EUROPEAN COMMISSION

Health & Consumer Protection
Directorate-General

Scientific Committee on Consumer Products

SCCP

# OPINION ON

# Hydrogen peroxide, in its free form or when released, in oral hygiene products and tooth whitening products

Scientific Committees
on consumer products
on emerging and newly identified health risks
on health and environmental risks

The SCCP adopted this opinion at its 14th plenary of 18 December 2007

About the Scientific Committees

Three independent non-food Scientific Committees provide the Commission with the scientific advice it needs when preparing policy and proposals relating to consumer safety, public health and the environment. The Committees also draw the Commission's attention to the new or emerging problems which may pose an actual or potential threat.

They are: the Scientific Committee on Consumer Products (SCCP), the Scientific Committee on Health and Environmental Risks (SCHER) and the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) and are made up of external experts.

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

SCCP

Questions concerning the safety of consumer products (non-food products intended for the consumer).
In particular, the Committee addresses questions related to the safety and allergenic properties of cosmetic products and ingredients with respect to their impact on consumer health, toys, textiles, clothing, personal care products, domestic products such as detergents and consumer services such as tattooing.

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## TABLE OF CONTENTS

## 1. BACKGROUND

The Scientific Committee on Cosmetics and Non Food Products intended for Consumers (SCCNFP), replaced by the Scientific Committee on Consumer Products (SCCP), by Commission Decision 2004/210/CE[1], has been consulted and expressed its view in several occasions on the safety of hydrogen peroxide, in its free form or when released, in oral hygiene products, and, in particular, in tooth whitening products.

Its latest opinion is from 15 March 2005 (SCCP/0844/04) in which it concluded that:

***"tooth whitening products containing up to 0.1% hydrogen peroxide***

* *The use of tooth whitening products up to 0.1% hydrogen peroxide is safe.*

***tooth whitening products containing > 0.1% to 6.0% hydrogen peroxide***

* *The proper use of tooth whitening products containing > 0.1 to 6.0 % hydrogen peroxide (or equivalent for hydrogen peroxide releasing substances) is considered safe after consultation with and approval of the consumer's dentist.*

- *The use of tooth whitening products is not recommended prior to or immediately after dental restoration.*

- *Particular care should be taken in using tooth whitening products by persons with gingivitis and other periodontal diseases or defective restorations. Conditions such as pre-existing oral tissue injury or concurrent use of tobacco and/or alcohol may exacerbate the toxic effects of hydrogen peroxide (see e.g. section 3.3.13.1).*

* *There is an absence of good clinical data and long-term epidemiological studies that assess the possible adverse effects within the oral cavity.*

* *The new additional data supplied does not provide the necessary reassurance in terms of risk assessment to support the safety of hydrogen peroxide up to 6 % in tooth whitening products freely and directly available to the consumer in various application forms (strips, trays, etc…). SCCP cannot quantify the risk of potential serious adverse effects in relation to the use of tooth whitening products".*

At the request of the Commission services, in order to ensure that industry would carry out relevant and good clinical data and long-term epidemiological studies that assess the possible adverse effects within the oral cavity, the SCCP delivered "a guidance document on epidemiological and clinical studies on tooth whitening products" on 28.03.2006 (SCCP/0974/06).

Commission services were then informed by a Member of the European Parliament and by the Council of European Dentists, formerly EU Dental Liaison Committee, of the existence of scientific articles which have not been considered by the SCCP. All relevant stakeholders were at that time asked to provide to the Commission services all available scientific articles on the matter. Norway and COLIPA[2] forwarded documents.

[1] OJ L 66, 4.3.2004, p.45
[2] COLIPA - European Cosmetics Toiletry and Perfumery Association

## 2. TERMS OF REFERENCE

### 2.1. Context of the question

The SCCP clarified in its opinion of March 2005, that for the purpose of its opinion, the terms "tooth whitening products" and "tooth bleaching products" define the same kind of products. In this request, neither do the Commission services draw a distinction.

In its opinion of 23.06.1999 (SCCNFP/0158/99), the SCCNFP has reviewed the use of hydrogen peroxide in tooth pastes and mouth rinses. It then concluded that "*an increase of hydrogen peroxide (and equivalent) in tooth paste and mouth-rinses to 3.6% is not permissible […]*". In parallel, in its opinion of 17.02.1999 (SCCNFP/0058/99), the SCCNFP considered the use of hydrogen peroxide (and equivalent) in tooth whitening products. It was this opinion which has been reviewed at several occasions.

Therefore, in order to ensure consistency, this request concerns the safe use of hydrogen peroxide, in its free form or when released, in oral hygiene products (tooth whitening products and tooth pastes and mouth-rinses).

Currently, Council directive 76/768/EEC on the approximation of the laws of the Member States relating to cosmetic products (the Cosmetics Directive) provides in Annex III, part 1, n°12 a limit of 0.1% of hydrogen peroxide (or equivalent for substances that release hydrogen peroxide) in oral hygiene products.

The Cosmetics Directive provides several options in order to ensure the safety of consumer when using cosmetic products. Those management options include:

- the requirement for a specific labelling;
- the requirement for the first use of a product by a professional;
- the requirement for the exclusive use by the professional.

In order to determine which of these options the appropriate one is, the Commission services need to know from the SCCP, within its scientific assessment, possible concerns and how scientifically these concerns can be handled.

### 2.2. Request

Considering all data, those already submitted in support of previous opinions and those identified since its last opinion (15 March 2005, SCCP/0844/04) and submitted to the SCCP, can the Committee assess the possible health risks associated with the use of hydrogen peroxide, in its free form or when released, in oral hygiene products?

In doing so, the Committee is asked, wherever it is scientifically justified, to make a distinction in the assessment of hydrogen peroxide and to identify any specific health risks regarding the use of hydrogen peroxide in oral hygiene products, or equivalent for substances that release hydrogen peroxide, taking account of:

- **Types** of oral hygiene products: mouth-rinses and tooth pastes on the one hand and tooth whitening products (strips, trays with gel…) on the other, as the SCCP has done in its previous opinions;

- **Concentration limits** (for example 0.1%, 6%, more than 6%);

- **Different usage conditions** which can create differences in the resulting exposure of consumers to hydrogen peroxide.

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## 2.4. Annex

Submission IV from COLIPA including:

– some of the articles mentioned above;

– review of all papers published on Hydrogen Peroxide (2004-Date);

– review "use of bovine samples as substitutes in studies evaluation the impact of bleaching agents on dental enamel microhardness" by Prof. Dr. Thomas Attin of December 2006.

## 3. OPINION

The present Opinion is to a large extend based on *"Opinion on Hydrogen Peroxide in Tooth Whitening Products (SCCP/0844/04)"* and *"Opinion on Hydrogen Peroxide and Hydrogen Peroxide Releasing Substances Used in Oral Care Products (SCCNFP/0158/99)"* and *Submission IV* from COLIPA including the articles mentioned under "2. TERMS OF REFERENCE" in the mandate**.** It should be noted that some of the papers included in the new material were previously submitted under the Public Consultation of SCCP/0844/04 and was already discussed. Additional material was received during a Call for Information following the release of this mandate. The references of the new articles included have been written *in italic.*

### 3.1. Chemical and Physical Specifications

| 3.1.1. | Chemical identity |
|---|---|

| 3.1.1.1. | Primary name and/or INCI name |
|---|---|

Hydrogen peroxide, dihydrogen dioxide, hydrogen dioxide, hydrogen oxide, oxydol, peroxide

Carbamide peroxide, urea peroxide, hydrogen peroxide carbamide, urea hydrogen peroxide, urea, compd. with hydrogen peroxide (1:1)

| 3.1.1.2. | Chemical names |
|---|---|

Hydrogen peroxide
Carbamide peroxide

| 3.1.1.3. | Trade names and abbreviations |
|---|---|

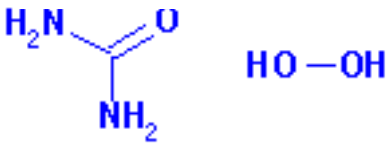
/

| 3.1.1.4. | CAS / EINECS number |
|---|---|

| | | |
|---|---|---|
| Hydrogen peroxide: | CAS: | 7722-84-1 |
| | EINECS: | 231-765-0 |
| Carbamide peroxide: | CAS: | 124-43-6 |
| | EINECS: | 204-701-4 |

| 3.1.1.5. | Structural formula |
|---|---|

Hydrogen peroxide 


Carbamide peroxide 

### 3.1.1.6. Empirical formula

Hydrogen peroxide: $H_2O_2$
Carbamide peroxide: $CO(NH_2)_2 \bullet H_2O_2$

### 3.1.2. Physical form

Hydrogen peroxide: Colourless liquid
Carbamide peroxide: White crystals or crystal powder

### 3.1.3. Molecular weight

Hydrogen peroxide: Mol. weight 34.0
Carbamide peroxide: Mol. weight 94.1

### 3.1.4. Purity, composition and substance codes

Commercial products:

**Hydrogen peroxide:** Hydrogen peroxide – water solutions. Commercially supplied as a 33 - 37% aqueous solution. Common stabilisers include phosphoric or other mineral acid (to keep the product acidic), pyrophosphate salts (complexing agents to inhibit metal-catalysed decomposition) and stannate (a colloid-forming inhibitor).

Commercial solutions contain low (<0.1%) levels of organic impurities (total organic carbon) and very low levels (<10 ppm) of inorganic impurities, with total heavy metals usually <2 ppm.

**Carbamide peroxide:** Products containing minimum 97% of the hydrogen peroxide – urea adducts are available.

### 3.1.5. Impurities / accompanying contaminants

/

### 3.1.6. Solubility

Hydrogen peroxide is miscible with water.
Carbamide peroxide is soluble in water.

### 3.1.7. Partition coefficient (Log $P_{ow}$)

/

| 3.1.8. Additional physical and chemical specifications |
|---|

**Hydrogen peroxide**

Pure $H_2O_2$ (not commercially available in EU)

| | | |
|---|---|---|
| Melting point | : | -0.4°C |
| Boiling point | : | 150-152°C |
| Density | : | 1.4425 g/cm$^3$ |
| Vapour pressure | : | 3 hPa |

**Carbamide peroxide**

| | | |
|---|---|---|
| Melting point | : | 75-85°C |
| Boiling point | : | not available |
| Density | : | 1.4 g/cm$^3$ |
| Vapour pressure | : | not available |

*Comment*
Possible impurities in hydrogen peroxide and carbamide peroxide are not known. Likewise is the stability of hydrogen peroxide and carbamide peroxide in oral hygiene unknown.

## 3.2. Function and uses

Hydrogen peroxide is capable of undergoing numerous reactions (e.g., molecular additions, substitutions, oxidations and reductions). It is a strong oxidant and can form free radical by homolytic cleavage. Carbamide peroxide is an adduct of urea and hydrogen peroxide which on contact with water break down to urea and hydrogen peroxide. For example, a 10% carbamide peroxide gel would yield a maximum of 3.6% hydrogen peroxide. 750,000 tonnes hydrogen peroxide (calculated as 100% $H_2O_2$) were produced in Europe in 1995. About 300 tonnes of carbamide peroxide were used.

The main usage of hydrogen peroxide is in production of chemicals (approx. 40%), bleaching pulp and paper (approx. 30%) and bleaching textiles (approx. 20%). Small quantities are used in cosmetics. Hydrogen peroxide is used for hair bleaching and for oxidation in permanent hair dyes and in oral hygiene products such as mouth-rinses and dentifrices as well as in tooth bleaching products.

While the content of hydrogen peroxide (and equivalent) in oral hygiene products available in EU should not exceed 0.1%, considerably higher concentrations are available in products sold in USA.

US Food and Drug Administration (FDA) approved both hydrogen peroxide and carbamide peroxide as oral antiseptic agents in 1983 (FDA, 1983). The products of 10-15% carbamide peroxide and 1.5-3% hydrogen peroxide preparations are classified in Category I, which includes agents that are generally recognised as safe (GRAS) and effective. A Subcommittee of *FDA (2003)* concluded that hydrogen peroxide is safe at concentrations of up to 3 percent, but there are insufficient data available to permit final classification of its effectiveness at 1.5 to 3 percent concentrations for long-term over the counter (OTC) use as an antigingivitis/antiplaque agent.

