c	auto spray closed booth	6	16	551	115	12

The difference between the results of two spraying sessions in open and closed booths respectively may be because the test persons were of different heights and they may have held the inlet slightly differently in relation to the nozzles.

The low DHA content in the air during manual treatment may be because there are extractor fans in the booth and the face was only sprayed for a small part of the total treatment time. After treatment, it was possible to see that the nose filter was coloured by the bronzer in the lotion. This colour indicates immediately where the customer has been treated. There is some reaction time for DHA's reaction with the skin.

Table 2 shows the results of a measurement of DHA in drops < 12 µm in diameter in rooms adjacent to the spraying booths. At both locations, measurements were taken after three treatments, which is worst case in relation to the clinics' normal treatment frequency.

Table 2. Concentration of DHA in rooms adjacent to spray booths after three treatments with self-tanning spray

		Collection time (min)	Flow rate ml/min	DHA in sample	Particle separation
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				µg/l air	µm
1	Method a and b	30.5	516	0.29	12
2	Method c	30.0	553	0.50	12

Distribution of drop sizes

Figure 8 shows the distribution of drop sizes (measured as mass) as a five-second average after spraying in a closed booth. Each curve represents five seconds of collection. The column to the right shows the relation between the colour of the curve and the measurement time. There is a large peak of drops in the inhalable area for particles (< 10 µm). After about one minute, the level is back down to the background level for particles.

Figure text:

Y-akse: Particle mass (dM/dlogdp)

X-akse: Droplet size (micrometer)

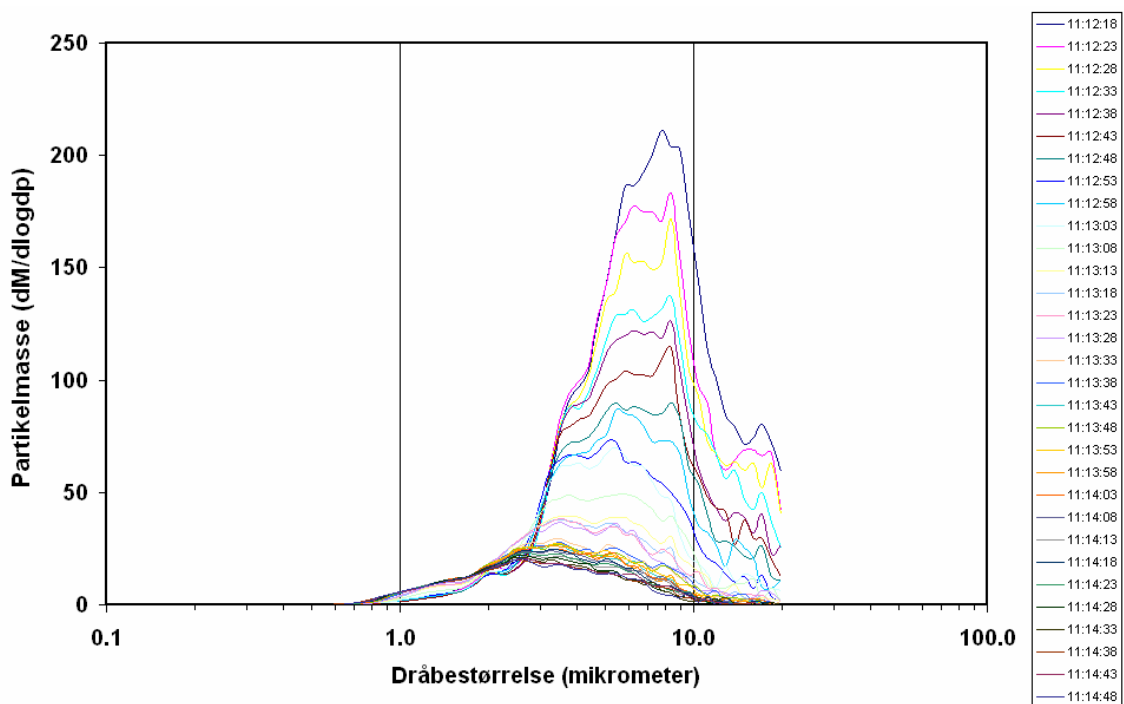


Figure 8. Size distribution for particles (measured as mass) as a five-second average during spray session in a closed booth. The drops are differentiated within the area 0.5 to 20 µm. The table to the right shows the time for each average measurement.

Figures 9-13 show the total concentration (mg/m³) of drops < 20 µm measured in periods of 250 seconds at various times during and after treatment. The density of the liquid has been set at 1.

The background concentration is lowest before the first spray treatment. It increases a little during treatment, but is still low. During the treatment proper, the mass increases significantly, while it remains at a constantly low level after the end of the last treatment.

