INTRODUCTION

Surfactants and water have been reported as being among the major causes of hand eczema due to occupational exposure (18). In many work situations it is difficult to avoid wet work, constant hand washing and use of surfactants. To prevent hand eczema it is important to identify the risk factors. In the present study one of the most common classes of chemicals used in such situations i.e. in detergents, ethoxylated non-ionic surfactants, was investigated. Their ability to be oxidized when exposed to air and form oxidation products with allergenic properties was investigated. An allergenic activity due to a change in their composition can contribute to and aggravate occupational contact dermatitis.

SURFACTANTS, THEIR CHEMISTRY AND PROPERTIES

Chemical composition of surfactants

The name amphiphile is sometimes used synonymously with surfactant. The term relates to the fact that all surfactant molecules consist of at least two parts, one part which is soluble in a specific fluid (the lyophilic part) and one which is insoluble (the lyophobic part). When the fluid is water one usually talks about the hydrophilic and hydrophobic parts, respectively. The hydrophilic part is often referred to as the polar head group and the hydrophobic part as the tail. The primary classification of surfactants as cationic, anionic, non-ionic and zwitterionic (amphoteric) is made on the basis of the charge of the hydrophilic group, which is either ionic or non-ionic (33). The hydrophobic group is normally a hydrocarbon chain and the majority of these are linear to meet the demands for biodegradability (Figure 1).

Physicochemical properties of surfactants

The word surfactant is an abbreviation for surface active agent. A surfactant is characterized by its tendency to adsorb at surfaces and interfaces. Examples of interfaces involving a liquid phase include suspension (solid-liquid), emulsion (liquid-liquid) and foam (liquid-vapour). In many formulated products several types of interfaces are present at the same time. Another general and fundamental property of surface active agents is that monomers in solutions tend to form aggregates, called micelles (33). Micelles form already at very low surfactant concentrations in water (Figure 2). The concentration at which micelles start to form is called critical micelle concentration (CMC). Micelle formation, or micellization, can be viewed as an alternative mechanism to adsorption at the interfaces for removing hydrophobic groups from contact with the water, thereby reducing the free energy of the system. It is an important phenomenon since surfactant molecules behave very differently depending on whether they are present in micelles or as free monomers. The micelles behave as large molecules and influence the solubility of organic hydrocarbons and oils in aqueous solution and also influence the viscosity. The size of the micelle is measured by the aggregation number which is the number of surfactant molecules associated with a micelle. Only surfactant monomers contribute to surface and interfacial tension lowering. Wetting and foaming are governed by the concentration of free monomers in solution. The micelles can be seen as a reservoir for surfactant monomers. At higher concentrations of the surfactant other aggregates are formed. Different phase structures give very different physicochemical properties. To understand the physicochemical behaviour of a surface active compound over a concentration range at different temperatures, phase diagrams of the surfactants in water are constructed. Depending on the temperature there is different solubility regions of the surfactant-water system, shown in the phase diagram as a homogeneous or a single-phase system and heterogeneous systems of two or more phases.

Ethoxylated non-ionic surfactants

The present study focuses on ethoxylated non-ionic surfactants. Non-ionic surfactants are compatible with all other types of surfactants and their physicochemical properties unlike those of ionic surfactants are not markedly affected by electrolytes. The physicochemical properties of ethoxylated

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**Fig. 1.** Structures of some representative surfactants: (a) Anionics have carboxylate, sulfate or phosphate surfactants as polar head groups, here illustrated with alkyl ether carboxylate. (b) Cationics are based on amine or quaternary ammonium groups, here illustrated with a fatty amine salt. (c) Non-ions have either ether or polyhydroxyl as polar group, here illustrated with an ethoxylated fatty alcohol. (d) Zwitterionics contain both an anionic and a cationic charge under normal conditions, here illustrated with betaine.

**Fig. 2.** Illustration of surfactant molecules (monomers) in a solution at low concentration. Above the CMC concentration (a) micelles is formed. At higher surfactant concentration, the amphiphiles form a variety of structures, here illustrated with (b) a liquid crystalline lamellar single-phase and (c) reversed micelles, an isotropic single-phase.
compounds are very temperature-dependent and a reverse solubility versus temperature behaviour is observed in water (33). The temperature at which the surfactant becomes insoluble i.e. phase separation occurs for a given surfactant concentration, is called the cloud point. Above this temperature the surfactant solution becomes turbid and separates in two phases in equilibrium. The cloud point is an important parameter of non-ionic surfactants and several properties, e.g. detergency, can be correlated with the cloud point. Figure 3 show a phase diagram of the one of the ethoxylated alcohols investigated with five oxyethylene groups in the structure in water over the temperature range 0–100°C (33). The phase diagram demonstrates the various aggregates present in single-phase or in heterogenous two-phase regions of the surfactant at increasing temperature and increasing percentage of surfactant. The single-phase systems can be divided into isotropic solutions, solid phases and liquid crystalline phases. In both isotropic and liquid crystals the state of the amphiphile alkyl chains can be denoted as “liquid-like” but in crystals the state is more or less “solid-like”.

Synthesis of ethoxylated non-ionic surfactants

Non-ionic surfactants are obtained by the addition of ethylene oxide to suitable materials and are complex mixtures. The most commonly used starting materials are fatty alcohol, alkyl phenols, fatty acids and fatty amines. The ethoxylation is usually carried out under alkaline conditions and any material containing an active hydrogen can be ethoxylated (33). The typical number of oxyethylene units in the polar chain is 5–20. Examples of polyhydroxyl based non-ionic surfactants are sucrose esters, sorbitan esters, alkyl glucosides and polyglycerol esters. A surfactant of technical quality intended for formulation of various products, normally contains a distribution of homologues with a varying chain length of the hydrophobe and a varying number of ethyleneoxide groups in the polar part of the molecule. Their properties and applications greatly depend on the chemical composition and the average degree of ethoxylation. Unlike the polyethoxylated non-ionic surfactants, the homogeneously ethoxylated ones, are uniform products with respect to the degree of ethoxylation and often the same holds true for the parent hydrophobe. These chemically well-defined products were introduced in order to control non-ionic surfactant production, test biodegradation and investigate the physicochemical properties of non-ionic surfactants.

Susceptibility of ethoxylated surfactants to oxidation on exposure to air

Ethoxylated surfactants are polyethers and as such susceptible to oxidation on exposure to air. This autoxidation is theoretically discussed in the surfactant literature (12–14, 48, 62). The proposed mechanism for autoxidation of the polyoxyethylene chain is a free radical mechanism initiated by minor amounts of free radicals present, or catalyzed by metal salts e.g. copper sulfate (12). Peroxides and hydroperoxides are primary oxidation products followed by formation of carbonyl compounds as secondary oxidation products (Figure 4). The susceptibility of ethoxylated surfactants to oxidation has been studied by some authors and several of the possible oxidation products that theoretically may be formed were discussed but have not been isolated and identified. The principal organic groups that have been detected were carboxylic acids, aldehydes, alcohols, lactones and esters (12, 48). In previous studies on mono- and diterpenes, various peroxides and hydroperoxides were shown to be potent contact allergens (27, 34, 35, 59).

Use of surfactants

Surfactants are widely used and many topical pharmaceutical formulations, cosmetics, antiseptics, shampoos, detergents, creams and lotions contain surfactants. Surface active products are for to their amphiphilic properties used as emulsifiers, suspending, wetting, solubilizing and stabilizing
agents (3). Anionic surfactants are used in greater volume than any other surfactant class and are used in most detergent formulations. One reason is the ease and low cost of manufacture. Non-ionic surfactants are the second largest surfactant class and have either polyether or polyhydroxyalkyl as polar group to increase water solubility. Non-ionic surfactants have long been recognized as compounds with a low irritating effect, and hence are widely used in topical products (18). Compounds such as cetomacrogol (polyethoxylated monooctadecanol) and cetostearyl alcohol are major components of non-ionic creams (3). Besides giving physical stability, non-ionic surfactant active agents may have a biological activity as membrane stabilizers or may influence the activity of other molecules (20). Non-ionic surfactants are known to have effects on the permeability of biological membranes, including the skin. Their comparatively low toxicity and irritation potential have made these compounds good candidates as potential penetration enhancers for use in transdermal drug delivery systems (68).

The single most important type of non-ionic surfactants is ethoxylated fatty alcohols with linear primary alcohol ethoxylates as predominant type. A major quantity of alcohol ethoxylates are used in household laundry detergents or further converted to ethoxysulfates, i.e. anionic surfactants, which are used in shampoos and other skin care products. Linear primary alcohol ethoxylates also find use in household cleaners, institutional and industrial cleaners, cosmetics, agriculture, and in textile, paper, oil and other process industries (54, 62). The consumption of ethoxylated alcohols in Western Europe was more than 300,000 tons in 1993 (44). Growth in the use of linear primary alcohol ethoxylates has been rapid over the past 20 years because of their many desirable qualities such as rapid biodegradation, low to moderate foaming ability, superior cleaning of man-made fibres, tolerance to water hardness, and ability to perform in cold water. Furthermore, the consumption of different surfactants used in occupational and household detergents is high with ethoxylated alcohols in majority (31).