The colour of the teeth is influenced by a combination of their intrinsic colour and the presence of any extrinsic stains that may form on the tooth surface. Intrinsic tooth colour is associated with the light scattering and adsorption properties of the enamel and dentine,

with the properties of dentine playing a major role in determining the overall tooth colour. Extrinsic stains tend to form in areas of the teeth that are less accessible to tooth brushing and the abrasive action of a toothpaste and is often promoted by smoking, dietary intake of tannin-rich foods (e.g. red wine) and the use of certain cationic agents such as chlorhexidine, or metal salts such as tin and iron. In the USA, 34% of an adult population reported that they were dissatisfied with their current tooth colour *(Odioso et at., 2000)* and in a survey of 3215 subjects from the UK 50% perceived they had some kind of tooth discoloration *(Alkhatib et al., 2004).*

Tooth colour can be improved by a number of methods and approaches including whitening toothpastes, professional cleaning by scaling and polishing to remove stain and tartar, internal bleaching of non-vital teeth, external bleaching of vital teeth, microabrasion of enamel with abrasives and acid, placement of crowns and veneers. Three bleaching approaches exist for external bleaching of vital teeth: 1) dentist supervised night guard bleaching, 2) in-office or power bleaching and 3) mass market bleaching products. Night guard bleaching typically uses a relatively low level of whitening agent applied to the teeth via a custom fabricated mouth guard and is worn at night for at least 2 weeks. In-office bleaching generally uses relatively high levels of whitening agents, for example 25–35% hydrogen peroxide containing products, for shorter time periods. The whitening gel is applied to the teeth after protection of the soft tissues and the peroxide may be further activated by heat or light. The in office treatment can result in significant whitening after only one treatment visit but may require multiple treatment appointments for optimum whitening. Mass market products typically contain low levels of whitening agent (e.g. 3–6% hydrogen peroxide) that are self-applied to the teeth via gum shields, strips or paint-on product formats and typically require twice per day application for up to 2 weeks *(Joiner, 2006)*. External bleaching of vital teeth is generally carried out with hydrogen peroxide or carbamide peroxide.

A tooth bleaching system based on sodium chlorite applied to the tooth surface and activated under acidic conditions has been described in the literature. Other potential vital tooth bleaching systems outlined in the literature includes peroxide plus metal catalysts, oxireductase enzymes, sodium percarbonate, sodium perborate and potassium peroxymonosulphate *(Joiner, 2006).* The use of the three latter chemicals will be briefly discussed in Appendix to the present Opinion. Sodium percarbonate and sodium perborate have been found in commercially available tooth bleaching products. Sodium perborate fulfils the criteria of a classification of toxic to reproduction category 2 (R61). Additionally, there is a current proposal that sodium perborate should be so classified (http://ecb.jrc.it/classification-labelling/search-classlab/ (Search Working Database)).

The first articles on bleaching teeth using night guard whitening bleaching were published in 1989 (Christensen, 1989a, b; Haywood and Heymann, 1989). The mechanism by which teeth are whitened by oxidizing materials such as hydrogen peroxide and carbamide peroxide are not fully understood. Evidence points towards the initial diffusion of peroxide into and through the enamel to reach the enamel dentine junction and dentine regions. *In vitro* experiments have demonstrated the penetration of low levels of peroxide into the pulp chambers of extracted teeth after exposure times of 15–30 min. The levels of peroxide measured in these experiments are considerably much lower than that needed to produce pulpal enzyme inactivation *(Joiner and Thakker, 2004).*

It is assumed that the whitening effects are primarily due to degradation of high molecular weight, complex organic molecules that reflect a specific wavelength of light and is responsible for the colour of the stain. The resulting degradation products are of lower molecular weights and are less complex molecules that reflect less light and result in a reduction or elimination of the discoloration (Flaitz and Hicks, 1996). Both the dentin and the enamel change colour as a result of the easy passage of the peroxide and urea through the tooth. Extended treatment times have been developed for difficult situations. Heavy

tobacco stains may require as much as three months of treatment. Tetracycline-stained teeth have responded in two to six months of nightly treatment, although not to the extent of normal teeth. Single dark teeth can also be bleached successfully.

Home-based chemically-induced whitening of teeth in adults has recently been reviewed by the Cochrane collaboration. The authors concluded: *"There is evidence that whitening products work when compared with placebo/no treatment. There are differences in efficacy between the products, mainly due to the levels of active ingredients, hydrogen peroxide and carbamide peroxide. All trials were however short term and the majority of the studies were judged to be at high risk of bias and were either sponsored or conducted by the manufacturers. There is a need for pragmatic long-term and independent clinical studies that include participants representing diverse populations. There is also a need to evaluate long-term harms. Several studies reported (where measured) the common side effects of tooth sensitivity and gingival irritation, and people should be informed of this" (Hasson et al., 2006).*

For the purpose of this Opinion the terms "tooth whitening products" and "tooth bleaching products" define the same kind of products.

## 3.3. Toxicological Evaluation

### 3.3.1. Acute toxicity

#### 3.3.1.1. Acute oral toxicity

**Hydrogen peroxide:**

* Oral $LD_{50}$ - values for rats vary between **600** and **1617 mg/kg bw** (Y.Li, unpublished; Ito et al., 1976).

* A 16-month-old boy (body weight 11.6 kg) was found playing with an empty bottle that had contained about 230 g of 3% hydrogen peroxide solution. The container had a cracked lid that allowed the contents to be sucked. White foam emerged from the child's mouth and nose. He then walked to bed and was found dead 10 hours later. In a post-mortem examination there was frothy blood in the right ventricle of the heart and the portal venous system. The gastric mucosa was red and the brain oedematous. Histopathological examination showed oedema in the lungs, and diffuse interstitial emphysema was evident. Gas emboli are found within the pulmonary vasculature and gastric and intestinal lymphatics. Clear vacuoles were also found within the walls of the gastrointestinal tract, in the spleen, kidney and myocardium (Cina et al., 1994). The maximum estimated dose of hydrogen peroxide ingested was 7 g (**600 mg/kg bw)**.

* An uncommon route of absorption from a cavity presumably lined by well-vascularized granulomatous tissue involved an obese 54-year-old male who underwent irrigation of an infected and fistulous herniorrhaphy wound with 5 x 20 ml volume of 3% hydrogen peroxide. Not all irrigating volume seemed to have drained from the wound. On the fifth irrigation, the patient suddenly lost consciousness, showed cardiac shock and fell to coma which lasted for 15 min. There was no indication of red cell damage. ECG showed signs of transient myocardial ischaemia. The patient made a full recovery within 3 days. The authors attributed this occurrence to widespread embolization of oxygen microbubbles, especially to the cerebral and coronary arteries (Bassan et al., 1982). If it is presumed that as much as one half of the volume of the irrigating

solution was absorbed, the hydrogen peroxide dose would have been 1.5 g implying for an obese person (assumed weight of 100 kg) about **15 mg/kg bw**.

* Oxygen embolism has been reported in several infants following intestinal irrigation with hydrogen peroxide to remove meconium (Danis et al., 1967; Shaw et al., 1967). In one case a 36-hour old infant died following use of 1% hydrogen peroxide to remove inspissated meconium from the bowel due to meconium ileus (Shaw et al., 1967).

**Tooth whiteners containing 10-22% carbamide peroxide:**

* Oral $LD_{50}$ - values for rats reported **> 5,000 mg/kg bw** (Rope [Report], 1993; Huang [Report], 1996; Adam-Rodwell et al., 1994; Cherry et al., 1993). (It appears that $LD_{50}$ studies with rats for doses less than 5,000 mg/kg bw has not been performed)

* Oral $LD_{50}$ - values for mice vary between **87.2-143.8 mg/kg bw** (Woolverton et al., 1993).

### 3.3.1.2. Acute dermal toxicity

**Hydrogen peroxide**

* Dermal $LD_{50}$ -values for rats vary between **700** and **7,500 mg/kg bw** (FDA, 1983).

* Dermal $LD_{50}$ –values in rabbits about **630 mg/kg bw** (FDA, 1983).

### 3.3.1.3. Acute inhalation toxicity

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### 3.3.1.4. Summary / Comment on Acute toxicity

The oral and dermal LD50 of hydrogen peroxide in rats is higher than 600 mg/kg bw. The dermal LD50 in rabbits is 630 mg/kg bw.

A 16-month-old boy (body weight 11.6 kg) died after ingestion of about 600 mg/kg bw.

### 3.3.2. Irritation and corrosivity

### 3.3.2.1. Skin irritation

**Hydrogen peroxide:**

* Skin irritation tests in rabbits with concentration of hydrogen peroxide of 3-8% were non-irritating to intact and abraded skin following exposure for 24 hours under occlusive dressing (cited in ECETOC, 1996). Irritation was slight following 4 hour exposure to 10% hydrogen peroxide and mild with 35% hydrogen peroxide. Desquamation occurred in 2 of 6 animals at day 14 with the latter concentration (Aguinaldo et al. [Abstract], 1992).

**Tooth whiteners containing 10-22% carbamide peroxide:**

* Primary irritation of the skin of rabbits was not found with tooth whitener (Rope [Report], 1993).

### 3.3.2.2. Eye irritation

**Hydrogen peroxide:**

* Eye irritation studies with rabbits indicate that a 5% hydrogen peroxide solution is non-irritant to mildly irritant (Weiner et al. [Abstract], 1990).

* Several drops of a 2-5% solution induced much clouding of the cornea and inflammation of the conjunctiva of rabbit eyes. A 1% solution applied repeatedly caused conjunctival hyperaemia and slight corneal haze, followed by recovery (Koster, 1921 as quoted by Grant, 1986).

* Testing of eye irritancy for hydrogen peroxide with the Draize method indicated that 5% solution was slightly irritating (FMC, 1987a), 8% solution was moderately irritating (EU classification irritating) (FMC, 1987b), and 10% solution was highly irritating (EU classification risk of serious damage to eyes) (FMC, 1985).

* A woman who had inadvertently stored a contact lens in a 3% hydrogen peroxide disinfectant solution experienced hyperaemia, tearing, and eyelid spasm (Knoph, 1984).

* In 10 human volunteers, the threshold of detection for irritation was about 0.1% when hydrogen peroxide was administered as drops directly to the eye (McNally, 1990).

* When a hydrogen peroxide solution was administered to the eye of human volunteers via soaking contact lenses, the threshold of detection for hydrogen peroxide irritation was less than 0.03% (McNally, 1990).

### 3.3.2.3. Mucous membrane irritation

**Hydrogen peroxide**

* 1 or 1.2% hydrogen peroxide applied to the gingivae or tongues of anaesthetised dogs by continuous drip caused oedema, followed by destruction and sloughing of the cornified epithelial layer of the gingivae (Martin et al., 1968, Dorman and Bishop, 1970).

**Tooth whiteners containing 10-22% carbamide peroxide**

* No evidence of oral mucosal irritation after applying tooth whiteners containing 10% or 22% carbamide peroxide for up to 6 week in experiments with rats, hamsters and rabbits has been reported (Rope [Report], 1993; Huang [Report], 1996; Adam-Rodwell et al., 1994; Li et al. [Abstract], 1996; Webb [Report], 1996).

* Stomach gavage of 15 and 50 mg/kg bw carbamide peroxide or 150 and 500 mg/kg bw of a tooth whitener agent (Opalescence) containing 10% carbamide peroxide produced ulceration of gastric mucosa. No ulceration was observed with 5 mg/kg bw carbamide peroxide. The lesions were clearly visible after 1 hour and seemed to be healing after 24 hours. The ulcerations of the gastric mucosa were more pronounced after exposure to the tooth-bleaching agents than those observed after a comparable

dose of carbamide peroxide. The authors point out that this may be attributed to the hydrophobic gel and the content of a carbopol (which increases the tissue adherence and retards the release of oxygen) in the bleaching agent (Dahl and Becher, 1995).

* Stomach gavage of doses up to 2,000 mg/kg bw of tooth whiteners containing 10% carbamide peroxide or 70 mg hydrogen peroxide was given weekdays for 15 weeks or 6 months to Chinese hamsters. Cyclophosphamide and water served as control substances. (Concentration and results with cyclophosphamide not stated). Histopathological findings of the gastroduodenal tissue were comparable among the groups (Li et al. [Abstract], 1993).