Figurtekster 9-13 (gentages for alle figure – testnr. ændres):
Test 1. Total conc. (mg/m³)

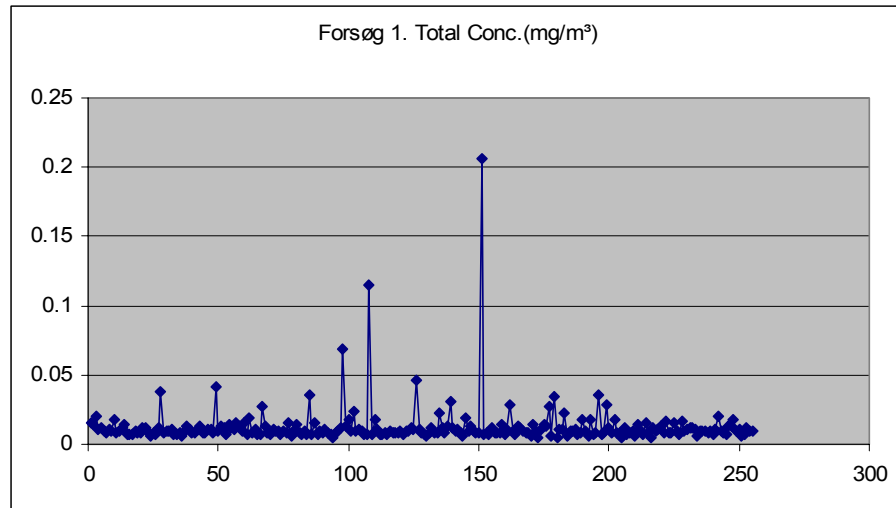


Figure 9. Total mass of drops < 20 μm measured in adjacent room before first treatment with self-tanning lotion in closed booth.

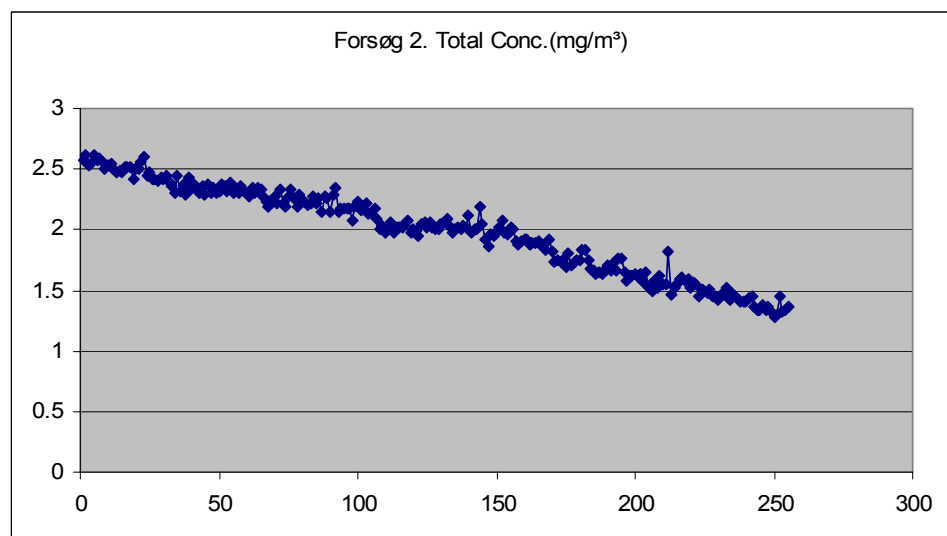


Figure 10. Total mass of drops < 20 μm measured in adjacent room during first treatment with self-tanning lotion in closed booth.