The prime uses of cationic surfactants relate to their tendency to absorb at interfaces i.e. metals, minerals, plastics, cell membranes since the majority of surfaces are negatively charged. The zwitterionic surfactants as a group are characterized by having excellent dermatological properties. The also exhibit low eye irritation and are frequently used in shampoos and other cosmetic products. They are compatible with all other classes of surfactants, but are the smallest surfactant class, partly due to high price.

OCCUPATIONAL HAND ECZEMA

Work related skin diseases are common and are among the most commonly occurring work-related illnesses. Of occupational skin diseases, eczema on the hands is by far the most common since in many jobs the skin of the hands is subject to damage caused by contact with skin irritants and contact allergens (56). Risk occupations for hand eczema are primarily those in which the skin on the hands is exposed to water and detergents to such degree that the skin barrier is harmed. In an epidemiological study in Göteborg, Sweden, Meding (42) found that hand eczema was twice as common among females as among males. Hand eczema was more common among people reporting some kind of occupational exposure. The exposed groups and risk groups often worked in different service occupations, such as cleaning, hairdressing, home-help service, kitchen, restaurant work and hospital care but also in different types of industrial production (42, 43). The most harmful exposure turned out to be to unspecified chemicals, water, detergents, dust and dry dirt. The risk of hand eczema is probably further increased if the exposure also involves substances that can cause allergic contact dermatitis.

In Sweden occupational injuries are reported to the Occupational Injury Information System (ISA) under the authority of the National Board of Occupational Safety and Health. The purpose of ISA is to provide the basic information required for injury prevention in industry. A compilation has been made of all reported cases of occupational skin disease in Sweden in 1980–92 (30). This indicates that the number of reported cases of skin disease per thousand paid employees during the whole period was higher for women than for men, and twice as many cases were reported in the 16–24 age group compared with the other age groups, see Figure 5. This indicates that young women constitute a clear risk group for work related skin disease.

The occupations in Sweden which have the highest number of reported cases of occupational skin disease in relation to the number of individuals employed in each occupation, are shown in Figure 6. Most cases occur within women’s occupations involving extensive wet work.

Allergic contact dermatitis

Allergic contact dermatitis, also referred to as delayed contact hypersensitivity, is an example of an undesirable consequence of the efficiency of our immune system (57). The immune system has evolved mainly as a host defence against infection and malignancy, but in the case of allergic contact dermatitis it also reacts to environmental chemicals. To date, over 3700 different substances which can cause contact allergy have been identified (11), but only about 100 chemicals frequently cause allergic contact dermatitis. These chemicals are able to react with proteins in the skin. Normally only low molecular weight

![Graph](https://example.com/graph.png)

**Fig. 5.** Frequency of skin disorders reported to the Occupational Injury Information System (ISA) in Sweden 1980-92. Source: Hedlin et al. 1994 (30).
compounds (Mw <1000) are able to induce contact allergy since larger molecules do not penetrate the skin barrier. The contact allergic reaction has two main phases: sensitization and elicitation. To cause sensitization, a compound (hapten) has to penetrate the skin and react with macromolecules in the skin to form complete antigens that are recognized as foreign by the immune system. It takes at least one week after exposure to develop contact allergy (sensitization). Specific memory T-cells produced by the immune system are able to recognize the antigen. At the following contacts with the low molecular weight compound the formed antigen is recognized by the specific memory T-cells. The immune system is activated and a cascade of reactions starts. Symptoms manifest themselves as an inflammation in the skin, normally 24–48 h after the exposure, Figure 7.

Some very potent contact allergens can sensitize at the first contact, while less potent contact allergens tend to sensitize only after years of exposure. Once an individual has become sensitized to a particular chemical, only minute amounts may be needed to elicit an allergic reaction. In general, the allergy remains throughout life. To avoid allergic contact dermatitis, one must avoid exposure to the allergenic compound. It is therefore important to identify chemical allergens and evaluate their sensitizing potency in order to make a proper risk assessment regarding their safe use.

Molecules which can function as haptens in allergic contact dermatitis either have electrophilic properties or have structures which can easily form free radicals (53). The electrophilic molecules are able to react with the nucleophilic skin proteins (-SH, -NH₂ and -OH groups in proteins) and form stable covalent bonds, which is generally accepted as the main hapten-protein interaction. A free radical mechanism has also been suggested (17), Figure 8.

Contact allergy is generally diagnosed by patch testing. At patch testing, test substances are applied on the skin of a patient’s back under occlusion, generally for 48 hours. The skin is examined for eczematous reactions generally after 2–4 days. In Sweden the tests have generally been read three days after application. In case of a high irritating capacity of a compound, the compound must be diluted to a non-irritating concentration at skin testing. The clinical appearance of acute irritant dermatitis is very variable and it may even be indistinguishable from the allergic type. Therefore, the prevalence of irritant contact dermatitis is likely to be overestimated since adequate patch testing may not have been performed or may have failed to identify relevant allergens (5).

Allergenicity of surfactants

In general, adverse reactions to soaps and synthetic surfactants are considered to be irritant effects of the surface

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**Fig. 6.** Occupational groups with an elevated incidence of reported skin disorders 1990–91. Source: Hedlin et al 1994 (30).

**Fig. 7.** Schematic representation of sensitization and elicitation in allergic contact dermatitis. During sensitization (a) the hapten is carried by Langerhans cells to the draining lymph node where the hapten-protein complex is presented to the T-cells. If the individual becomes sensitized to the hapten, there will be specific memory T-cells circulating in the body within 5–7 days after contact with the hapten. At following contacts with the hapten (b) the specific T-cells recognize the hapten-protein complex on the Langerhans cells and become activated to release inflammatory cytokines. An inflammation (c) is seen in the skin 24–48 h after contact with the hapten.

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active agents present for to their amphiphilic properties. Hand eczema due to work with water and surfactants is mainly reported to be irritative and an additional allergenic contribution has not been thoroughly studied. Although surface active agents were described in 1993 by Flyvholm (23) as potentially important contact allergens, only a few cases of contact allergy have been reported in the literature (29, 63). In some cases weak positive reactions of allergic nature were observed, although irritancy also occurred (65). The ethoxylated alkylphenolic compounds (nonoxynols) are widely used in industrial and household detergents as well as in cosmetic and pharmaceutical preparations. In a review by Dooms-Gossens and Blockeel (15) some cases of allergic contact dermatitis were reported, in majority due to antiseptic agents used in wound care (16). Contact dermatitis to nonoxynol-10 in a liquid household detergent and to nonoxynol-6 in an industrial hand cleanser has been reported (16, 45). A domestic cleaner had an acute severe dermatitis in his face, neck and forearms caused by nonoxynol-12 in a polish (70). In a case report by Meding (41) a patient became sensitized to nonoxynol-6 by contact with a fluid used to indicate cracks in metallic objects; he later suffered relapses of his dermatitis after contact with detergents when washing his car. Since the nonoxynols have been shown to have low biodegradability, these surfactants are gradually being replaced by the ethoxylated alcohols (62). The irritating properties of surfactants may facilitate sensitization to other substances in a formula, such as preservatives, perfumes, and, more rarely, coloring agents, lipids, and metal salts (15).

Research approach in allergenicity studies on a complex material

For the full investigation of materials with suspected allergenic properties a multidisciplinary approach is needed. Clinical experiences in occupational dermatology give rise to questions to be studied more carefully. In studies on the allergenic activity of a complex material, chemical analyses and syntheses are tools of crucial importance, in order to make it possible to isolate and identify an offending agent. Experimental sensitization studies in animals are so far the only tool available to determine the allergenic potency of an isolated compound. Finally, the results from the experimental work have to be evaluated in exposed groups to be able to verify the human relevance of the animal findings.

Experimental testing in animals

The allergenic activity was studied experimentally in young outbred guinea pigs with the albino phenotype in common, following a generally accepted procedure since no alternative method is presently available. About 15 different guinea pig methods for studying the sensitizing potential are described in the literature. In the updated version (1992) of OECD guideline 406 for Testing of Chemicals two guinea pig methods are given in preference to others to provide information on the sensitization potential of a chemical (2). These are the Guinea Pig Maximization Test (GPMT) (38) and the Buehler Test (6, 52). The GPMT method combines the use of intradermal administration of the chemical and topical application of the chemical under occlusion with addition of Freund’s complete adjuvant (FCA) to sensitize the animals. FCA is used to enhance the non-specific immune response to contact allergens. The GPMT method is considered to be the most sensitive procedure to reveal the sensitizing capacity of a chemical and is preferred in Europe. The Buehler test, which is the method of choice in the USA, uses only repeated short epicutaneous exposure (3 x 6 h) without adjuvant.

Three weeks after the induction of allergy the test animals and sham-treated controls are patch tested (challenged) with the compound of interest and the reactions of the two groups are compared. The skin is examined for eczematous reactions generally 48–96 h after application of the test material, and the response is statistically evaluated using Fischer’s exact test (26). The reactions are scored according to a 4-step scale ranging from 0 to + + + +: 0 = no visible reaction, + = patchy erythema, + + = confluent erythema, + + + + = erythema and oedema. Since there is no definite way to distinguish the allergic from irritant reactions, reactions graded + + should not be regarded as a positive challenge reaction (1, 37). Furthermore, pilot studies on FCA treated guinea pigs to determine the non-irritant challenge test concentration are recommended (67) together with the performance of a proper rechallenge to obtain information on the reproducibility and persistence of a reaction (25). Reproducible reactions are generally allergic in nature, while irreproducible reactions are more likely to be irritant (36).