#### 3.3.2.4. Summary / Comment on Irritation and Corrosivity

4 hour exposure to 10% hydrogen peroxide caused slight skin irritation in rabbits. A 5% solution of hydrogen peroxide was slightly irritating to the eye while a 10% solution was highly irritating. The threshold of detection for irritation was about 0.1% when hydrogen peroxide was administered as drops directly to the human eye.

Stomach gavage of 15 mg/kg bw of carbamide peroxide (5.4 mg/kg bw of hydrogen peroxide) produced ulceration of gastric mucosa in rats observed after 1 hour; the lesions appeared to be healing after 24 hours. No effects were observed with 5 mg/kg bw of carbamide peroxide (1.8 mg/kg bw of hydrogen peroxide) (Dahl and Becher, 1995).

### 3.3.3. Skin sensitisation

**Hydrogen peroxide**

* Ten guinea pigs were exposed to 3 or 6% hydrogen peroxide on intact or abraded skin and by intradermal injections of 0.1 ml of test solution in saline. Test solutions were re-applied 9 times over a 2 week period prior to a challenge to evaluate sensitisation. The final reactions did not indicate induction of skin sensitization with either solution (DuPont [Report], 1953).

* A case report observed skin sensitisation reaction from two women who had been exposed to hydrogen peroxide as an ingredient in commercial hair dyes. Both women tested positively to 3% hydrogen peroxide and numerous other ingredients in the hair dyes (Aguire et al., 1994).

* In a study of 156 hairdressers patch tested with the hairdressers series of chemicals were all negative to hydrogen peroxide (3%). The Dermatological Department at the Finnish Institute of Occupational Health has since 1985 tested dermatitis patients having had exposure to hairdressing chemicals with a series of test substances containing 3% hydrogen peroxide in water. 130 patients have been tested with no allergic reactions. One patient exhibited an irritant reaction. The Finnish Register of Occupational Diseases which was searched from 1975 through 1997 did not contain any cases of allergic dermatosis caused by hydrogen peroxide. The Dermatology Department of the University Central Hospital in Turku, Finland, patch tested 59 patients with 3% hydrogen peroxide during 1995-96. No positive reactions were found (*Kanerva et al., 1998*).

#### 3.3.3.1. Summary / Comment on Sensitisation

Hydrogen peroxide is not considered to cause skin sensitisation.

### 3.3.4. Dermal / percutaneous absorption

After the application of 5-30% solutions of hydrogen peroxide on rat skin *in vivo*, some $H_2O_2$ could be localised in the excised epidermis within a few minutes. By contrast, with human cadaver skin *in vitro,* $H_2O_2$ was detectable in the dermis only after the application of high $H_2O_2$ concentrations for several hours, or after pretreatment with hydroxylamine (inhibitor of catalase). Based on histochemical analysis, $H_2O_2$ was not metabolised in the epidermis, and the passage was transepidermal, avoiding the "preformed pathways" of skin appendages. The localisation of dermal emphysema, caused by liberation of oxygen, correlated for the most part with the distribution of catalase activity within the tissue (ECB, 2003).

#### 3.3.4.1. Absorption from mucous membranes

Administration of hydrogen peroxide solutions to body cavities lined by mucous membranes, (e.g. sublingually, intraperitoneally and rectally) resulted in increased oxygen content of the draining venous blood and, if the amounts of hydrogen peroxide were sufficiently high, formation of oxygen bubbles. Mongrel dogs were treated with colonic lavage, or the lavage of small and large bowel was performed through an enterotomy with dilute saline solutions of hydrogen peroxide. Small amounts of the more concentrated solution (1.5% or higher) produced immediate whitening of the mucosa, with prompt appearance of bubbles in the circulation. More dilute (0.75-1.25%) solutions had the same effect when left in contact with the bowel for a longer time or when introduced under greater pressure or in greater volume for a given length of bowel. Venous bubbling was never observed at concentrations less than 0.75% $H_2O_2$. In none of the animals did mesenteric thrombosis or intestinal gangrene develop. Application of 1% hydrogen peroxide to the serosal membrane caused whitening due to gas filled small vessels; higher concentrations (up to 30%) on the skin and mucous membranes (of various species) caused lasting damage when subcutaneous emphysema and disturbances of local blood circulation impaired tissue nutrition (ECB, 2003).

#### 3.3.4.2. Summary / Comment on Absorption

Biological membranes are highly permeable to hydrogen peroxide. Thus, hydrogen peroxide is expected to be readily taken up by the cells constituting the absorption surfaces, but at the same time it is effectively metabolised, and it is uncertain to what extent the unchanged substance may enter into blood circulation. Moreover, red blood cells have an immense metabolic capacity to degrade hydrogen peroxide and will remove hydrogen peroxide that might enter blood circulation.

### 3.3.5. Repeated dose toxicity

#### 3.3.5.1. Repeated Dose (28 days) oral / dermal / inhalation toxicity

***Inhalation***

***Rats***

Groups of five male and female Alpk:APfSD (Wistar-derived) rats were exposed whole-body for 6 hours per day to 0 (control), 2.9, 14.6 or 33 mg/m$^3$ hydrogen peroxide vapour for 5 days per week, for a period of 28 days. Clinical signs which demonstrated respiratory tract irritation were seen at the exposure levels of 14.6 and 33 mg/m$^3$, but not at 2.9 mg/m$^3$. Concentration related necrosis and inflammation of the epithelium in the anterior regions of the nasal cavity was found at the two higher levels. Mononuclear cell infiltration was seen in two females at the highest exposure concentration in the larynx. Moreover, in the lungs,

one male rat in each exposure group and two female rats in the top dose group exhibited perivascular neutrophil infiltration, and there was haemorrhage in some animals at the two lower dose levels. Control animals did not exhibit changes. The nasal localisation of the primary injury by peroxide is what can be expected from a water soluble oxidant vapour. As regards pathology in the lungs, the authors of the study considered it unlikely that the effects were treatment related due to the absence of a relationship with exposure concentration and the low incidence, and hence the NOAEL of the study would be 2.9 mg/m$^3$ *(CEFIC, 2002)*

### 3.3.5.2. Sub-chronic (90 days) oral / dermal / inhalation toxicity

***Oral***

**Hydrogen peroxide**

***Mice***

*Study 1*

Mice drinking 0.15% hydrogen peroxide (about 150 mg/kg/day) *ad libitum* grew normally and developed no visible abnormalities during a 35-week test period (FDA, 1983). Necropsy results show changes in the liver, kidney and stomach and small intestine. Hydrogen peroxide solutions at >1% (> 1 g/kg/day) caused pronounced weight loss and death of mice within 2 weeks (FDA, 1983).

*Study 2*

Mice (C57BL/6N, catalase deficient) (groups of 15/sex) received solutions of 0, 100, 300, 1000 or 3000 ppm hydrogen peroxide in distilled water for 13 weeks. Control animals were given distilled water. At term, ten males and ten females from each group were anesthetised, blood samples were collected and the animals were killed for macroscopic and histopathological examination. Five animals/sex/group continued on untreated distilled water for an additional 6-week recovery period.

*Treatment period (Days 0-90):* Clear treatment-related, dose-dependent effects were noted among both females and males receiving 300, 1,000 or 3,000 ppm of $H_2O_2$. Body weights were significantly reduced only in male and female animals receiving 3,000 ppm. Dose-related reductions in both food and water consumption were observed in female animals receiving 300 ppm and greater, while among the males consistent reductions were observed at the top dose level. Among females 300 ppm (103 mg/kg/day) was a LOAEL based on significant reduction in water consumption.

*Recovery period (Days 91-134):* The most notable effect was increased water consumption observed among males that had received 3,000 ppm, and among females that had received 300, 1,000 or 3,000 ppm.

*Histopathology:* Histological examinations were performed on all gross lesions, on the tongue, oesophagus, stomach, duodenum, ileum, jejunum, caecum, colon, and rectum from all animals in all groups, and on all major organs including the sex organs in the high dose and control animals.

Hydrogen peroxide related changes were observed only in the duodenum at terminal sacrifice in the 1,000 and 3,000 ppm groups of males and females, and in a single 300 ppm group male. Although the general architecture of the affected duodenum was normal, there was an increase in cross sectional diameter and a larger mucosal area with broader, more substantial villi when compared to those of control mice. The change was assessed as mucosal hyperplasia because of the increase in mucosal thickness and size of the villi. Mucosal hyperplasia was not found in 100 ppm group mice, neither among controls.

*Mortality:* There were no treatment-related deaths. One male mouse died in the control group (the cause of death was undetermined), and one male mouse in the 3,000 ppm group died on study day 43 (no histopathological findings). After the recovery period no hyperplasia was observed in any dose group.

*Conclusion*
NOAEL was 100 ppm (26 and 37 mg/kg/day) for males and females, respectively, based on dose-related reductions in food and water consumption, and on the observation of duodenal mucosal hyperplasia (Weiner et al., 1998).

***Rats***

*Study 1*
When Wistar rats were administered 5% hydrogen peroxide by oral gastric tube 6 days weekly for 90 days with a dose range 56.2 to 506 mg/kg bw/day, the dose of 506 mg/kg suppressed bodyweight gain, decreased food consumption, and caused changes in haematology, blood chemistry, and organ weights. Principal organ affected was gastric mucosa, and the effect was local. The no-observed-effect-level (NOEL) of hydrogen peroxide was 56.2 mg/kg/day (Ito et al., 1976).

*Study 2*
In another Wistar rat study (Kawasaki et al., 1969) the rats were administered a 0.06 to 0.6% hydrogen peroxide by gavage for 100 days with a dose range 6 to 60 mg/kg bw/day. The top dose was associated with effects: a significant reduction of the body weight gain after day 20 of administration, a slightly higher spleen weight on day 40 (but not at termination on day 100), a decreased haematocrit and plasma proteins on day 100. Plasma catalase was significantly decreased at the termination on day 100 in the 30 and 60 mg/kg bw/day dose groups. Thus, NOAEL was 20 mg/kg bw/day.

*Study 3*
Groups of 10 male and female F344 rats were given 0, 0.15, 0.3, 0.6, 1.2, or 2.4% hydrogen peroxide in drinking water for 10 weeks *(Takayama, 1980)*. Prominent weight losses and nasal bleeding were noted in the rats on the 2.4% solution starting immediately after initiation of the treatment. Also in the 1.2 and 0.6% dose groups, weight losses were noted from an early stage of hydrogen peroxide treatment. Regarding body weight gain, a gain rate of 66.1% was achieved in the male controls, whereas a maximum gain of 53.3% was achieved in the hydrogen peroxide treated groups (0.6%), and a 45.9% weight loss occurred in males on 2.4% hydrogen peroxide. A gain rate of 37.2% was found in female controls, whereas a maximum gain of 29.7% was achieved in the low dose (0.15%) hydrogen peroxide group, and a weight loss of 30.4% in the top dose group. Nine of the males on the 2.4% solution and all rats at other dose levels survived the 10-week treatment. As in the male groups, nine of the 10 females at the top dose level and all animals at the other dose levels survived the treatment. Histopathology was performed on 5 rats in each group. Pathological findings were made only at the top dose level: all males and females exhibited multiple gastric erosions and ulcer, 2 males showed atrophy of testis (in the whole group testis weights were reduced by 60% compared to controls), one rat showed congestion of the liver (died at week 7). The losses in weight of tissues other than the brain in the top dose males roughly corresponded to the body weight loss, the same applied for females. In view of the apparent effect on the weight gain even at the lowest dose level, no NOAEL can be determined.

*Conclusion*
A LOAEL can be calculated to 75 and 86 mg/kg bw/day for male and female rats, respectively.

### 3.3.5.3. Chronic (> 12 months) toxicity

See section 3.3.7 Carcinogenicity

### 3.3.5.4. Summary / Comment on Repeated dose toxicity

In a 90 day study in mice (Mouse study 2) with hydrogen peroxide in the drinking water, a NOAEL of 100 ppm was found based on dose-related reductions in food and water consumption, and on the observation of duodenal mucosal hyperplasia. This corresponds to 26 and 37 mg/kg bw/day for males and females, respectively. In a 100 days rat gavage study (Rat study 2), a NOAEL of 20 mg/kg bw/day was found based a significantly reduced plasma catalase level at higher dose levels.

A NOAEL of 20 mg/kg bw/day may be used for calculation of MOS.

## 3.3.6. Mutagenicity / Genotoxicity

**Hydrogen peroxide**

The *in vitro* and *in vivo* genotoxic potential of $H_2O_2$ is summarised in Table 3.1 and 3.2, respectively.