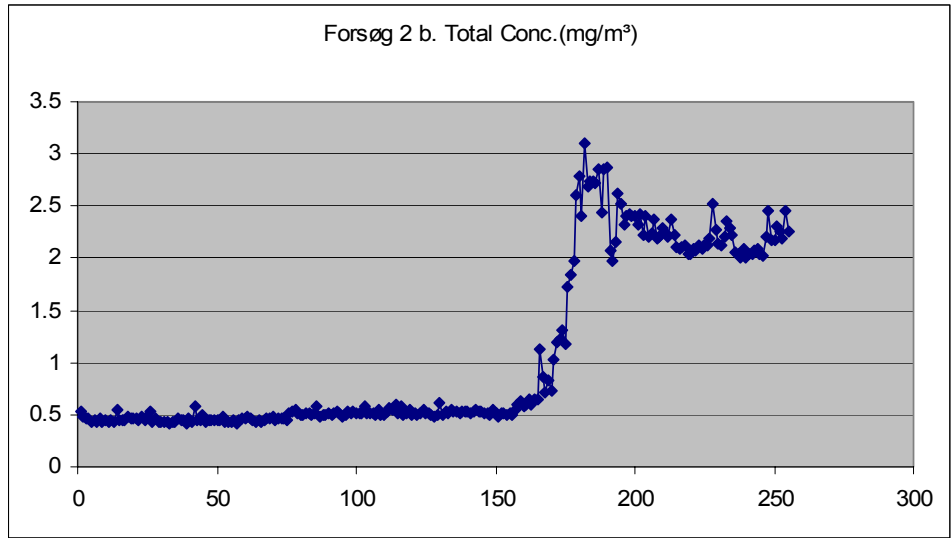


Figure 11. Total mass of drops < 20 μm measured in adjacent room during second treatment with self-tanning lotion in closed booth.

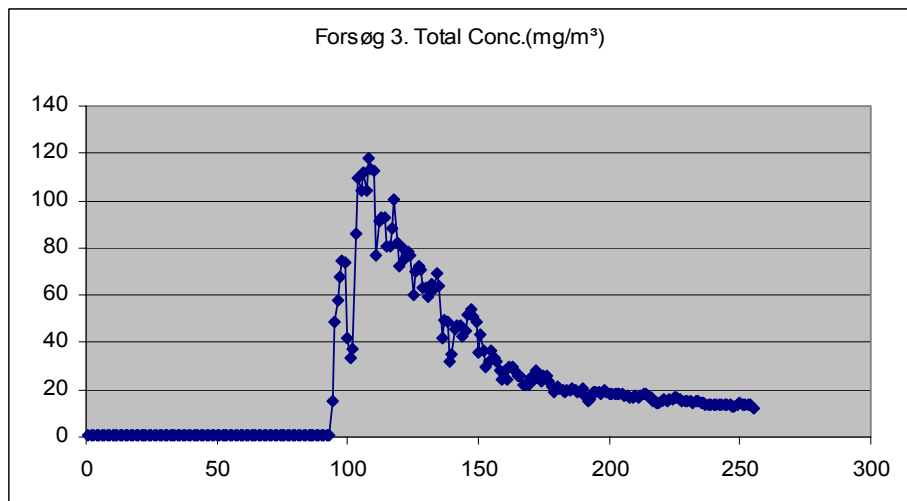


Figure 12. Total mass of drops < 20 μm measured in closed booth during treatment with self-tanning lotion.

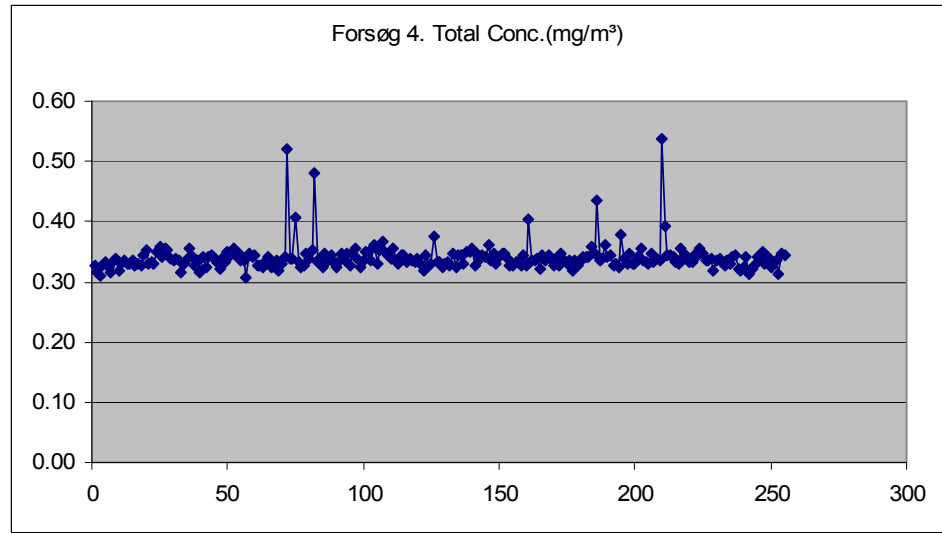


Figure 13. Total mass of drops < 20 μm measured in adjacent room after three treatments with self-tanning lotion.

Conclusion

Methods have been developed for collection and analysis of dihydroxyacetone in self-tanning lotion. Measurements have been taken during three types of treatment. The highest exposure is found during treatment in closed booths. The measurements should be regarded as minimum concentrations, as unreacted DHA may have passed the last purifying flask. During treatment in closed booths, a large proportion of drops in the inhalable area $< 10 \mu\text{m}$ in diameter was detected. The instrument for measurement of distribution of drop sizes was not available during measurements in open booths and for manual turbine spray.

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