In previous studies at our laboratory we found the Cumulative Contact Enhancement Test (CCET) (64) in a modified version (4) to be a valuable alternative to the officially recommended predictive methods, Figure 9 (see also Table 1 in paper II).
According to the original CCET method the induction procedure consists of four closed epicutaneous applications and two intradermal (i.d. in the figure) injections of 0.1 ml FCA. Open testing is performed at first challenge, and if negative, closed challenge testing is performed one week later. The controls are non-treated except for the FCA injections. In our modified version we use sham-treated controls and the chemicals are challenge tested with closed challenge for the first challenge test since we find closed application to be more reliable than open application for elicitation of eczematous reactions (4). In paper II in this thesis, the suitability of a modified CCET method was further evaluated after the initial experiences with the method in the studies of the ethoxylated surfactant, Tween<sup>®</sup> 80 (paper I), for use in the following allergenicity studies of surfactants.

The experiments performed were approved by the local ethics committee.

AIMS OF THE STUDY

The aims of this study were:

(1) to investigate if ethoxylated surfactants are oxidized after air exposure,
(2) to isolate and identify some of the compounds formed,
(3) to study the contact allergenic activity of previously unidentified oxidation- and degradation products,
(4) to investigate if the physicochemical properties of the surfactant, i.e. the solution behavior, are changed when a polyoxyethylene-based surfactant undergoes gradual oxidation.

SURFACTANTS INVESTIGATED AND THEIR AIR OXIDATION (PAPERS I-VI)

In order to study a possible allergenic activity of surfactants, it is preferable to use compounds with a low irritating potential so that it is possible to distinguish allergic reactions from irritant reactions at skin testing. The diagnosis of irritant contact dermatitis is in most cases made by exclusion of allergic contact dermatitis, since it is not possible to morphologically distinguish irritant contact dermatitis from allergic contact dermatitis (71). Skin irritating potential and cytotoxic properties of some frequently used surfactants were ranked in a recent review article (18). Non-ionic surfactants are known to cause less skin irritation than anionic and cationic surfactants and ethoxylated non-ionic surfactants were therefore the choice of surfactants to investigate since their use have increased during the recent years.

In the first study (paper I) an easily available ethoxylated surfactant of technical quality, Tween<sup>®</sup> 80 (sorbitan mono-oleate), was studied. The ethoxylated sorbitan ester has about 20 ethyleneoxy units in the structure (Figure 10). The ethoxylated sorbitan esters make excellent foamers, dispersing and wetting agents and detergents. However, in the majority of cases more cost effective non-ionic surfactants i.e. ethoxylated alcohols, are used. In the following studies (paper III, IV, V) chemically well-defined and pure homologues ethoxylated alcohols were chosen, with five (C<sub>12</sub>E<sub>5</sub>) and eigth (C<sub>12</sub>E<sub>8</sub>) ethyleneoxy groups in the structure respectively, to be able to detect and identify formed oxidation products (Figure 10). In papers III and IV the abbreviations C<sub>12</sub>E<sub>5</sub>OH and C<sub>12</sub>E<sub>8</sub>OH were used in order to assign the free hydroxyl group in the chemical structure. Since C<sub>12</sub>E<sub>5</sub> is the most commonly used abbreviation for these types of compounds, e.g. in the technical surfactant literature, this abbreviation was used in papers II, V, VI and in this thesis. C<sub>12</sub>E<sub>5</sub> and C<sub>12</sub>E<sub>5</sub>OH are thus used synonymously in the context of this thesis, and both contain the hydroxyl group. The following generic names and abbreviations are commonly used for ethoxylated esters and alcohols: ethoxylated esters, sorbitan ester ethoxylates, Tween<sub>s</sub> (Atlas trade name), and alkyl polyoxyethylene glycols, fatty alcohols ethoxylated (FAE), monoalkylpolyethylene glycol ethers, polyoxyethylene alcohols, polyoxylether fatty alcohols, polyoxyethylene straight chain alcohols, C<sub>m</sub>E<sub>n</sub>, C<sub>m</sub>EO<sub>n</sub>.

**Induction (sensitization)**

**Day** | 0, 2, 9 | 7 | 21
---|---|---|---
**Exposed** | Application of 0.2 ml test material in vehicle, epidermal occlusion for 24 h | 0.1 ml FCA i.d. x 2 | Patch testing on the flanks
**Controls** | Application of 0.2 ml vehicle, epidermal occlusion for 24 h | 0.1 ml FCA i.d. x 2 | Patch testing on the flanks

![Fig. 9. The Cumulative Contact Enhancement Test (CCET) in a modified version with sham-treated controls and closed challenge testing (64, 4).](acta-derm-venereol_79_10-fig9)

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The susceptibility of Tween®80 and the ethoxylated alcohols to oxidation on exposure to air was investigated in water solutions of the surfactants. The water solutions in open flasks were stored and handled in daylight at room temperature in a manner mimicking normal handling of a surfactant-containing product or the undiluted surfactant before use in formulations. The surfactant sample was gently stirred for 1 h, 4 times a day. To increase the rate of reaction and the amounts of formed oxidation products, water solutions of Tween®80 were also exposed to pure oxygen at elevated temperature (40°C). The oxidation of undiluted C12E5 and C12E8 was also examined after closed storage in the refrigerator, closed storage in darkness at room temperature, open storage in daylight at room temperature and open storage in daylight at room temperature in a manner mimicking normal handling. See the description given above for the water solutions. The storage in daylight was performed without direct exposure to sunlight and the top of open flasks were covered with aluminium foil to prevent contamination and diminish evaporation.

Chemical analyses of the content of peroxides, using an iodometric titration method (19), and aldehydes as their 2,4-dinitrophenyl hydrazine derivatives, using a high performance liquid chromatography method (HPLC) (58), were performed after certain time intervals. These methods were used to detect an oxidation in progress since peroxides (13, 50) and formaldehyde (7, 10) are reported to be oxidation- and degradation products in the autoxidation process of ethoxylated surfactants. More details of the conditions are given in papers I and III.

ALLERGENIC ACTIVITY OF TWEEN®80 BEFORE AND AFTER OXIDATION (PAPER I)

The aim of paper I was to study if an ethoxylated non-ionic surfactant was easily oxidized. If oxidation occurs, will an oxidized surfactant show an increased allergenic activity, in experimental sensitization studies, compared to an unoxidized surfactant (a sample that had not been actively air exposed). Another aim was to detect and identify formed oxidation products.

Low amounts of peroxides and formaldehyde were detected already in Tween®80 delivered from the producer. According to the producer, no formaldehyde was added to the Tween®80 studied here. At oxygen- and air exposure there was an increase in the contents of peroxides and formaldehyde. After prolonged air exposure the content of peroxides decreased, while the content of formaldehyde steadily increased (Figure 11). Another low molecular weight aldehyde, acetaldehyde, was detected in the newly prepared water solutions of Tween®80. The content of acetaldehyde decreased with time at exposure to air and oxygen (Figure 11). The chemical analyses revealed that a complex mixture of oxidation products was formed at oxidation of Tween®80. Due to the complex nature of the starting material, no attempts were made to further identify these compounds.

The contact allergenic activity of Tween®80 before and after air exposure was determined in experimental sensitization studies in guinea pigs, using the modified cumulative contact enhancement test (CCET). In the first experiment the sensitizing capacity of Tween®80 before air exposure was examined (Table 1).

The results in Table 1 show a significant response in the animals induced with 50% Tween®80 in saline compared to the control group when all animals were tested with Tween®80, 12.5, 50 and 100%. No significant response was observed in the group induced with 12.5% Tween®80, and neither group responded significantly to oxidized...
Tween®80 at concentrations of 12.5% and 50%. The result was confirmed at a rechallenge of the animals with 6.25, 12.5 and 50% of Tween®80 in saline 64 days after the start of the experiment. In the rechallenge a response to Tween®80 12.5% was found in both exposed and control animals (paper I).

In order to compare the sensitizing capacity of Tween®80 with oxidized Tween®80 at different concentrations a dose-response experiment was performed. A sample of Tween®80 exposed to oxygen was used since it contained the highest amount of detected oxidation products. No obvious dose-response relationship was seen using different induction concentrations according to the design of Andersen and Vølund (1). However, significant reactions were seen in some of the subpopulations according to Fischer’s exact test. At rechallenge all animals induced with Tween®80 were pooled into one group and all animals sensitized with oxidized Tween®80 to another group. In this experiment 13/19 animals induced with Tween®80 reacted when tested with Tween®80, and 12/20 animals induced with oxidized Tween®80 reacted when tested with oxidized Tween®80 (Table 2).