**Table 3.1:** *Summary of in vitro genotoxicity of hydrogen peroxide (IARC, 1999)*

| Test system | Result [a] | | Dose[b] (LED or HID) | Reference [c] |
|---|---|---|---|---|
| | Without exogenous metabolic activation | With exogenous metabolic activation | | |
| PRB, Prophage, induction/SOS response/strand-breaks/or cross-links | + | NT | 0.5 | *Müller & Janz (1993)* |
| PRB, Prophage, induction/SOS response/strand-breaks/or cross-links | + | NT | 1 | *Northrop (1958)* |
| PRB, Prophage, induction/SOS response/strand-breaks/or cross-links | + | NT | 45 | *Nakamura et al. (1987)* |
| BRD, Escherichia coli, differential toxicity | + | NT | 20 | *Hartman & Eisenstark (1978)* |
| BRD, Escherichia coli, differential toxicity | + | NT | 340 | *Ananthaswamy & Eisenstark (1977)* |
| SAF, Salmonella typhimurium BA13, forward mutation | + | NT | 0.2 | *Ariza et al. (1988)* |
| SAF, Salmonella typhimurium (SV50), forward mutation | + | NT | 0.22 | *Xu et al. (1984)* |
| SA0, Salmonella typhimurium TA100, reverse mutation | – | NT | 340 | *Stich et al. (1978)* |
| SA0, Salmonella typhimurium TA100, reverse mutation | (+) | NT | 136 | *Norkus et al. (1983)* |
| SA0, Salmonella typhimurium TA100, reverse mutation | – | – | 0.9 | *Xu et al. (1984)* |
| SA0, Salmonella typhimurium TA100, reverse mutation | (+) | NT | 5 | *Fujita et al. (1985)* |
| SA0, Salmonella typhimurium TA100, reverse mutation | (+) | – | 5780 | *Kensese & Smith (1989)* |
| SA2, Salmonella typhimurium TA102, reverse mutation | (+) | – | 5780 | *Kensese & Smith (1989)* |
| SA2, Salmonella typhimurium TA102, reverse mutation | (+) | NT | 20.4 | *Abu-Shakra & Zeiger (1990)* |
| SA4, Salmonella typhimurium TA104, reverse mutation | + | NT | 10 | *Abu-Shakra & Zeiger (1990)* |
| SA7, Salmonella typhimurium TA1537, reverse mutation | + | – | 4046 | *Kensese & Smith (1989)* |
| SA8, Salmonella typhimurium TA1538, reverse mutation | (+) | – | 5780 | *Kensese & Smith (1989)* |
| SA9, Salmonella typhimurium TA98, reverse mutation | – | NT | 340 | *Stich et al. (1978)* |
| SA9, Salmonella typhimurium TA98, reverse mutation | – | – | 0.9 | *Xu et al. (1984)* |
| SA9, Salmonella typhimurium TA98, reverse mutation | (+) | – | 5780 | *Kensese & Smith (1989)* |
| SAS, Salmonella typhimurium hisC3108, reverse mutation | + | NT | 30 | *Ames et al. (1981)* |
| SAS, Salmonella typhimurium TA96, reverse mutation | + | NT | 50 | *Levin et al. (1982)* |
| SAS, Salmonella typhimurium TA97, reverse mutation | (+) | – | 2890 | *Kensese & Smith (1989)* |
| SAS, Salmonella typhimurium TA97, reverse mutation | + | NT | 4.25 | *Abu-Shakra & Zeiger (1990)* |
| SAS, Salmonella typhimurium SB1106p, reverse mutation | + | NT | 5.1 | *Abu-Shakra & Zeiger (1990)* |
| SAS, Salmonella typhimurium SB1111, reverse mutation | (+) | NT | 10 | *Abu-Shakra & Zeiger (1990)* |
| SAS, Salmonella typhimurium SB1106, reverse mutation | + | NT | 10 | *Abu-Shakra & Zeiger (1990)* |
| ECF, Escherichia coli (excluding K12), forward mutation | + | NT | 3 | *Abril & Pueyo (1990)* |
| ECR, Escherichia coli WP2, reverse mutation | + | NT | 2160 | *Demerec et al. (1951)* |
| BSM, Bacillus subtilis, multigene test | + | NT | 7.2 | *Sacks & MacGregor (1982)* |
| MAF, Micrococcus aureus, forward mutation | + | NT | 6 | *Clark (1953)* |
| SCF, Saccharomyces cerevisiae ade2, forward mutation | + | NT | 100 | *Thacker (1976)* |
| SCF, Saccharomyces cerevisiae ade2, forward mutation | + | NT | 2000 | *Thacker & Parker (1976)* |
| SGR, Streptomyces griseoflavus, reverse mutation | – | NT | 1440 | *Mashima & Ikeda (1958)* |
| ANR, Aspergillus chevalieres, reverse mutation | (+) | NT | 1440 | *Nanda et al. (1975)* |
| NCF, Neurospora crassa, forward mutation | (+) | NT | 9180 | *Han (1997)* |
| NCR, Neurospora crassa, reverse mutation | + | NT | 7140 | *Dickey et al. (1949)* |
| NCR, Neurospora crassa, reverse mutation | + | NT | 6800 | *Jensen et al. (1951)* |
| DMX, Drosophila melanogaster, sex-linked recessive lethal mutations | – | | 43200 inj | *Dipaolo (1952)* |

**Table 3.1 (contd)**

| Test system | Result[a] | | Dose[b] (LED or HID) | Reference[c] |
|---|---|---|---|---|
| | Without exogenous metabolic activation | With exogenous metabolic activation | | |
| DIA, DNA single-strand breaks, Chinese hamster lung V79 cells *in vitro* | (+)d | NT | 12 | *Bradley et al. (1979)* |
| DIA, DNA single-strand breaks, rat hepatocytes in vitro | + | NT | 3.4 | *Olson (1988)* |
| DIA, DNA single-strand breaks, Chinese hamster ovary CHO cells *in vitro* | + | NT | 3.4 | *Cantoni et al. (1989)* |
| DIA, DNA single-strand breaks, Chinese hamster lung V79-379A fibroblasts *in vitro* | + | NT | 0.34 | *Prise et al. (1989)* |
| DIA, DNA single-strand breaks, Chinese hamster ovary CHO cells *in vitro* | + | NT | 0.85 | *Cantoni et al. (1992)* |
| DIA, DNA single-strand breaks, Chinese hamster ovary CHO cells *in vitro* | + | NT | 0.68 | *Iliakis et al. (1992)* |
| G9H, Gene mutation, Chinese hamster lung V79 cells, hprt locus *in vitro* | – | NT | 12 | *Bradley et al. (1979)* |
| G9H, Gene mutation, Chinese hamster lung V79 cells, hprt locus *in vitro* | – | NT | 20 | *Bradley & Erickson (1981)* |
| G9H, Gene mutation, Chinese hamster lung V79 cells, hprt locus *in vitro* | – | NT | 3.4 | *Tsuda (1981)* |
| G9H, Gene mutation, Chinese hamster lung V79 cells, hprt locus *in vitro* | – | NT | 7 | *Nishi et al. (1984)* |
| G9H, Gene mutation, Chinese hamster lung V79 cells, hprt locus *in vitro* | – | NT | 13.6 | *Speit (1986)* |
| G9H, Gene mutation, Chinese hamster lung V79 cells, hprt locus *in vitro* | + | NT | 17 | *Ziegler-Skylakakis & Andrae (1987)* |
| G9O, Gene mutation, Chinese hamster lung V79 cells, ouabain resistance in vitro | – | NT | 3.4 | *Tsuda (1981)* |
| G51, Gene mutation, mouse lymphoma L5178Y cell subline LY-R, *hprt locus in vitro* | (+) | NT | 0.17 | *Kruszewski et al. (1994* |
| G51, Gene mutation, mouse lymphoma L5178Y cell subline LY-S, *hprt locus in vitro* | (+) | NT | 0.34 | *Kruszewski et al. (1994* |
| SIC, Sister chromatid exchange, Chinese hamster lung V79 cells *in vitro* | (+) | NT | 12 | *Bradley et al. (1979)* |
| SIC, Sister chromatid exchange, Chinese hamster ovary CHO cells *in vitro* | + | NT | 0.13 | *MacRae & Stich (1979)* |
| SIC, Sister chromatid exchange, Chinese hamster ovary CHO cells *in vitro* | (+) | NT | 17 | *Wilmer & Natarajan (1981)* |
| SIC, Sister chromatid exchange, Chinese hamster lung V79 cells *in vitro* | + | NT | 3.4 | *Speit et al. (1982)* |
| SIC, Sister chromatid exchange, Chinese hamster lung V79 cells *in vitro* | + | (+) | 0.34 | *Mehnert et al. (1984a)* |
| SIC, Sister chromatid exchange, Chinese hamster ovary CHO cells *in vitro* | + | (+) | 0.34 | *Mehnert et al. (1984a)* |
| SIC, Sister chromatid exchange, Chinese hamster lung V79 cells *in vitro* | (+) | NT | 7 | *Nishi et al. (1984)* |
| SIC, Sister chromatid exchange, Chinese hamster lung V79 cells *in vitro* | + | NT | 0.68 | *Speit (1986)* |
| SIC, Sister chromatid exchange, Chinese hamster ovary CHO AU × 91 cells in vitro | + | NT | 1.4 | *Tucker et al. (1989)* |
| MIA, Micronucleus test, C57BL/6J mouse splenocytes in vitro | – | NT | 0.68 | *Dreosti et al. (1990)* |
| CIC, Chromosomal aberrations, Chinese hamster ovary CHO cells | (+) | NT | 10 | *Stich et al. (1978)* |

**Table 3.1 (contd)**

| Test system | Result[a] | | Dose[b] | Reference[c] |
|---|---|---|---|---|
| | Without exogenous metabolic activation | With exogenous metabolic activation | (LED or HID) | |
| CIC, Chromosomal aberrations, Chinese hamster DON-6 cells *in vitro* | + | NT | 34 | *Sasaki et al. (1980)* |
| CIC, Chromosomal aberrations, Chinese hamster ovary CHO-K1 cells *in vitro* | + | NT | 3.4 | *Tsuda (1981)* |
| CIC, Chromosomal aberrations, Chinese hamster lung V79 cells *in vitro* | + | NT | 3.4 | *Tsuda (1981)* |
| CIC, Chromosomal aberrations, Chinese hamster ovary CHO cells *in vitro* | (+) | NT | 340 | *Wilmer & Natarajan (1981)* |
| CIC, Chromosomal aberrations, Chinese hamster ovary CHO cells *in vitro* | (+) | NT | 1 | *Hanham et al. (1983)* |
| CIM, Chromosomal aberrations, newborn BALB/c mouse back-skin cells *in vitro* | + | NT | 0.34 | *Tsuda (1981)* |
| CIS, Chromosomal aberrations, Syrian hamster lung cells *in vitro* | + | NT | 3.4 | *Tsuda (1981)* |
| DIH, DNA single-strand breaks, transformed human WI-38 & XP cells *in vitro* | (+) | NT | 3.4 | *Hoffmann & Meneghini (1979)* |
| DIH, DNA single-strand breaks, human D98/AH2 cells *in vitro* | + | NT | 2 | *Wang et al. (1980)* |
| DIH, DNA single-strand breaks, human epithelioid P3 cells *in vitro* | + | NT | 0.21 | *Peak et al. (1991)* |
| DIH, DNA single-strand breaks, human cells *in vitro* | + | NT | 0.85 | *Meyers et al. (1993)* |
| DIH, DNA single-strand breaks, human leukocytes *in vitro* | + | NT | 17 | *Rueff et al. (1993)* |
| DIH, DNA damage, human bronchial epithelium (HBEI) cells *in vitro* | + | NT | 1.7 | *Spencer et al. (1995)* |
| DIH, DNA damage, human bronchial epithelium (BEAS and NHBE) cells *in vitro* | + | NT | 0.68 | *Lee et al. (1996)* |
| DIH, DNA damage, human lymphobastoid (GM1899A) cells *in vitro* | +[e] | NT | 0.34 | *Duthie & Collins (1997)* |
| UHF, Unscheduled DNA synthesis, human fibroblasts *in vitro* | + | NT | 20 | *Stich et al. (1978)* |
| UHF, Unscheduled DNA synthesis, human fibroblasts *in vitro* | + | NT | 9 | *Coppinger et al. (1983)* |
| SHL, Sister chromatid exchange, human lymphocytes *in vitro* | + | (+) | 2.7 | *Mehnert et al. (1984b)* |
| CHF, Chromosomal aberrations, human fibroblasts *in vitro* | + | NT | 0.07 | *Parshad et al. (1980)* |
| CHL, Chromosomal aberrations, human lymphocytes *in vitro* | – | NT | 0.17 | *Smith et al. (1990)* |
| CIH, Chromosomal aberrations, human embryonic fibroblasts *in vitro* | + | NT | 0.34 | *Oya et al. (1986)* |
| CIH, Chromosomal aberrations, human lymphocytes *in vitro* | + | NT | 510 | *Rueff et al. (1993)* |
| AIH, Aneuploidy, human lymphocytes *in vitro* | ? | NT | 0.17 | *Smith et al. (1990)* |
| CBA, Chromosomal aberrations, rat bone-marrow cells *in vivo* | – | | NG | *Kawachi et al. (1980)* |
| CVA, Chromosomal aberrations, mouse ascites tumour cells *in vivo* | + | | 340 µg/mouse | *Schöneich (1967)* |
| CVA, Chromosomal aberrations, mouse ascites tumour cells *in vivo* | + | | 170 µg/mouse | *Schöneich et al. (1970)* |
| BID, DNA binding (covalent), 8-hydroxydeoxyguanosine, BALB/c mouse keratinocytes *in vitro* | – | NT | 680 | *Beehler et al. (1992)* |
| ICR, Inhibition of cell communication, WB-Fischer 344 rat liver epithelial cells *in vitro* | + | NT | 3.4 | *Upham et al. (1997)* |

[a] +, positive; (+), weakly positive; –, negative; NT, not tested; ?, inconclusive [b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw/day; inj, injection; NG, not given
[c] The full references of the articles in Tables 3.1 and 3.2 are given separately at the end of the Opinion [d] Negative for DNA–DNA and DNA–protein cross-links [e] Positive at 50 µM (1.7 µg/mL) for HeLa, CaCo-2 colon cells and HepG2 liver cells.

**Table 3.2:** *Summary of in vivo genotoxicity of hydrogen peroxide (ECB, 2003)*

| Species and strain | Type of study<br>Measured endpoint | Exposure data<br>Test conditions | Result | Remark | Reference [a] |
|---|---|---|---|---|---|
| Mouse Swiss HIM/OG1 | Host mediated assay with intraperitoneally inoculated Salmonella typhimurium strains TA1530, G46 | dosing: 0.003, 0.3 or 3.0% $H_2O_2$ in milk for one week. 0.5 ml 0.3% $H_2O_2$ twice by gavage with a 2 h interval | negative; $H_2O_2$ in milk positive; pure H2O2 | a strong positive response for TA1530, a weak one for G46. | *Keck & Binder (1980)* |
| Mouse inbread strain AB Jena Gat. | cytogenetic assay with intraperitoneally inoculated tumour cells (S2 sarcoma, Ehrlich ascites, sarcoma 180) | dosing: 1 ml of 0.01, 0.05, 0.1 or 0.5 M $H_2O_2$ i.p. 48 h after the implantation of the tumour cells. chromosomes were studied 48 h after the treatment. | increased chromatid aberrations | local effect; response presumed to depend on the presence or absence of RBCs. | *Schöneich (1967)* |
| Rat Wistar, male | *In vivo - in vitro hepatocyte unscheduled DNA synthesis (UDS)* | $H_2O_2$ dosing: 0, 25 or 50 mg/kg by intravenous infusion of 0%, 0.1% or 0.2% water solution at a rate of 0.2 ml/min during approximately 30 min (=MTD) | Negative | exposure duration limited to 30 min. | *CEFIC (1997)* |
| Mouse Swiss HIM/OF1 | micronucleus assay of bone marrow polychromatic erythrocytes | dosing: 0.003, 0.3 or 3.0% $H_2O_2$ in milk for 32 h (apparently also in water, % not given) | Negative | oral route, reporting unclear and incomplete. | *Keck & Binder (1980)* |
| Mouse strain unkown | micronucleus assay of bone marrow polychromatic erythrocytes | single intraperitoneal injection of ½, 1/5, 1/25 or 1/100 LD50 dose of $H_2O_2$ | Negative | no experimental details given | *Liarskii et al. (1983)* |
| Mouse C57BL/6NCr1BR | micronucleus assay of bone marrow polychromatic erythrocytes | $H_2O_2$ in drinking water at 0, 200, 1,000, 3,000 or 6,000 ppm for 2 weeks. Doses males: 0, 42.4, 164, 415 or 536 mg/kg bw/day; females: 0, 48.5, 198, 485 or 774 mg/kg bw/day. | negative, P/N ratio was not changed | oral route | *Du Pont (1995)* |
| Mouse Swiss OF1/ICO:OF1 (IOPS Caw) | micronucleus assay of bone marrow polychromatic erythrocytes | dosing: 0, 250, 500 or 1,000 mg/kg i.p. (25 ml/kg: 0, 1, 2 or 4% $H_2O_2$, respectively) Time of harvest 24 or 48 h | negative, P/N was lower at 24 h, and at 48 h in the 250 and 1,000 mg/kg groups | single i.p. injection | *CEFIC (1995)* |
| *Drosophila melanogaster* | Drosophila SLRL test | single dose of 3% $H_2O_2$ injected into male larvae | Negative | | *DiPaolo (1952)* |

## Table 3.2 (contd)

| Species, strain, sex | Type of study, measured endpoint | Test conditions | Result | Remark | Reference [a] |
|---|---|---|---|---|---|
| Mouse Sencar Female | Pre-screen for carcinogenicity in target tissue (mouse skin)<br><br>quantity of 8-OH-2'-deoxyguanosine (DNA damage)<br><br>mutations in codon 61 of c-Ha-ras gene<br><br>epidermal hyperplasia and dermal cellularity changes | Hydrogen peroxide 70% was applied to the skin of 10 female Sencar mice per dose group at dose levels of 10, 100, or 200 µmol in 200 µl of ethanol (i.e. 0.2-3.2% solutions) twice weekly for 4 weeks. Mice treated under the same conditions with DMBA (10 or 100 µmol/animal) or ethanol (200 µl) acted as positive and negative controls, respectively. The animals wer killed on days 2 or 4 after the last administration (5 mice on each day). The application sites were removed and after fixation and staining, epithelial and dermal thickness and dermal cellularity were determined visually by light microscopy. Non-phenol extraction of fresh frozen tissue was used to isolate DNA from animals killed 2 days after last dosing, and following digestion to nucleosides, 8-OH-2'-deoxyguanosine (8-OH-dG) was quantified by HPLC. mutations in codon 61 of c-Ha-ras gene were determined using DNA isolated from paraffin blocks of whole skin. | negative for all enpoints | at the relatively low concentrations used hydrogen peroxide did not induce local in vivo genotoxicity and mutagenicity in the skin. | *Society for Plastic Industry (1997)* |

[a] The full references of the articles in Tables 3.1 and 3.2 are given separately at the end of the Opinion

In addition to the studies reported above it has been found that hydrogen peroxide in concentrations of 0.2 μg/ml induces cell transformation in the Syrian hamster embryo assay (Mikalsen et al., 1990). Hydrogen peroxide enhanced N-methyl-N-nitrosourea (MNU)-initiated transformation of MYP3 cells, an anchorage-dependent non-tumorgenic rat bladder epithelial cell line. Moreover, hydrogen peroxide treatment alone also caused transformation. The transformants induced by MNU plus hydrogen peroxide or hydrogen peroxide alone formed high-grade transitional cell carcinomas when injected into nude mice (Okamato et al., 1996). Hydrogen peroxide inhibited gap junction intercellular communication (GJIC) in WB-F-344 rat liver epithelial cells with an $I_{50}$ of 6.8 μg/ml. The results indicated that the effects were not caused by free radical damage (Upham et al., 1997). In other systems it has been found that hydrogen peroxide enhances GJIC (Mikalsen and Sanner, 1994).

**Tooth whiteners**

The genotoxicity of tooth whiteners has been investigated in a number of studies. Two studies (Adam-Rodwell et al., 1994; Lee [Report], 1996) found that tooth whiteners containing 10% carbamide peroxide were not mutagenic in the Salmonella test. Other studies showed a dose response effect of tooth whiteners containing 10% carbamide peroxide in TA102 when tested without S9 (Li et al. [Abstract], 1992; Li [Report], 1997). In the test with S9, the tooth whiteners were not mutagenic. When comparing data obtained from hydrogen peroxide and carbamide peroxide examined in the same test, the observed effect of tooth whiteners appears to be associated with their peroxide contents (Li et al. [Abstract], 1992; Li [Report], 1997). The effect of four bleaching agents containing hydrogen peroxide or carbamide peroxide was studied in different *E. coli* strains with various capabilities to repair damages to DNA. The bleaching agents tested decreased the survival fractions of all strains studied and the effect was greatest on the strains with the lowest ability to repair DNA damage. The authors conclude that the results on dental bleaching agents generate biological effect like the ionising radiations and that their use must be strictly controlled by a dentist in order to prevent any contact with gingival and mucous tissues (Zouain-Ferreira et al., 2002).

*Ribeiro and co-workers (2005, 2006)* have recently assessed the genotoxicity of six commercial dental bleaching agents by the single cell gel (comet) assay *in vitro* with mouse lymphoma cells and Chinese hamster ovary (CHO) cells. All compounds tested induced DNA damage as depicted by the mean tail moment. The strongest effect was observed with the highest dose of hydrogen peroxide (35%). The authors concluded that dental bleaching agents may be a factor that increases the level of DNA damage.

Whitening gel containing hydrocarbon-oxo-borate complex were compared with commercial hydrogen peroxide and carbamide peroxide products. The effects of human epithelial cell line for induction of DNA damage and subsequent induction of apoptosis and necrosis has been studied. The study was used in MCF-7 (human breast cancer cells). The results show that the two hydrogen peroxide and the one carbamide peroxide based products induce significant DNA breakdown in MCF-7 cells while the hydrocarbon-oxo-borate complex showed much less DNA breakdown even at the highest concentration. While the hydrogen peroxide and carbamide peroxide bleaching agents induced massive necrosis at both 1 mg/ml and 10 mg/ml and no induction of apoptosis, the borate gel induced physiological cell death (apoptosis), both at 1 mg/ml and 10 mg/ml, while virtually no necrosis was found (Li and Ramaekers, 2004).

Several *in vivo* studies on peroxide containing tooth whiteners detected no genotoxicity. No increased frequency of micronuclei was observed in bone marrow cells of mice that were gavage-fed with two solutions containing 10% carbamide peroxide (Woolveton et al., 1993). Three tooth whiteners containing 10% carbamide peroxide did not increase the SCE

frequency in bone marrow cells of Chinese hamsters and mice after the animals received doses up to 10 g/kg (Li et al. [Abstract], 1992, 1993; Lee [Report], 1996). Also using the SCE assay, a tooth whitener paste containing 10% carbamide peroxide was found to be non-genotoxic when administered to rats at doses ranging from 0.1 to 1.0 g/kg for 5 days (Adam-Rodwell et al., 1994). A long term study showed that oral administration of tooth whiteners of 10% carbamide peroxide up to 2 g/kg daily on week days for 3 or 6 months did not affect the SCE frequency of bone marrow cells of Chinese hamsters (Li et al.[Abstract], 1993).

**Conclusion of the European Chemicals Bureau on the mutagenicity of hydrogen peroxide** (ECB, 2003)

Hydrogen peroxide is a mutagen and genotoxicant in a variety of *in vitro* test systems. The responses observed were modified by the presence of degrading enzymes (catalase), the extent of formation of hydroxyl radicals by Fenton reaction, and the cells repair abilities.