The animals induced with oxidized Tween®80 also reacted when challenged with Tween®80. Formaldehyde and acetaldehyde were also tested in the animals, but gave no significant response. Significantly fewer reactions ($p<0.01$) were seen to oxidized Tween®80 in the animals sensitized with non- 

### Table 1. Sensitizing capacity of Tween®80 of technical quality in guinea pigs using a modified CCET

<table>
<thead>
<tr>
<th>Guinea pigs</th>
<th>Tween®80 of technical quality</th>
<th>Ox. Tween®80</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6.25%</td>
<td>12.5%</td>
</tr>
<tr>
<td>group 1 ($n=15$)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>48 h</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>72 h</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>group 2 ($n=15$)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>48 h</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>72 h</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>controls ($n=15$)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>48 h</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>72 h</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

1The figures are the number of animals with confluent erythema 48 and 72 h after application of the test material.
2Oxidized Tween®80, 12.5% w/w in water. Exposed to pure oxygen for 24 days.
3Induction: Tween®80, 12.5% w/w in water (taken from producers vessel and diluted).
4Induction: Tween®80, 50% w/w in water (taken from producers vessel and diluted).
5Significantly different from the controls, $p=0.021$.
6Significantly different from the controls, $p=0.049$.
7Significantly different from the controls, $p=0.037$.

### Table 2. Allergenic activity of Tween®80 and oxidized Tween®80 using a modified CCET. Results from the rechallenge 78 days after start of the experiment

<table>
<thead>
<tr>
<th>Guinea pigs</th>
<th>Tween®80</th>
<th>Ox. Tween®80</th>
<th>Formaldehyde</th>
<th>Acetaldehyde</th>
<th>Carbopol® gel</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>12.5%</td>
<td>12.5%</td>
<td>0.3%</td>
<td>0.1%</td>
<td>1.5%</td>
</tr>
<tr>
<td>group 1 ($n=19$)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>48 h</td>
<td>7</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>72 h</td>
<td>13</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>group 2 ($n=20$)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>48 h</td>
<td>6</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>72 h</td>
<td>11</td>
<td>12</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>controls ($n=5$)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>48 h</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>72 h</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

1The figures are the number of animals with confluent erythema 48 and 72 h after application of the test material.
2Oxidized Tween®80, 12.5% w/w in water. Exposed to pure oxygen for 24 days.
3Pooled group induced with Tween®80 of technical quality (12.5, 25, 50 and 80% w/w in Carbopol® gel).
4Pooled group induced with oxidized Tween®80, exposed to oxygen for 24 days (12.5, 25, 50 and 80% w/w in Carbopol® gel).
5The same controls were used as in the first challenge.
6Significantly different from the controls, $p=0.011$.
7Significantly different from the controls, $p=0.038$.

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SENSITIZING POTENTIAL OF ACETALDEHYDE AND VERIFICATION OF THE ANIMAL ASSAY FOR USE IN THE INVESTIGATION OF SURFACTANTS (PAPER II)

The aim of paper II was to investigate the contact allergenic activity of acetaldehyde since this aldehyde was detected in Tween® 80 previously studied and may be present as an impurity also in other ethoxylated surfactants. Formaldehyde, was tested in an initial experiment with the modified CCET method to verify the suitability of this animal model, since formaldehyde was used as one of the substances to validate the method in the original description (64). The original method was found to be rather insensitive regarding formaldehyde compared to the GPMT method (38), since a high concentration was required to get a response. The use of an experimental sensitization method in the allergenicity studies which uses only epidermal administration resembles common exposure to a chemical. This may enable a proper risk assessment to be made regarding exposure in man so as to avoid the induction of sensitization. Another aim was to study a possible cross-reactivity between formaldehyde and acetaldehyde.

More details about the conditions is given in paper II.

A clear dose-response relationship was obtained for formaldehyde and acetaldehyde in the sensitizing experiment using the modified CCET method (Table 3). The contact allergenic effect of formaldehyde is well known. It is one of the ten most common causes of patch test reactions in the standard series used for diagnosis of allergic contact dermatitis (39). Acetaldehyde was for the first time shown to be a potent contact allergen using the modified version of the CCET method. Acetaldehyde is a very rare sensitizer in man, which may be explained by the limited exposure in the population. Only one case report on allergic contact dermatitis to acetaldehyde was found in the literature (60). Contact urticaria caused by acetaldehyde has been described in case reports (51, 69). No cross-reactivity was observed between formaldehyde and acetaldehyde at cross-challenge with the two aldehydes. The sensitizing capacity of acetaldehyde was lower than that of formaldehyde, since higher concentrations of acetaldehyde were needed for induction and elicitation compared to formaldehyde (Table 3). The difference in response observed between acetaldehyde and formaldehyde could be due to differences in penetration, metabolism and chemical reactivity. The content of acetaldehyde and formaldehyde in the tested products was too low to cause reactions as can be seen from the results obtained for the pure compounds tested in corresponding concentrations (Table 3). As the CCET protocol involves epicutaneous induction and challenge, the modified version was regarded to be well suited for a further evaluation of the allergenicity of potentially allergenic compounds in ethoxylated surfactants expected to come in contact with human skin.

ALLERGENIC ACTIVITY AND AIR OXIDATION OF CHEMICALLY WELL-DEFINED, HOMOLOGOUS ETHOXYLATED ALCOHOLS (PAPER III)

The aims of paper III were to investigate the sensitizing potential of chemically well-defined homologues ethoxylated alcohols as models for technical products and to investigate the susceptibility of the homologous ethoxylated alcohols to oxidation on exposure to air. The sensitizing potential of C₁₂E₅, diluted in water, was investigated using a modified CCET protocol without adjuvant treatment of the animals (Table 4). The oxidation of C₁₂E₅ and C₁₂E₈ at different

Table 3. Allergenic activity of formaldehyde and acetaldehyde in guinea pigs using a modified CCET

<table>
<thead>
<tr>
<th>Guinea pigs</th>
<th>Formaldehyde</th>
<th>Acetaldehyde</th>
<th>T 80³</th>
<th>Ox. T 80²</th>
<th>saline</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.3%</td>
<td>0.1%</td>
<td>10%</td>
<td>5%</td>
<td>2.5%</td>
</tr>
<tr>
<td>group 1⁵(n=15)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>48 h</td>
<td>6 ³</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>72 h</td>
<td>11⁶</td>
<td>8⁷</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>group 2⁵(n=15)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>48 h</td>
<td>0</td>
<td>1</td>
<td>13⁸</td>
<td>7⁹</td>
<td>4⁵</td>
</tr>
<tr>
<td>72 h</td>
<td>0</td>
<td>0</td>
<td>13⁸</td>
<td>9⁹</td>
<td>5⁹</td>
</tr>
<tr>
<td>controls (n=15)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>48 h</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>72 h</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

¹The figures are the number of animals with confluent erythema 48 and 72 h after application of the test material.
²Oxidized Tween® 80, 12.5% w/w in water containing 0.01% (100 ppm) formaldehyde. Exposed to pure oxygen for 24 days.
³Induction: 5% w/w (1.7 mmol/g) formaldehyde in saline.
⁴Significantly different from the controls, p<0.05.
⁵Significantly different from the controls, p<0.001.
⁶Significantly different from the controls, p<0.01.
⁷Induction: 15% w/w (3.3 mmol/g) of acetaldehyde in saline.
storage and handling conditions was studied as described previously (Table 5).

Some irritation was found but no significant allergic activity of the homologous ethoxylated alcohol was observed in the allergenicity experiment.

The increase in content of peroxides and formaldehyde was obvious at storage and handling of the ethoxylated fatty alcohols at room temperature. The autoxidation was observed not only after storage in daylight but also after storage in darkness (Table 5). Only a minor increase in the content of the detected oxidation products was observed when the products had been stored in the refrigerator.

Surfactants are normally stored at room temperature, since they most often become semisolid when stored at low temperatures. Because we used chemically well-defined homologues ethoxylated alcohols as model compounds, the autoxidation of the ethoxylated surfactants, i.e. the observed formation of peroxides and formaldehyde, can be attributed mainly to oxidation of the ethylenoxy chain and not to impurities and other easily oxidized compounds that may be present in surfactants of technical quality with a complex chemical composition. The autoxidation seems to vary between the batches and also between samples of the same batch (Table 5). The result indicates that if allergenic oxidation products can be formed at exposure to air of ethoxylated alcohols this may be a potential health risk that has not been considered before.

SYNTHESIS OF REFERENCE COMPOUNDS AND ANALYTICAL PROCEDURES USED FOR STRUCTURAL ELUCIDATION (PAPERS IV AND V)

The detected oxidation- and degradation products, peroxides and formaldehyde, may contribute to the sensitizing capacity of oxidized samples of surfactants. Various other carbonyl compounds, detected in the complex oxidation mixture as their hydrazone derivatives in the HPLC analysis (58) and with Fourier transform infrared spectroscopy (FT-IR) analysis, could also contribute to an allergenic activity.

To be able to isolate and identify oxidation products from a

Table 4. Allergenic activity of C12E5 assessed using a modified CCET without adjuvant treatment

<table>
<thead>
<tr>
<th></th>
<th>no. of animals with positive reaction after exposure³</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C12E5 (% w/w in water)</td>
</tr>
<tr>
<td>Guinea pigs</td>
<td></td>
</tr>
<tr>
<td>Exposed²</td>
<td></td>
</tr>
<tr>
<td>48 h</td>
<td>0 1 0 0</td>
</tr>
<tr>
<td>72 h</td>
<td>0 2 0 0</td>
</tr>
<tr>
<td>96 h</td>
<td>1 0 0 1</td>
</tr>
<tr>
<td>Controls³</td>
<td></td>
</tr>
<tr>
<td>48 h</td>
<td>0 2 0 1</td>
</tr>
<tr>
<td>72 h</td>
<td>0 1 0 2</td>
</tr>
<tr>
<td>96 h</td>
<td>1 0 0 1</td>
</tr>
</tbody>
</table>

¹The figures are the number of animals with confluent erythema 48, 72 and 96 h after application of the test material.
²Induction: 10% w/w of C12E5 (0.25 mmol/g) in water, no adjuvant stimulation.
³Peroxide test strips (Merck).

Table 5. Formation of peroxides and formaldehyde when C12E5 and C12E8 were stored under various conditions

<table>
<thead>
<tr>
<th></th>
<th>storage condition</th>
<th>peroxide content (PN)¹</th>
<th>formaldehyde content (µg/g)²</th>
</tr>
</thead>
<tbody>
<tr>
<td>C12E5, Batch A</td>
<td>freshly opened ampoule</td>
<td>neg.³</td>
<td>0.4</td>
</tr>
<tr>
<td>Sample 1 (100 %)</td>
<td>dark, 8°C, 16 months</td>
<td>24</td>
<td>3.7</td>
</tr>
<tr>
<td>Sample 2 (100%)</td>
<td>daylight, 20–22°C, 4 months</td>
<td>193</td>
<td>30</td>
</tr>
<tr>
<td>Sample 3 (100%)</td>
<td>daylight, handled, 4 months</td>
<td>269</td>
<td>138</td>
</tr>
<tr>
<td>Sample 4 (10% aq)</td>
<td>daylight, handled, 10 months</td>
<td>218⁵</td>
<td>106⁶</td>
</tr>
<tr>
<td>C12E5, Batch B</td>
<td>freshly opened ampoule</td>
<td>neg.³</td>
<td>5</td>
</tr>
<tr>
<td>Sample 1 (100%)</td>
<td>dark, 8°C, 18 months</td>
<td>22</td>
<td>9</td>
</tr>
<tr>
<td>Sample 2 (100%)</td>
<td>dark 20–22°C, 10 months</td>
<td>107</td>
<td>1289</td>
</tr>
<tr>
<td>Sample 3 (100%)</td>
<td>daylight, handled, 4 months</td>
<td>pos.³</td>
<td>655⁶</td>
</tr>
<tr>
<td>Sample 4 (10% aq)</td>
<td>daylight, handled, 10 months</td>
<td>1087</td>
<td>2950⁶</td>
</tr>
<tr>
<td>C12E5, Batch C</td>
<td>freshly opened ampoule</td>
<td>11</td>
<td>6</td>
</tr>
<tr>
<td>Sample 1 (100%)</td>
<td>dark 8°C, 3 months</td>
<td>12</td>
<td>8.7</td>
</tr>
<tr>
<td>C12E5, Batch D</td>
<td>freshly opened ampoule</td>
<td>neg.³</td>
<td>n. a.⁴</td>
</tr>
<tr>
<td>Sample 1 (100%)</td>
<td>dark 20–22°C, 16 months</td>
<td>pos.³</td>
<td>1178</td>
</tr>
<tr>
<td>C12E8</td>
<td>freshly opened ampoule</td>
<td>neg.³</td>
<td>2</td>
</tr>
<tr>
<td>Sample 1 (100%)</td>
<td>dark, 8°C, 24 months</td>
<td>3.6</td>
<td>12</td>
</tr>
<tr>
<td>Sample 2 (10% aq)</td>
<td>daylight handled, 10 months</td>
<td>345⁵</td>
<td>276⁶</td>
</tr>
</tbody>
</table>

¹The peroxide number (PN) obtain after iodometric titration (19).
²The formaldehyde content obtained after HPLC analysis (58).
³Peroxide test strips (Merck).
⁴n.a. = not analyzed.
⁵See also Figure 2 in paper III.
⁶See alsoFigures 1 and 3 in paper III.

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complex material, a non-destructive chromatographic method is preferable since fractions corresponding to the chromatographic peaks can be collected as samples for further structure elucidation and for use in allergenicity studies. Therefore, HPLC and thin layer chromatography (TLC) was used as a preparative clean-up step prior to FT-IR and gas chromatography mass spectrometry (GC-MS) analyses (papers I, III and IV). Gas chromatography (GC) was used for quantification since aliphatic ethoxylated alcohols have no chromophores in their structure and therefore cannot be detected by use of a LC-UV detection system without a derivatization prior to analysis.

To facilitate the identification work of other compounds in the oxidation mixture, theoretical proposed oxidation products, ethoxylated aldehydes 5–9 and formates 10–14, were synthesized and used as reference compounds in the chromatographic separation and spectroscopic identification of isolated degradation products of the surfactants.

The ethoxylated alcohols used in the synthesis of the aldehydes and formates (below) were all obtained by monoalkylation of the corresponding ethyleneoxyglycol using 1-bromodecane (C_{12}H_{25}Br) and dimethyl sulphoxide (DMSO). The products 1–4 were purified by chromatography and obtained in about 60% yields (Scheme 1).

The aldehydes, 5–9 (paper IV) were obtained from the appropriate alcohol which was oxidized by DMSO in the presence of dicyclohexylcarbodiimide (DCC). The products were isolated and purified by chromatography and were obtained as oils in 23–40% yields (Scheme 2).

An alternative method was also used for the synthesis of aldehyde 9. The alcohol, C_{12}H_{25}(OCH_{2}CH_{2})_{4}OH was allowed to react with bromoacetaldehydediethylacetal to form the diethylacetal of aldehyde 9. This product was then hydrolyzed using trifluoroacetic acid in dichloromethane with a minute amount of water present. The overall yield was 24% (Scheme 3).

For the preparation of the formates 10–14 (paper V) the appropriate ethoxylated alcohol was esterified in formic acid using p-toluenesulphonic acid as catalyst. The products were obtained in 80–100% yields (Scheme 4).

Structural elucidation of the synthetic compounds was performed with FT-IR, with ^1H and ^13C nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry with electron impact ionization (MS-EI) and chemical ionization (MS-CI), in both positive ion (PICl) and negative ion (NICl) mode.

The FT-IR spectra were recorded with a Perkin-Elmer 16 PC FT-IR instrument using a sealed liquid cell with potassium bromide (KBr) windows. NMR spectroscopy was performed on a Jeol EX 270 instrument equipped with a Varian 3400 gas chromatograph with a Varian SPI on-column injector and a direct insertion probe. In chemical ionization mode, methane of >99.995% purity was utilized as reagent gas and the instrument was tuned by optimizing the reactant ions, CH_{5}^{+}, C_{2}H_{5}^{+} and C_{3}H_{5}^{+}, to an approximate ratio of 5:4:1. The MS analyses were performed in full scan and masses in the range from m/z 42 – 600 were scanned. More details about the conditions are given in papers IV and V.

Quantitative analyses were performed using GC with a flame ionization detector (FID). Methyl stearate was added as internal standard and quantification was performed using the synthesized reference compounds as external standards. Sample introduction was performed using both a split/splitless injector (paper IV) and an on-column injector (paper V). More details about the conditions are given in papers IV and V.
IDENTIFICATION AND ALLERGENIC ACTIVITY OF ETHOXYLATED ALDEHYDES IN THE MIXTURE AFTER OXIDATION OF AN HOMOLOGOUS ETHOXYLATED ALCOHOL, C12E5 (PAPER IV)

By use of the synthetic reference compounds 5–9 (scheme 2) the ethoxylated aldehydes were detected in the oxidation mixture of air-exposed C12E5 (Figure 12). Small amounts of the identified aldehydes 7–9 were detected in a sample from a freshly opened ampoule.

To obtain structural information, the major aldehyde, denoted 9 in the chromatogram, was isolated from C12E5 with preparative TLC and analyzed with FT-IR (paper IV). The isolated compound had an FT-IR spectrum identical to that of the synthetic reference compound 9. The ethoxylated aldehydes in oxidized C12E5 were later detected with GC-MS in NICI mode using on-column injection technique (unpublished result) since the responses were very low in the PICI mode. The NICI analyses were applied to obtain a better sensitivity. Intense [M-1] ions and less fragmentation were obtained in the NICI spectra of the homologous ethoxylated aldehydes in the mixtures after oxidation of C12E5. A MS-NICI spectrum of 9 is shown in Figure 13. After 7 months of air exposure the oxidation mixture contained 1.2% of the aldehyde corresponding to compound 9. The total amount of the identified aldehydes after 7 months of air exposure was about 2% in a sample of C12E5 stored in daylight at room-temperature and 3% in a sample of C12E5 handled in daylight at room temperature in a way mimicking normal use. The GC quantification was performed with standard addition of known aliquots of reference compounds 5–9 to the oxidation mixtures of C12E5. Linear relationships were obtained in the concentration range up to 10 µg/ml with regression correlation coefficients better than 0.99 for each aldehyde.

In the sensitization experiments performed the major aldehyde 9, dodecyltetraoxyethyleneoxyacetaldehyde, showed a significant allergenic activity in using a modified CCET test protocol with and without adjuvant treatment in two exposed groups with corresponding controls (Table 6).