Hydrogen peroxide has been studied for possible *in vivo* genotoxicity. Studies employing modern methodologies have explored DNA repair in liver cells of rats administered hydrogen peroxide by intravenous infusion for 30 minutes (CEFIC, 1997), as well as micronucleus formation in mice in the context of a 2-week drinking water exposure (Du Pont, 1995), or after a single intraperitoneal injection (CEFIC, 1995), all with a negative outcome. Intravenous administration of hydrogen peroxide in the *in vivo-in vitro* unscheduled DNA synthesis study ensured that the substance had a fair chance to reach the target (liver) cells, although the duration of exposure was limited (CEFIC, 1997). In the micronucleus study by oral drinking water exposure (Du Pont, 1995), the systemic fate of hydrogen peroxide was uncertain, and there was no decrease in the ratio of polychromatic/normochromatic erythrocytes in the bone marrow. In the other micronucleus study (CEFIC, 1995), a single intraperitoneal injection of a large dose of hydrogen peroxide somehow affected the bone marrow (because the PE/NE decreased), but the absence of micronucleus formation must be viewed with caution because of the presumably very short lifetime of hydrogen peroxide. With a view to exploring target tissue *in vivo* genotoxicity and mutagenicity as a pre-screen for carcinogenicity, hydrogen peroxide 0.2-3.2% solutions in ethanol were applied to the skin of Sencar mice twice weekly for 4 weeks (Society for Plastic Industry, 1997). There was no indication of induced DNA damage (increased 8-OH-dG), c-Ha-ras mutations, epidermal hyperplasia and dermal cellularity changes. Thus, at low concentrations, and with a low application frequency, hydrogen peroxide did not induce local mutagenicity in this tissue model.

In conclusion, the available studies are not in support of a significant genotoxicity/mutagenicity for hydrogen peroxide under *in vivo* conditions. A wider database of genotoxicity and mutagenicity observations on other relevant target tissues in direct contact with hydrogen peroxide is, however, desirable. Mechanistic studies suggest that cells are adapted to repair DNA damage caused by oxidants; on the other hand there is some evidence that hydrogen peroxide may inhibit the repair of DNA lesions inflicted by other types of reactive chemicals (Churg et al., 1995, Pero et al., 1990, Hu et al., 1995).

According to the principles followed in the EU, hydrogen peroxide is not classified as a mutagen.

#### 3.3.6.1. Comment on the Conclusions of the European Chemicals Bureau

The SCCP endorses the conclusions of the European Chemicals Bureau on hydrogen peroxide.

3.3.7. Carcinogenicity

**Hydrogen peroxide**

***Mice***

Oral

C57BL/6J mice, groups of 50 males and 50 females (eight weeks old) were given 0 (control), 0.1, and 0.4% hydrogen peroxide in drinking water for 100 weeks. The bodyweight of the hydrogen peroxide treated groups were comparable to those of control mice except for a slight decrease in the bodyweight of females of the 0.4% group at 15 months of age. Survival among control mice (54%) was lower than for mice treated with hydrogen peroxide (63% for high dose and 61% for low dose). An increased frequency of tumours in the duodenum was found (Table 3.3) (Ito et al., 1981).

**Table 3.3:** *Incidence of gastro-duodenal lesions in C57BL/6J mice after receiving hydrogen peroxide in the drinking water (Ito et al., 1981)*

| Concentration in drinking water (%) | Duodenum | | | | | |
|---|---|---|---|---|---|---|
| | Hyperplasia | | Adenoma | | Carcinoma | |
| | Males | Females | Males | Females | Males | Females |
| 0 | 2 (14%) | 7 (14%) | 0 (2%) | 1 (2%) | 0 (0%) | 0 (0%) |
| 0.1 | 16 (48%) | 24 (48%) | 2 (4%) | 4 (8%) | 1 (2%) | 0 (0%) |
| 0.4 | 30 (63%) | 31 (63%) | 2 (4 %) | 0 (0%) | 1 (2%) | 4 (8%) |

When the data for male and female mice were combined (Ito et al., 1981), there was a statistically significant increase in the incidence of duodenal carcinomas, but when treated separately and analysed statistically with Fisher's Exact Test, there was no significant difference between dosage groups. Ito et al. (1981) reported invasion of the duodenal carcinomas into the muscular layer and small vessels, but no metastatic tumours were evident. No treatment-related tumours were noted elsewhere. The latency of tumour induction was decreased in the treated mice, the first lesion occurring at about 42 weeks in mice treated with 0.4% $H_2O_2$. The decreased latency was based on animals that died and not those from interim kills. The authors suggested that the neoplastic nodules developed mainly in the duodenum because $H_2O_2$ is unstable under alkaline conditions.

A group of 138 male and female C57BL/6N mice was treated with 0.4% hydrogen peroxide in the drinking water. Groups of 5-17 mice were killed sequentially at 30-day intervals up to 210 days and then every 60, 70 or 90 days up to 630 days; 29 mice were killed on day 700, when the experiment was terminated. Gastric erosions appeared at the first kill (30 days) and were present consistently at each subsequent kill. "Nodules" (hyperplastic lesions, adenomas and carcinomas) were found in both duodenum and stomach from 90 days until the end of the experiment, but not on days 210 and 360 in the stomach. The lesions did not appear to increase in frequency, but atypical hyperplasia appeared late in the experiment, and 5% of the animals developed duodenal adenocarcinoma. No such lesion was observed in controls. The reversibility of the lesions was investigated in groups of mice treated with 0.4% hydrogen peroxide for 120, 140, 150 or 180 days after a treatment-free period of 10-30 days. The stomach lesions regressed completely, irrespective of length of treatment, but some of the duodenal lesions persisted. Groups of 22 DBA/2N, 39 BALBcAnN and C57BL/6N mice of both sexes were given 0.4% hydrogen peroxide in drinking water. The mice were examined sequentially from 90 to 210 days of treatment for strain differences in the development of gastric and duodenal "nodules" (hyperplastic lesions, adenomas and carcinomas). The incidences of gastric nodules were 2/22 (9%), 1/39 (3%) and 12/34

(35%), and those of duodenal nodules were 14/22 (64%), 7/39 (18%) and 22/34 (65%) in DBA, BALB/c and C57BL mice, respectively. The duodenal nodules appeared at 90 days in all three strains (Ito et al., 1982).

Groups of 18-24 female C3H/HeN, B6C3F1 and C3H/$C_s$ mice with different levels of catalase activity in the duodenal mucosa were given 0.4% hydrogen peroxide in drinking-water for 6 or 7 months. The incidence of duodenal "nodules" (hyperplastic lesions, adenomas and carcinomas) is shown in Table 3.4 (Ito et al., 1984). (The IARC Group noted that the pathology of the tumours was not well documented.)

**Table 3.4:** *Incidence of duodenal tumours in 4 strains of female mice treated with 0.4% $H_2O_2$ in drinking water (Ito et al., 1984)*

| Strain | Number of mice | Catalase activity ($10^{-4}$ k/mg protein) | Number of mice with tumours (% incidence) | Total number of tumours |
|---|---|---|---|---|
| **C3H/HeN** | 18 | 5.3 | 2 (11.1%) | 2 |
| **B6C3F1** | 22 | 1.7 | 7 (31.8%) | 8 |
| **C57BL/6N** | 21 | 0.7 | 21 (100 %) | 82 |
| **C3H/C** | 24 | 0.4 | 22 (91.7%) | 63 |

***Rats***
Oral

Hydrogen peroxide was administered to Fischer 344 rats in drinking water at concentrations of 0%, 0.3% or 0.6% for 78 weeks followed by a 6-month recovery phase. Survival was similar to that of the controls (41/50), except for male rats in the 0.3% group (approximately 30% mortality; 36/50 alive at 97 weeks). Tumours of the testes, mammary gland and skin were observed in rats that died during the study; there were no differences in tumour incidence between control and treated rats. After 45 weeks of administration, body weight was decreased by about 6% in male and female rats in the 0.3% group and 10% in the 0.6% group. Nasal bleeding was observed in the treated groups; the significance of this is uncertain. At the end of the study (104 weeks), all surviving animals were killed. No significant differences were observed between treated rats and controls relative to the incidence and types of tumours. The authors concluded that, under the conditions of this study, hydrogen peroxide was not carcinogenic to Fischer 344 rats. Because this study was not published in detail, its quality cannot be assessed. Furthermore, no account was taken of other measurements made during the study, and a full characterisation of the pathological changes was not given (Ishikawa and Takayama [Abstract], 1984).

In other studies, forestomach papillomas were observed in rats exposed to hydrogen peroxide in drinking water (1%) (see Takahashi et al., 1986 [below]).

**Hydrogen peroxide in initiation – promotion experiments**

***Mice***
Skin painting

Groups of 60 female Sencar mice, aged 7 to 9 weeks, were used to test the tumour-promoting (A), tumour-initiating (B) and complete carcinogenic (C) activity of hydrogen peroxide on the skin. Mice in experiment (A) received a single topical application of 10 nmol

DMBA in 0.2 ml acetone, followed one week later by applications of a 30% solution of hydrogen peroxide diluted 1:1 (15%) (once and twice weekly), 1:2 (10%) or 1:5 (5%) in 0.2 ml acetone twice weekly for 25 weeks. Controls received acetone alone. The proportions of mice with papillomas at 25 weeks in experiment A (tumour-promoting) were 0/60 (0%) (controls), 3/58 (5%; 5%HP), 5/59 (9%; 10%HP), 6/59 (10%; 15%HP once weekly) and 6/60 (10%; 15%HP twice weekly), respectively. Mice in experiment B (tumour-initiating) received a single topical application of hydrogen peroxide diluted 1:1 (15%) in 0.2 ml acetone, or acetone alone (controls), followed one week later by twice-weekly applications of 2 μg 12-O-tetradecanoylphorbol 13-acetate (TPA) in acetone for 25 weeks. Papillomas were found after 25 weeks in 3/56 (5%) and 6/58 (10%) control and hydrogen peroxide-treated animals, respectively. Mice in experiment C (complete carcinogen) received twice-weekly topical applications of hydrogen peroxide diluted 1:1 (15%) in 0.2 ml acetone for 25 week; 3/57 (5%) had papillomas at that time. No squamous-cell carcinoma was found when these animals were observed up to 50 weeks (Klein-Szanto and Slaga, 1982) (The IARC Working Group noted the absence of a DMBA-treated control group for the promotion experiment and the short duration of the experiment for complete carcinogenicity evaluation).

*Comment*
*Mahony et al (2006)* note that the authors of the report had stated that *"hydrogen peroxide has extremely weak tumour promoting activity"* Due to the lack of a DMBA-treated control group it is not possible to determine to potency of hydrogen peroxide as a promoter.

In similar studies, mice were treated dermally for up to 58 weeks with 3% or 5% hydrogen peroxide following initiation with DMBA (Shamberger, 1972; Bock et al., 1975; Kurokawa et al., 1984). In these studies there were no significant increases in the incidence of skin tumours, although epidermal hyperplasia was evident in most of the mice treated.

***Rats***
Oral

Takahashi et al. (1986) examined the potential of hydrogen peroxide to promote N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) initiated gastric tumours in rats. Two groups of rats (n=30 and 21) received MNNG-treated drinking water and food supplemented with 10% sodium chloride, the water of one group being supplemented with 1% hydrogen peroxide for 7 weeks *ad libitum* after which the animals were maintained on normal food and tap water. A third group (n=10) was not given MNNG or a sodium chloride supplemented diet, but was administered 1% hydrogen peroxide in the drinking water.

**Table 3.5:***Effect on gastro-duodenal carcinogenesis induced by MNNG (Takahashi et al, 1986)*

| Treatment group (n) | Forestomach papillomas | Fundus hyperplasia[a] | Glandular stomach | | Duodenum adeno-carcinoma |
|---|---|---|---|---|---|
| | | | Pylorus adeno-carcinoma | Preneoplastic hyperplasia | |
| MNNG controls (30) | 0 (0%)[b] | 0 (0%) | 1 (3.3%) | 7 (23.3%) | 3 (10%) |
| MNNG-H2O2 (21) | 21 (100%) | 8 (38.1%) | 2 (9.5%) | 6 (28.6%) | 0 (0%) |
| H2O2 (10) | 5 (50%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) |

[a] Adenomatous hyperplasia
[b] Number of rats with tumours (%)

Adenocarcinomas were observed in the pyloric stomach and duodenum of the MNNG-treated rats, and "preneoplastic hyperplasia" was observed in the pylorus (Table 3.5). In rats treated with MNNG and hydrogen peroxide, there was no enhancement in the number of gastrointestinal tumours, although all treated animals exhibited forestomach papillomas; these also occurred in rats treated only with hydrogen peroxide in the drinking water. No carcinoma development was noted in the stomach or duodenum. Erosions and ulcerations also occurred in the fundic mucosa of the stomach of the hydrogen peroxide treated rats. The authors concluded that, in contrast to the study of Hirota and Yokoyama (1981, see below), no enhancement of duodenal tumours occurred, although characteristic diffuse lesions, showing fusion of the villi, were observed throughout the duodenum.

In a study (Hirota and Yokoyama, 1981) of promoting effects in intestinal carcinogenesis, groups of 3 or 8 male F344 rats were administered 1.5% $H_2O_2$ in drinking water either with or without methylazoxymethanol acetate (MAM) treatment (three i.p. injections of 25 mg/kg bw every other week) for 10 or 21 weeks; 3 control rats received water. Rats given $H_2O_2$ four weeks prior to MAM injections, during intervals between injections, and until the termination of the study showed higher incidences of duodenal (8/8, 100%) and jejunal (5/8, 63%) carcinomas when compared to rats otherwise similarly treated but not given $H_2O_2$ subsequent to MAM injections (2/8, 25% and 2/8, 25%, respectively). The three rats given $H_2O_2$ alone throughout the study period did not develop carcinomas in the studied organs; there was no group of animals receiving MAM alone. Only gross tumours of the g-i tract were reported. The authors concluded that hydrogen peroxide had a tumour promoting effect on MAM-initiated intestinal tumours. Because of the lack of a MAM control group and details of the method, it is not possible to evaluate this study.

***Hamsters***

Buccal pouch painting

DMBA and/or hydrogen peroxide was painted onto the left buccal pouch of 4 groups of male Syrian golden hamsters twice weekly for 19 or 22 weeks. Animals in Group A were painted twice weekly with a 0.25% solution of DMBA in heavy mineral oil. Animals in Group B were painted twice weekly with DMBA and twice weekly (on days other than the DMBA painting) with 3% hydrogen peroxide. Group C was painted in exactly the same way as Group B animals except that the concentration of hydrogen peroxide used was 30%. Group D animals were painted twice weekly with 30% hydrogen peroxide alone. Cheek pouches from animals that had not been painted and from animals that had been painted twice weekly with only the mineral oil vehicle, served as controls. Six of 11 hamsters (55%) treated with DMBA and 3% $H_2O_2$ developed epidermoid carcinomas by 22 weeks, whereas all 5 (100%) hamsters treated with DMBA and 30% hydrogen peroxide developed epidermoid carcinomas by 22 weeks. No carcinomas were observed in hamsters treated with 30% hydrogen peroxide alone, but 3/7 (43%) of the hamsters treated with DMBA alone developed carcinomas. In all hamsters, chronic inflammation, hyperchromatic cells and dysplasia were also noted at 19 weeks. The authors concluded that long term, twice weekly application of 3% or 30% hydrogen peroxide could induce inflammatory changes, but that pathological changes associated with preneoplastic lesions and augmentation of the oral carcinogenesis of DMBA were observed only with 30% hydrogen peroxide. The experiment demonstrated a promoting effect of hydrogen peroxide (Weitzman et al., 1986).

*Marshall et al. (1996)* used groups of 25 hamsters of each sex. The cheek pouches of one group were exposed to a solution containing 0.75% hydrogen peroxide together with 5% baking soda 5 times per week for 20 weeks. Another group received the 0.5% DMBA together with 5% baking soda. A third group received a commercial dentifrice containing 3% hydrogen peroxide together with 0.5% DMBA. A control group received 0.1 ml mineral oil. At the end of the treatment 0/50 (0%) of the first group developed tumours, while

40/50 (80%) of the second group developed tumours. 37/50 (74%) of the third group developed tumours.

In a second phase of the previous study, *Marshall et al. (1996)* applied 1.5% hydrogen peroxide in dentifrice formulation (single or dual phase) mixtures with sodium bicarbonate (7.5%) to the cheek pouches of groups of 25 male and 25 female hamsters. Another group of hamsters was exposed to a solution of 3% hydrogen peroxyde/7.5% baking soda. In each of these groups, the cheek pouches were co-treated with DMBA at concentrations of either 0.25% or 0.5%. DMBA applications were made 3 times per week while the dentifrice/solutions were administered 5 times per week. In this second phase, there was no hydrogen peroxide/baking soda exposure only group. Since the cheek pouch carcinoma incidence was close to 100% in the DMBA-only groups as well as in the DMBA/hydrogen peroxide groups, this phase of the study was not capable of detecting any potential enhancing effect of hydrogen peroxide.

**Carbamide peroxide**

***Mice***

Skin painting

In a skin painting experiment 30 female Swiss mice (55-69 days old) were painted once with 125 μg DMBA and after 3 weeks treated 5 times weekly with 5% carbamide peroxide in water. No tumours were found when the experiments were terminated after 56 weeks of promoting stimulus. In a similar experiment with 0.1% perbenzoic acid in acetone, about 40% developed skin tumours and 10% skin cancer (Bock et al., 1975).

***Hamster***

Buccal pouch painting

A hamster cheek pouch study was done by *Collet et al. (2001)* with a 10% carbamide peroxide (~3.3% hydrogen peroxide) tooth bleaching gel. Tumours were apparent in all male hamsters (10/10; 100%) that received an oral cavity application of 0.5% DMBA three times a week for 14 weeks. No tumours were observed in hamsters treated with a combination of 0.5% DMBA twice a week and the 3.3% hydrogen peroxide gel once a week for 34 weeks or in hamsters treated with only the 3.3% hydrogen peroxide gel three times a week for 34 weeks.

**Tooth whiteners**

***Human case report***

In a press release from American Head and Neck Society a study presented at the 6th International Conference on Head and Neck Cancer is reported. Patients with primary oral cancer diagnosed at Georgetown University Medical Center between 1997 and 2003 were identified. Nineteen patients agreed to participate in the study. Three (16 percent) of patients reported a history of tooth whitener use in the past. There was no significant difference in age at diagnosis between the patients who used and did not use tooth whiteners, however the tooth whitener users tended to be younger (mean age 34.3 vs. 52.4, $p = 0.11$). Alcohol use and smoking history were similar in the two groups. The patients who used tooth whiteners were more likely to present with regional lymph node disease, than those who did not use tooth whiteners. All three patients presented with node positive disease as opposed to 3 of 16 (19 percent) patients without a history of tooth whitener use. The authors point out that the data do not necessarily suggest a causative relationship between the use of these products and the development of oral cancer. However, free radicals generated in the whitening process have carcinogenic potential, and

therefore the use of these products in this patient population should be studied further (Burningham et al., 2004).

### 3.3.7.1. Discussion on carcinogenicity

Dermal exposure may be a useful model for addressing carcinogenicity in the oral cavity. The dose expressed as mg/cm$^2$ has been established for risk assessments focused on local effects following topical application of a chemical, e.g., contact sensitisation.

Dose per unit area in dermal tumour promotion assays (assuming application to 6 cm$^2$ area) is presented in Table 3.6 (data were given in Submission III and in *Mahony et al (2006)*. The experiment by Klein-Szanto and Slaga (1982) was not included in the submission and was added later by SCCP).

**Table 3.6:** Hydrogen peroxide exposure in mouse dermal and hamster check pouch tumour promotion assays following initiation by with DMBA

| Study | Application | Results | mg/cm$^2$/day |
|---|---|---|---|
| **Mice**<br>Bock et al., 1975 | 3% in 0.2 ml applied 5x/wk for 56 weeks | Negative | ~ 0.71 |
| Kurokowa et al., 1984 | 5% in 0.2 ml applied 2x/wk for 51 weeks | Negative | ~ 0.48 |
| Shamberger, 1972 | 3% in 0.25 ml applied daily for 40 weeks | Negative | ~ 1.25 |
| Burnett and Goldenthal, 1988 | 3% (+ hair dye base) in 0.5 ml applied twice weekly /two years). Successive applications made to adjacent areas to minimise skin irritation | Negative | ~ 0.71 |
| Klein-Szanto and Slaga, 1982 | 5% in 0.2 ml applied 2x/wk for 25 weeks, no tumours in the control. | 5% tumours | ~ 0.47 |
| **Hamster**<br>*Marshall et al., 1996* | 3% in 0.1 ml applied 5x/wk for 16 weeks | Inadequate | 0.43 |
| *Collett et al., 2001* | 3.3% [10% carbamide peroxide] in 0.5 ml applied 1x/wk for 34 weeks | Negative | 0.47 |

The doses shown refer to dermal applications in non-clinical studies evaluating tumour promotion potential. No carcinogenic effects were observed in the three first experiments. In the first hamster study it is not possible to determine if hydrogen peroxide had any promoting effect as the tumour frequency with DMBA alone was very high (80%). No tumours were found in the second hamster experiment.

In the study by Klein-Szanto and Slaga (1982) the tumour frequency was 5% in Sencar mice after initiation with the initiator DMBA followed skin painting with hydrogen peroxide for 25 weeks corresponding to about 0.47 mg/cm$^2$/day. The peak exposure in the saliva after teeth bleaching with 5% Whitestrip was 0.1% hydrogen peroxide (Hannig et al., 2003) corresponding to about 5 μg/cm$^2$ which is only about 100 times lower that the concentration inducing a tumour frequency of 5% in the mice skin painting study.

*Mahony et al (2006)* claim that 0.71 and 0.41 mg/cm$^2$ represent a NOEL for tumour promotion in mouse skin and hamster cheek, respectively. Based on an assumed maximal human exposure of 1.5 μg/cm$^2$, they calculated a MOS of 473 and 287 based on the mouse and hamster study, respectively. Based on the results of Klein-Szanto and Slaga (1982) as discussed above, these calculations can be questioned.

*Munro et al. (2006a,b)* are of the opinion that the available genetic toxicity and animal toxicology data do not indicate that hydrogen peroxide poses a carcinogenic risk to the human oral mucosa. They claim that their conclusion is supported by the results of the dosimetric exposure analyses from tooth whitening product users. *Tredwin et al. (2006)* and

*Naik et al. (2006)* on the other hand point out that several carcinogenesis studies, including the hamster cheek pouch model, indicate that hydrogen peroxide might possibly act as a promoter and that urgent clinical studies are required on the genotoxic and tumour-promoting effects of hydrogen peroxide bleaching agents. They conclude that in the light of the concern regarding the possible tumour-promoting ability of $H_2O_2$ with the tobacco carcinogen DMBA, patients should be advised to avoid smoking and alcohol.

ECB (2003) note that the weak effect found in complete carcinogenesis studies in mice as well as in some promotion studies suggest promotion type of activity and possible underlying genotoxic mechanisms.

A drinking water study in mice showed that hydrogen peroxide caused duodenal hyperplasia at a high frequency and localised duodenal carcinomas at a low frequency. A subsequent study with different strains of mice showed a strong negative correlation between incidence of duodenal tumours and catalase activity in duodenal mucosa. In one study with rats a high incidence of forestomach papillomas were found after receiving 1% hydrogen peroxide in the drinking water. While humans do not have a forestomach, they do have comparable squamous epithelium tissues in the oral cavity and the upper part of the oesophagus. Thus, in principle, carcinogens targeting the forestomach squamous epithelium rodents are relevant for humans. Also, the target tissues for carcinogens may differ between experimental animals and humans and a forestomach carcinogen in rodent may target a different tissue in humans (IARC, 2003). Some tumour promotion studies indicate that hydrogen peroxide may act as a promoter.

Hydrogen peroxide has a weak potential to induce local carcinogenic effects. The mechanism is unclear, but a genotoxic mechanism cannot be excluded. As regard to tumour promotion, several mechanisms might be operative; direct genotoxicity, impairment of DNA repair, and chronic inflammation.