A significant response was observed to 1% in both groups, challenge-tested with 0.02–1% aldehyde in water, and there was no difference in reactivity due to adjuvant treatment between the two exposed groups. The result was confirmed in a rechallenge two weeks later with higher concentrations, 1, 5 and 10%, of the aldehyde in water (paper IV, Table 2). A significant reactivity was observed to 5% and a dose-response relationship was seen in both groups. Positive reactions were seen to the higher concentrations in some of the controls, probably due to irritation.

The results were again confirmed at a second rechallenge using a new control group in a cross-reactivity study with the synthesized homologues, 6–9. No response was observed at challenge with formaldehyde. Positive reactions were observed to the homologues, but a significant reactivity (p<0.01) was...
found only to 9 at 72 h (Figure 14). A significant reactivity to 8 ($p < 0.05$) was seen after 96 h (paper IV).

The decreasing rate of response at challenge with 9–6 in animals sensitized to 9 at challenge indicates a cross-reactivity pattern between the homologues and was a further verification of an allergenic effect of the identified ethoxylated aldehydes.

A liquid chromatography mass spectrometry (LC-MS) analysis of 9 in water:methanol (1:1) on a Autospec MS instrument from Micromass using electrospray ionization (ESI) technique was performed since 9 was dissolved in water in the allergenicity study. Acetic acid (1%) was added to promote the ionization and detection was made with a time-of-flight mass analyzer. The analysis showed that the molecular ion of the aldehyde, $m/z = 405$ [M+1]$^+$, and its corresponding geminal diol after addition of water, $m/z = 422$ [M+18]$^+$, were in equilibrium in the test solution of 9.

**IDENTIFICATION AND ALLERGENIC ACTIVITY OF ETHOXYLATED FORMATES IN THE MIXTURE AFTER OXIDATION OF AN HOMOLOGOUS ETHOXYLATED ALCOHOL, C$_{12}$E$_5$ (PAPER V)**

Ethoxylated esters were identified in the mixture after oxidation of the homologous ethoxylated alcohol, C$_{12}$E$_5$, with GC-MS-PICI by use of the synthetic, theoretically proposed reference compounds 10–14, scheme 4 (paper V). Figure 15 shows the formation of ethoxylated formates with time in a sample stored in darkness at room-temperature and in a sample stored and handled in daylight according to GC-analyses every fourth week. The total content of formates in C$_{12}$E$_5$ was 3.3% in the sample stored in a closed vessel in darkness and 4.0% in the sample handled in daylight after 12 months. A very low allergenic activity was observed in the sensitization studies performed using 10% of compound 14 in water for sensitization (paper V, Table 1).

The ethoxylated alcohols, C$_{12}$H$_{25}$[OCH$_2$CH$_2$]$_n$OH (where $n = 1–4$), were also identified in the air oxidized C$_{12}$E$_5$ with GC-MS-PICI using commercial and synthesized ethoxylated alcohols 1–4 as reference compounds (scheme 1). The GC chromatogram in Figure 16 illustrates the complex composition C$_{12}$E$_5$ after air exposure for 12 months after introduction with on-column injection technique.

Most of the formed degradation products seen in the GC-analysis still remain to be identified. However, highly polar compounds formed in the autoxidation process, i.e. formed acids and other polar components, could not be analyzed and detected by the used method.

**PHYSICOCHEMICAL PROPERTIES OF AN ETHOXYLATED ALDEHYDE AND HOMOLOGOUS ETHOXYLATED ALCOHOLS AFTER OXIDATION (PAPER VI)**

To increase the understanding of the relation between the biological activity and the physicochemical properties, the cloud point, phase behavior and aggregation characteristics of dodecyltetraoxymethyleneoxycetaldhyde, 9, were studied in co-operation with the Institute for Surface Chemistry, Stockholm, Sweden and compared with the corresponding data on the pure ethoxylated alcohol, C$_{12}$E$_5$ (paper VI). The physicochemical behavior of an homologous ethoxylated alcohol, hexaethylene mono n-dodecyl ether (C$_{12}$E$_6$) was also studied. The phase behavior was studied in the same concentration range as those used in the sensitization experiments with 9 in order to possibly relate the phase behavior to the observed biological effect. Since the aldehyde...
will be present together with the parent surfactant, the micellization behavior of mixtures of 9 and C₁₂E₅ was investigated. Pulsed-gradient-spin-echo NMR was used in order to measure the size and shape of the micelles of 9 in solution. Figure 17 shows the cloud point of 9 for samples kept at 8, 22 and 40 °C. The cloud point of 9 was almost as high as that of the homologous ethoxylated alcohol, C₁₂E₅, indicating that 9 and C₁₂E₅ have similar properties, i.e. a similar hydrophilic-lipophilic balance, and that a considerable portion of the aldehyde is present as its geminal diol which contribute to the hydrophilicity of the molecule. The presence of the diol was also detected in the mass spectrometric electrospray ionization (MS-ESI) analysis of 9 in water (paper IV). The cloud point of 9 decreased rapidly with time even when it was stored at low temperature.

In figure 18 is shown the variation of cloud point with time for the ethoxylated alcohol, C₁₂E₅, and its homologues, C₁₂E₆. Above 30 °C there is a separation into two phases, a heterogeneous two-phase system is formed. The appearance of a single-phase region, a sponge phase, denoted L₃, at higher temperature in the phase diagram was probably due to the aldehyde being more hydrophilic at elevated temperatures, corresponding to an increased percentage of the geminal diol.

Approximately the same CMC value was achieved for individual components and the different combinations of 9 and C₁₂E₅ (paper VI, Figure 4). This, together with the observed phase behavior, indicated a similar hydrophilic-lipophilic balance of 9 compared to the homologous ethoxylated alcohol, C₁₂E₅. In general the phase behavior of the ethoxylated aldehyde was similar to that of C₁₂E₅, but the decrease of cloud point with an increasing concentration was not a normal behavior for polyoxyethylene surfactants (Figure 19).

The shape and size of the micelles of 9 could not be determined using pulsed-gradient-spin-echo NMR since there was a remarkable difference in cloud point when the aldehyde was dissolved in D₂O compared water (paper VI, Figure 5). Normal ethoxylates show practically the same cloud points in the two media.

GENERAL DISCUSSION

This study shows that oxidation products were rapidly formed from the ethoxylated non-ionic surfactants examined. Formaldehyde was detected and identified among the formed oxidation products and the major compound among the identified ethoxylated aldehydes was shown to have a contact allergenic potential in the experimental studies on laboratory animals. The ethoxylated non-ionic surfactants are commonly used in detergents and in a variety of products. Use of these surfactants has increased during the last years due to their biodegradability and low skin irritating potential. In 1993 the total consumption of ethoxylated alcohols was estimated to about 313 000 tons in Western Europe (44). According to data from the Swedish National Chemicals Inspectorate, ethoxylated alcohols were the surfactants most commonly used as raw material for products used in the consumer sector in 1995. This increase of the use of ethoxylated alcohols was also shown in their report (31) of the needs for measures regarding the use of detergents for laundry, dishwashing and cleaning for consumer and professional use in 1994 commissioned by the Swedish government. The autoxidation of ethoxylated surfactants is theoretically discussed in the technical literature (14, 48) but a possible allergenic activity of formed degradation products has not been previously considered. The mechanism of formation is probably a free radical chain reaction initiated by the presence of hydroxyl radicals or other radical species. The subsequent chain reaction probably occurs through a hydrogen abstraction at one of the ethoxylated units or at the carbon specific α to the oxygen of the aliphatic chain (12) (Figure 4). This oxidation may also cloud point drop of the ethoxylated alcohols after an initial induction period indicating a gradual oxidation process.

A partial phase diagram, constructed from measurements of cloud points in the concentration range 1–10% of 9 and C₁₂E₅, respectively, in water, is shown in Figure 19 (see also the complete phase diagram of C₁₂E₅, 1–100% in Figure 3).

Both C₁₂E₅ and 9 form a single-phase micelle solution, denoted L₁. Above 30°C there is a separation into two phases, a heterogeneous two-phase system is formed. The appearance of a single-phase region, a sponge phase, denoted L₃, at higher temperature in the phase diagram was probably due to the aldehyde being more hydrophilic at elevated temperatures, corresponding to an increased percentage of the geminal diol.

Fig. 15. The content (%) of the identified ethoxylated formates (a) in a sample of C₁₂E₅ stored in a closed vessel in darkness and (b) in a sample stored and handled in daylight at room temperature for 12 months.

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affect the technical properties of the surfactants, since changes were observed in the physicochemical studies performed (Figure 17, paper VI).

To study the allergenicity of a complex material is difficult, since the raw material mixture can contain both irritants and allergens. The commercial products containing surface active agents are mixtures with similar but not identical properties. Surfactants have a complex chemical composition and all surfactants, without exception, will contain by-products which are mostly unknown (48). The major source of information on surfactants is the manufacturers. They have practical information on the composition on the raw material used and on the chemistry involved. The detailed knowledge on the chemical composition of surfactants is becoming more important in a discussion of occupational health and consumer safety.

An allergenic activity of Tween® 80 of technical quality was found before and after oxidation of water solutions of the surfactant (paper I). The results indicated that allergens were present in Tween® 80 before oxidation and that new allergens

![Fig. 16. GC chromatogram of identified oxidation/degradation products: 1–4, the ethoxylated alcohols, 5–9, the ethoxylated aldehydes and 10–14, the ethoxylated formates, in air exposed C_{12}E_5. Methyl stearate (*) was used as internal standard. The peaks are denoted with the same number as given to the corresponding synthetic reference compounds.](image1)

![Fig. 17. Cloud point versus time for storage of 1% aqueous solutions of 9 at three different temperatures.](image2)

![Fig. 18. Cloud point versus time for storage of 1% aqueous solutions of the homologues ethoxylated alcohols, C_{12}E_6 and C_{12}E_5 at storage at three different temperatures.](image3)
were formed in the oxidation process. Formaldehyde was detected in concentrations that may elicit a reaction in allergic individuals (22, 32). Positive patch test reactions have been described in concentrations that may elicit a reaction in allergic sensitizing experiments. A comparison can also be made to the complete phase diagram of C12E5 in Figure 3, in which the single-phase region mic corresponds to L1 here, and the one phase-region SPO corresponds to L3. In between the two single-phase regions, L1 and L3, there is separation into two phases.

The autoxidation rate in Tween®80 and the ethoxylated alcohols, here measured as peroxide- and formaldehyde formation, varied between different batches, and also between different samples (Table 5). The content of formaldehyde exceeded in some samples the limit for risk labelling of cosmetics, which is 500 ppm (μg/g), within the European Union. Technical products containing more than 2000 ppm (0.2%) of formaldehyde are classified as allergenic (8). Since these surfactants have wide applications, exposure to formaldehyde could be more frequent than is generally considered, contributing to the persistence of the allergic dermatitis in individuals allergic to formaldehyde. Elicitation due to very low concentrations, 30–250 ppm in formaldehyde sensitized individuals has been reported (22, 32). This may occur following repetitive damage to the skin, enabling low concentrations of allergens to penetrate the skin barrier. Contact eczema caused by formaldehyde in a liquid soap has been described (72). Formaldehyde donors, commonly used as preservatives in cosmetics and skin care products are other sources of formaldehyde exposure (21). According to our investigation, formaldehyde formed by autoxidation from surfactants present in the formula may contribute to a higher content of formaldehyde in such products than expected. Only one previous paper was found which discusses the risk of contact dermatitis in formaldehyde allergic individuals due to the content of formaldehyde in autoxidized ethoxylated surfactants (10). Similar results, i.e. a higher content of formaldehyde in cosmetics and other consumer products than expected, were reported in a Danish study (49). Formaldehyde has also been described to be an important allergen in women with hand eczema caused by occupational and domestic exposure (9). Since no reactions were seen in the animals sensitized to oxidized Tween®80 when patch tested with a pure formaldehyde solution using the same concentration, the amount of formaldehyde found in oxidized Tween®80 (paper I) could, however, not be considered to cause the sensitization. According to the allergenicity studies of formaldehyde (paper II) higher concentrations of formaldehyde are needed to induce a formaldehyde sensitivity and elicitation of eczematous reactions in the guinea pigs.

The majority of methods reported for analysis of the complex composition of surfactants in raw materials furnish only quantitative results, and no structure elucidation is obtained. As the chemical composition of surfactants strongly influences the physicochemical and end-use properties of the raw material, adequate analytical methods are needed for rapid and reproducible determinations of differences in the distribution of homologous compounds. The release of surfactants in the environment has necessitated the development of sensitive analytical methods for their determination at trace levels and their biodegradation products. Gas chromatography-mass spectrometry (GC-MS) methods with electron impact (EI) (66) or chemical ionization (CI) (61) using methane or ammonia as reactant gases have been shown to be the most accurate methods for structure identification of ethoxylated non-ionic surfactants. However, GC analysis is limited to materials containing relatively few ethylene oxide units because of the lack of volatility of most high molecular weight non-ionic surfactants. High-temperature GC can be extended to the analysis of higher molecular mass weight components but normally the formation of volatile derivatives prior to GC analysis is required. Another approach to determine the oligomer distribution in a mixture of high molecular weight compounds was the distillation of the mixture directly into the ion source of the mass spectrometer using a direct inlet probe (DIP) (55). The separation of the components during the distillation was relatively poor, but by applying chemical ionization with an appropriate choice of reagent ions, abundant ions corresponding to the molecular weights could be determined, and thus the distribution in the complex mixtures.

The structural elucidation of the products formed due to autoxidation of the fatty ethoxylated alcohol, C12E5, could be performed with GC-MS analysis using EI and CI with methane as reagent gas and the synthesized reference compounds 1–14. Aliphatic ethers normally exhibit weak molecular ion peaks in EI (40). The EI mass spectra were dominated by unspecified ions of the general formula (CH2CH2O)nH+ and molecular ions corresponding to the molecular weight were not detected. The characteristic fragments of aliphatic aldehydes, [M-1]+, [M-29]+ and [M-43]+, corresponding to α-cleavage, β-cleavage and McLafferty rearrangement, were observed for the ethoxylated aldehydes together with the [M+1] ion in the PICI analyses. Regarding the structural elucidation of the ethoxylated aldehydes present in the oxidation mixtures of C12E5, they were initially identified with FT-IR after isolation with TLC.

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Fig. 19. Partial phase diagram showing the solubility regions for 9 and C12E5 in the same concentrations (1–10%) as used in the sensitizing experiments. A comparison can also be made to the complete phase diagram of C12E5 in Figure 3, in which the single-phase region mic corresponds to L1 here, and the one phase-region SPO corresponds to L3. In between the two single-phase regions, L1 and L3, there is separation into two phases.
and by addition of the synthesized reference compounds 5–9, to the oxidation mixture of \( \text{C}_{12}\text{E}_5 \) prior to GC analysis (paper IV). Structural information was later obtained by GC-MS-NICI. As for many other electrophilic compounds, a higher sensitivity was observed when monitoring negative ions. Furthermore, NICI led to less fragmentation compared to PICI. Intense \([M-I]^-\) ions were obtained as demonstrated in Figure 13 for the major ethoxylated aldehyde formed in the oxidation mixture. Ions corresponding to \( \text{McLafferty} \) rearrangement were also detected in the NICI analyses.

The autooxidation seems to proceed with parallel and different mechanisms, since the major formate 10 was apparently formed due to cleavage between the polyoxyethylene groups. The alkyl chain and the ethoxylated aldehydes were probably formed by oxidation of the terminal hydroxyl group, yielding the dominating ethoxylated aldehyde 9, and also by cleavage of the polyoxyethylene chain resulting in loss of oxyethylene units. The amount of peroxides was determined with the unspecific iodometric titration method (19), since no method to isolate and structurally elucidate the peroxo compounds formed was found. Peroxy compounds are most often thermally labile and the peroxy compounds in the oxidation mixtures were probably destroyed in the GC analyses. The ethoxylated alcohols that were identified in the degradation mixture with GC-MS may also be formed by thermal decomposition of the corresponding peroxides or hydroperoxides during the analysis, since alkyl hydroperoxides are reported to decompose to alcohols at temperatures above 90°C (24). The boiling point of \( \text{C}_{12}\text{E}_5 \) is in the range of 202–216°C. Therefore, the commonly used GC-technique involves injection via a split/splitless injector at a temperature of 280°C. Despite the use of on-column injection technique in paper V to avoid degradation of thermally unstable compounds at injection, the hydroperoxides were not detected with GC-MS, even when negative ions chemical ionization was applied. NICI was previously found to be suitable in the structure elucidation of the \( d \)-limonene hydroperoxides since relative intense \([M-I]^-\) ions were formed (46). Furthermore, in the PICI analyses most of the limonene hydroperoxides showed very low molecular ion intensities since water and hydrogen peroxide was eliminated from the \([M+1]^+\) ion. The presence of the alcohol homologues to \( \text{C}_{12}\text{E}_5 \) in the oxidation mixture were also indicated from the cloud point measurements (paper VI). Their formation could be due to loss of oxyethylene units in the autooxidation process or to decomposition of formed hydroperoxy compounds.

The content of the formed oxidation products, i.e. ethoxylated aldehydes and formates, in undiluted \( \text{C}_{12}\text{E}_5 \) reached a few percent after 6 months of air oxidation. A complex oxidation mixture was formed as seen in the chromatogram from GC-analysis (Figure 15). Some of these unidentified compounds may also contribute to an allergenic activity. About 70% of homologous \( \text{C}_{12}\text{E}_5 \) remained after 6 months. Furthermore, only a rough quantification of the content of ethoxylated aldehydes in the mixtures could be performed by GC analysis, since changes of the responses were observed with time, leading to a decreased reproducibility. These effects were probably due to thermal decomposition during analysis. To obtain an accurate quantification of the content of the aldehydes, standard addition was applied. The standard addition method compensates for matrix effects in complex material since known amounts of the standard solutions are added to sample aliquots of the same size prior to analysis. These problems caused by decomposition demonstrate the need of a suitable analytical method for complex degradation mixtures of ethoxylated surfactants. The application of a non-destructive LC-MS method may be an appropriate alternative for quantification of the ethoxylated aldehydes present in complex mixtures since a high sensitivity of the protonated molecular ion of ethoxylated aldehyde 9 was obtained in the MS-ESI analysis performed (paper IV). MS is also suitable for detection since no UV-absorbing functional groups are required. The use of refractive index (RI) detection is unsuitable for small amounts of compounds due to the low sensitivity obtained by this method. Furthermore, gradient elution often leads to problems due to extensive changes in the refractive index of the mobile phase during LC separation.

The use of the homologous and chemically well-defined ethoxylated alcohols, as model compounds for the technical products, made it possible to perform allergenicity studies using a pure material and to isolate and structurally characterize formed oxidation- and degradation products. No significant allergenic activity was found to the pure ethoxylated alcohol, \( \text{C}_{12}\text{E}_5 \), using the modified CCET without adjuvant addition (Table 5). The identified oxidation products were synthesized in amounts needed for use in the allergenicity studies. The Cumulative Contact Enhancement Test (CCET) involves solely epidermal applications of the test substance for induction and was chosen since it more accurately reflects common exposure to surfactants. The fact that we were able to synthesize the test compounds made it possible to use the CCET method, since this method requires a larger amount of test substance for the epidermal sensitization.

In the allergenicity studies, the ethoxylated aldehyde 9 was shown to have an allergenic effect of the same magnitude as that of formaldehyde using the modified CCET test protocol with and without adjuvant treatment (paper IV). The induction concentration of 9 was 0.25 mmol/g and challenge concentrations were 0.0025, 0.025, 0.12, and 0.25 mmol/g. It should be noted that a lower induction concentration of 9 compared to that of formaldehyde (1.7 mmol/g) gave an elicitation of the same magnitude, when using approximately equimolar challenge concentrations of formaldehyde, 0.033 and 0.1 mmol/g (Tables 3 and 6). No allergenic activity was observed in response to the homologous \( \text{C}_{12}\text{E}_5 \) and the ethoxylated formate 14 studied in the sensitizing experiments using a concentration equimolar to that of 9. This is probably due to their low reactivity as electrophiles, and their low ability to form free radicals.

Since surfactants have amphiphilic properties, they form a variety of aggregates in solution depending on the concentration. The different behavior of these aggregates in solution may therefore affect the irritating properties. Thus, the bioactivity of surfactants may not show a strict dose-dependent relationship. In theory, only the monomers should be active regardless of the total concentration, and the concentration of monomers is constant above CMC. However, the data from the allergenicity studies of 9 indicate a more complex situation (Table 6, paper IV). This indicates that an allergenic response depends not only on the
concentration of free aldehyde monomers. Also the presence of different aggregates may be of importance for the observed biological response. In these tests, the concentration of the surfactants tested was well above CMC and no elicitation was observed when the CMC concentration (determined in the physicochemical studies) of compound 9 (0.002%) was tested on the guinea pigs (Table 6). The measurement of the size and shape of the aldehyde micelles may however not be appropriate micelle parameters to 9 in water since a difference in behavior in the D$_2$O media of 9 compared to water using pulsed-gradient-spin-echo NMR was observed. The observed difference can be attributed to differences in the degree in hydration of the aldehyde group in the two media. Formaldehyde exists in a reversible equilibrium with its diol (98%) in water solutions and the corresponding geminal diol of 9 in water was also seen in the MS-ESI analysis (paper IV).

Since surface active compounds are amphiphilic they are often used as solubilizers and emulsifiers in lipophilic formulations, and non-ionic surfactants, e.g. ethoxylated esters, are often used in topical creams (3). This was a reason to use water (saline) and Carbopol$^\text{1}\text{8}$ gel in the initial experiments with Tween$^\text{9}\text{8}$80. When the test material is applied in a solvent, it is necessary to absorb the solution on a piece of filter paper in the test chamber. Thus, there is a risk that the test substance adheres to the filter and does not reach the skin. This possibility was considered, but the presence of the filter paper did not seem to affect the observed allergenic response to the ethoxylated aldehyde 9, since there was a good correlation at repeated challenge testing with the same concentration (Tables 1 and 2, paper IV).

The gradual oxidation of surfactants leads to a product with considerably lower cloud point as shown in Figure 18. The effect of autoxidation on cloud point and CMC of non-ionic surfactants has been recognized before (14, 28). However, earlier studies on solution behavior have been qualitative in nature. The solution behavior was measured on the entire oxidized product mixture and no attempts have been made to isolate surface-active oxidation products and investigate the physicochemical properties of each individual product. Such measurements could have important practical implications, and might be used together with chemical analysis of peroxide- and formaldehyde content to detect an ongoing autoxidation. The surfactant used in a formulated product could have been stored above or at room temperature, for a long time before the formulation of the product. The practical consequence will be that a product containing ethoxylated alcohols might have quite a different chemical composition after storage and handling compared to the original product. Since the oxidative instability concerns the polyether chain the oxidation might also occur in all other types of ethoxylated surfactants, e.g. in the amide ethoxylates and the nonoxynols widely used in skin care products. So far, this has not been studied. A considerable autoxidation also was observed at storage in darkness at room temperature (Table 5). When the ethoxylated alcohols were stored in the refrigerator the deterioration was limited. The results from the cloud point measurements indicated that the presence of 9 does not influence the physicochemical properties considerably compared to pure C$_{12}$E$_3$, and oxidation to 9 would not show as a change in cloud point.

Predictive animal tests are used for classification of a substance. A modified CCET without adjuvant stimulation was deemed to be appropriate since the CCET protocol involves epicutaneous induction and challenge and resembles common exposure to an offending agent. The omission of adjuvant in these experiments may increase their predictive value since it is difficult to determine the sensitizing potential of chemicals, such as surfactants, that also have irritating properties. If skin reactions are seen in animals that have not been in contact with the specific chemical before, i.e. the controls, this must be due to a non-immunological irritating reaction. However, irritation in some of the control animals can normally not be avoided even when the patch test material is considered to be non-irritating. Although, pre-tests in the animals prior to start of the experiments were performed to determine non-irritating challenge concentrations, irritation in the controls were observed in sensitization experiments.

However, these animal results cannot stand alone and should be validated in man. To investigate the clinical significance in man an appropriate diagnostic patch testing in exposed humans is required, since challenge testing must be performed with the chemical in non-irritating concentrations, which might then be too low to detect an allergenic effect. Exposure to skin irritants can in some cases contribute to a deterioration of the condition. Since many female-dominated occupations involve extensive wet work, women are affected by irritant dermatitis to a greater extent than men as demonstrated in Figures 5 and 6. The most common type of hand eczema is irritant dermatitis, an effect of repeated damage to the skin, which makes it easier for skin irritants and contact allergens to penetrate the skin and give rise to eczema. When testing for allergic contact dermatitis it is desirable to use identified allergens. However, the chemical composition of surfactants is complex. Variable mixtures are processed until the desired technical properties have been achieved. A complete identification of all oxidation- and degradation products in such mixtures is not a realistic goal. An augmented allergenic effect can be expected since a cross-reactivity pattern was indicated when guinea pigs, sensitized to the major aldehyde 9, were challenge with the homologues ethoxylated aldehydes, 6–8.

The observed autoxidation of commonly used ethoxylated surfactant and formation of a complex degradation mixture indicates a potential health risk that has not been considered previously. Previously unknown breakdown/oxidation products were identified, some of which had allergenic properties. This oxidation may also affect the technical performance of the surfactants. It has not been a topic of this study to investigate this in detail, but our data support that a stability study is requested. The contact allergenic oxidation products examined in this thesis, i.e. the identified ethoxylated aldehydes and formaldehyde, might cause allergic contact dermatitis in individuals occupationally exposed to these ethoxylated surfactants and water.

**CONCLUSIONS**

The commonly used ethoxylated surfactants investigated are easily oxidized after air exposure forming a complex degradation mixture. The observed autoxidation has not been considered to be of some importance previously, although theoretically discussed in the literature. These
surfaceants are regarded by the producers to be stable at normal handling conditions.

Some of the formed oxidation products were isolated and identified in the complex oxidation mixture, both earlier known compounds and previously undescribed oxidation products. Formaldehyde was formed in concentrations known to elicit allergy in formaldehyde sensitized individuals. Not earlier known compounds, ethoxylated aldehydes and formates, were identified for the first time in the degradation mixture of an homologous ethoxylated alcohol used as model compound.

The ethoxylated aldehydes showed a allergenic potential in an experimental sensitization study. The allergenic potential was of the same magnitude as that of formaldehyde. A cross-reactivity pattern was indicated at testing with corresponding aldehyde homologues. No statistically significant sensitization was found in an experimental sensitization study on the ethoxylated formates.

The studies on physicochemical behavior showed an effect of autooxidation. The oxidation leads to a product with a considerably lower cloud point. The gradual change in solution behavior can have important practical implications. The individual physicochemical behavior of the major aldehyde investigated was similar to that of the pure ethoxylated alcohol investigated.

COMPREHENSIVE SUMMARY IN SWEDISH


Kända och tidigare inte beskrivna förningar med kontakttallergen effekt identifierades i de komplexa oxidationbandsnamingarna som bildades vid luftexponering av de studerade etoxilerade tensiderna. Detta är ny kunskap som visar att vanliga tensider som tidigare betraktats som hållbara vid hantering och lagring i rumstemperatur oxideras vid kontakt med luft och bildar nya allergiframkallande förningar. Oxidationprodukterna som bildas kan således ge en allergen pålagring till det irritativa handeksemnet. Genom luftoxidering ändras även de ytaktiva och fysikaliska egenskaperna, vilket kan innebära att de egenskaper som hittills anges är okända istället för vissa tensider inte gäller efter en tids förvaring.

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