### 3.3.8. Reproductive toxicity / oral

***Hydrogen peroxide***

***<u>Fertility</u>***

***Mice***

Wales et al. (1959) gave 0.33, 1 or 3% hydrogen peroxide in drinking water to three groups of 12 male albino mice. Solutions were changed twice weekly. The mice on the high level of peroxide (3%) refused to drink and after 5 days were removed from the experiment having lost about 20% of their body weight. The remaining two groups were each divided at random into four subgroups of 3 animals. Two female mice were placed with each male of the first subgroup on day 7 and again (with two other females) on day 28 after starting hydrogen peroxide. Two subgroups of males were placed with females on day 21: the animals in one of the groups continued on hydrogen peroxide, for the other group hydrogen peroxide was replaced with tap water (ensuring no consumption of hydrogen peroxide by the females). The fourth subgroup of three male mice was killed on day 21 and the epididymal spermatozoa were examined. The drinking water of three albino rabbits was also replaced with 0.33, 1 or 3% hydrogen peroxide and the semen was examined at weekly intervals for 6 weeks. All female mice mated to treated males became pregnant within a few days and in each case healthy young were born in litters of normal size. Pregnant mice that continued to consume 1% $H_2O_2$ in water up until near term showed some delay in parturition compared to dams using tap water (the effect was, however, small and inconsistent). The concentration, morphology and motility of the mouse spermatozoa (in three mice) after 3 weeks of treatment appeared normal. There were no detectable

abnormalities in the sperm of the three rabbits exposed for 6 weeks either. No firm conclusions can be drawn from this limited study which did not use any control animals, although any major deleterious effects by the treatment on reproduction seem to be excluded.

***Rats***

Three weanling Osborne-Mendel female rats were given 0.45% $H_2O_2$ in drinking water and maintained on it for 5 months. Thereafter they were given tap-water and mated with normal males. Six normal male litter mates were divided into two equal groups: one received 0.45% $H_2O_2$, while the other received tap water. These animals were maintained on their respective regimens for 9 months. Normal litters were produced, and thus long-term treatment with peroxide did not appear to affect the reproduction in female rats. Regarding observations made on the six male offspring that were followed for 9 months, the only noticeable effect was a difference in body weight: an average of 521 g for those on tap water against 411 g for those on $H_2O_2$ *(Hankin, 1958)*. No firm conclusions can be drawn from this restricted study with few animals.

Male and female rats were administered hydrogen peroxide daily by gavage at doses of 1/10-1/5 $LD_{50}$ (which was not specified) for 45 days. At the high dose, females showed modifications of the oestrus cycle and males reduced mobility of spermatozoa, without effects on the weight of the testicles. In a second experiment male and female rats received daily doses of 0.005, 0.05, 0.5, 5 and 50 mg hydrogen peroxide/kg bw by gavage for 6 months and were mated. Variations of the oestrous cycle in females were observed at 50 and 0.5 mg hydrogen peroxide/kg bw, but not at 5 mg/kg bw. Reduced mobility of spermatozoa in males was observed at 50 mg hydrogen peroxide/kg bw. No changes were observed in the morphology and weight of the testes. Among the high dose females, only 3/9 produced litters, compared to 7/9 in the control group. In addition, litter size and body weight gain of the offspring of the high dose females were reduced relative to those of control females (Antonova, 1974). The results of the study should be considered with caution because the information on the experiment is incomplete.

***Developmental***

One study which addresses developmental toxicity has been conducted with Wistar rats Moriyama et al., 1982). Aqueous solutions of hydrogen peroxide were mixed with powdered feed to 10, 2, 0.1, or 0.02% and administered to groups of 5-8 pregnant rats for one week during "the critical period of pregnancy". The foetuses were removed on day 20 for examinations (Study A). Separate dose groups of 2-3 rats were similarly treated, but the rats were allowed to go through normal delivery, and the offspring were followed-up for about four weeks (Study B). In Study A, at the high dose level the dam body weight did not increase markedly. Food consumption was reduced to about one third as compared to the other dose groups, for which there was no difference from controls. Foetal resorptions were increased and the foetal body weight was decreased; most of the foetuses were close to death. No external malformations were found in any of the dose groups. Haemorrhaging of internal organs (eye, parietal region of the brain, cardiopulmonary region, torso) was dose dependently increased in the dose range 0.1-10% $H_2O_2$. Skeletal hypoplasias occurred dose dependently at the two highest levels. In Study B, all the neonates of the 10% treatment group died within 1 week post partum, the body weights were low and the number of live births was decreased. In the other dose groups there were no major effects on the development of neonates. There are major uncertainties about the exposure and effect mechanism which cast doubt on the relevance of the study. $H_2O_2$ concentration in feed was reported to decrease to 1/10 after 24 hours and to virtually nil by 72 hours. The authors state that "the amount of residue was determined and consumption was estimated"; however, it is not stated how frequently fresh feed was prepared. Nevertheless, it seems likely that the dams indeed ingested hydrogen peroxide, and there was not much of an

increase in dam body weight at the top dose level. There was no marked difference between the groups in placental weight. The authors proposed that the observed effects on foetal development were due to the breakdown of essential nutrients in food by hydrogen peroxide.

***Tooth whitener***

In order to test the effect of ingested tooth whitener on early embryo development and growth, rats were intubated with 500 mg/kg whitener on day 2 of pregnancy. It was concluded that a) ingestion of tooth whitener containing 35% carbamide peroxide causes a loss of embryos sometimes between day 2 (treatment) and day 5 (collection), but that b) day 5 embryos have the same cell number both prior to and after 24 hour culture, and c) have the same ability to implant *in vitro* (Redmond et al. [Abstract], 1998).

### 3.3.8.1. Summary / Comment on Reproductive toxicity

No appropriate animal studies were available for a complete evaluation of reproductive and developmental toxicity. Limited studies with mice and rats exposed to hydrogen peroxide in drinking water suggested no grave disturbances on the male or female reproductive functions. The only available developmental toxicity study in Wistar rats which were fed on powdered feed mixed with hydrogen peroxide did show foetotoxic effects (Moriyama et al., 1982), but the study contains major uncertainties about the exposure and effect mechanisms. Although raising some further questions, the study cannot be used for an evaluation.

### 3.3.9. Toxicokinetics

Hydrogen peroxide is a normal metabolite in the aerobic cells. It is produced from superoxide anion spontaneously or as a result of the activity of superoxide dismutase (SOD) (EC1.15.1.1). Superoxide radical undergoes dismutation quickly and spontaneously, but the enzymatic process occurs at a rate that is $10^{10}$-fold faster. Eukaryotic cells contain two kinds of SOD that are highly specific for superoxide ($O_2$) as a substrate. Hydrogen peroxide occurs under most conditions at submicromolar concentrations.

$O_2 + e^- \rightarrow O_2^-\bullet$ (superoxide anion)

$O_2^-\bullet + e^- + 2H^+ \rightarrow H_2O_2$ (spontaneous dismutation to hydrogen peroxide)

$2O_2^-\bullet + 2H^+ \rightarrow H_2O_2 + O_2$ (SOD:superoxide dismutase)

Hydrogen peroxide passes readily across biological membranes. Because it reacts slowly with organic substrates, it can diffuse considerable distances in biological systems. There are two main hydrogen peroxide metabolising enzymes, catalase and glutathione peroxidase which control the hydrogen peroxide concentration. Catalase deals with large amounts of $H_2O_2$ that may be generated in peroxisomes. Glutathione peroxidase (GSH peroxidase) metabolises $H_2O_2$ in both the cytosolic and mitochondrial compartments.

The overall decomposition reaction of hydrogen peroxide in the present of catalase is as followes:

$H_2O_2 + H_2O_2 \rightarrow 2H_2O + O_2$ (catalase)

Catalases are present at a wide range of concentrations in nearly all mammalian cells. Catalases are located in subcellular compartments, mainly in peroxisomes. Soluble catalases are found in erythrocytes. The highest catalase activity is observed in cells of the

duodenum, liver, spleen, kidney, blood, mucous membranes and other highly vascularised tissues.

Glutathione peroxidases decompose hydrogen peroxide through the reaction:

$H_2O_2 + 2GSH \rightarrow 2H_2O + GSSG$ (GSPx: glutathione peroxidase)

Glutathione peroxidase can react with both hydrogen peroxide and organic hydroperoxides. Glutathione peroxidase is more efficient at low concentrations of hydrogen peroxide compared to catalase. Glutathione peroxidase reduces hydrogen peroxide to water with formation of oxidised glutathione which is regenerated by glutathione reductase by consuming NADPH.

Relatively high peroxidase activities occur in human adrenal medulla, liver, kidney, leukocytes and saliva. In the oral cavity, salivary peroxidase and myeloperoxidase are the primary defences against bacterially derived peroxide. Salivary peroxidase activity, resulting in the conversion of hydrogen peroxide to water, is coupled with the conversion of thiocyanate to hypothiocyanate, which has bacteriostatic activity and reduces the formation of peroxide and dental plaque acid by bacteria. In the absence of salivary peroxidase and thiocyanate, the rate of production of hydrogen peroxide by bacteria in saliva is approximately 100 nmol/ml/hr and would lead to a steady-state level of 0.1 mM hydrogen peroxide in one hour (Thomas et al, 1994). In the presence of salivary peroxidase and thiocyanate, the steady-state level of peroxide was predicted to be maintained below 0.01 mM.

Significant amounts of topically applied hydrogen peroxide can penetrate the epidermis or mucous membranes followed by rapid spontaneous or enzyme-catalysed decomposition to oxygen and water in the underlying tissue. The formation of gaseous oxygen causes capillary microembolism and prevents irrigation of tissues by blood resulting in a visible, reversible bleaching of the exposed tissue area. The local spontaneous or enzymatic-catalysed breakdown prevents it to enter the general circulation and thus its systemic distribution.

The oxidative reactivity of hydrogen peroxide with biological molecules such as carbohydrates, proteins, fatty acids or nucleic acids is not pronounced in the absence of transition metals, except for a few nucleophilic reactions.

In the organism the highly reactive (and thus toxic) hydroxyl radical can be produced non-enzymatically from superoxide anion and hydrogen peroxide through catalysis by transition metal ions like $Fe^{2+}$ and $Cu^{+}$ (the so-called Haber-Weiss- and Fenton reactions):

metal ions
$H_2O_2 + O_2^{-}\bullet \rightarrow OH\bullet + OH^{-} + O_2$ (Haber-Weiss reaction)

$H_2O_2 + Fe^{2+}/Cu^{+} \rightarrow OH\bullet + OH^{-} + Fe^{3+}/Cu^{2+}$ (Fenton reaction)

In all likelihood the "full" Haber-Weiss reaction (i.e., the reduction of $H_2O_2$ by $O_2^{-}\bullet$) is as follows (showing that the Fenton reaction is representing one particular part of the Haber-Weiss reaction):

$O_2^{-}\bullet + Fe^{3+}/Cu^{2+} \rightarrow O_2 + Fe^{2+}/Cu^{+}$

$H_2O_2 + Fe^{2+}/Cu^{+} \rightarrow OH\bullet + OH^{-} + Fe^{3+}/Cu^{2+}$

Iron is normally bound and the level of free iron in the plasma is very low. The cellular iron is thus not available to mediate a Fenton reaction *in vivo.* Biological reducing or chelating

agents, or acidic pH, may however promote the release of iron from transport and storage proteins. (ECB, 2003).

The hydroxyl radical is highly reactive and oxidises all organic chemicals, including biomolecules, when present in very close proximity to the place where the hydroxyl radical is formed. Superoxide and $H_2O_2$ are less reactive and can diffuse away from their site of formation, leading to OH• generation whenever they meet a "spare" transition metal ion. Oxygen radical formation can lead to lipid peroxidation, destruction of proteins, including enzyme inactivation, or to DNA damage.

***Groups at extra risk***

Genetically determined traits (acatalasaemia, glucose-6-phosphate dehydrogenase (G6PD) deficiency) render humans more susceptible to peroxide toxicity.

Acatalasemic individuals are more susceptible to hydrogen peroxide exposure because of a hereditary disorder in their hydrogen peroxide metabolising enzymes, i.e. the blood catalase activity level is below normal (hypocatalasemia). Acatalesemia is a rare (frequency 0.2-0.4%) genetic defect occurring particularly in the Orient (Ogata, 1991). It has been found that approximately half of the Japanese acatalasemic patients developed progressive gangrene of the mouth called Takahara's disease. This condition is characterised by small, painful ulcers in the gingival crevices and tonsillar lacunae, attributed to excess levels of hydrogen peroxide generated by various microorganisms in the mouth without normal destruction by catalase. The total number of reported patients of acatalasemia worldwide in 1989 was 107 belonging to 52 families.

Two Hungarian acatalasaemic subjects were reported (Góth, 1992). There appears to be two types of acatalasaemia. The Japanese type is the result of a splice mutation resulting in defective catalase synthesis (Góth and Páy, 1996). The Swiss type of acatalasaemia type is caused by point mutation resulting in catalase that is rapidly degraded. Swiss type acatalasaemic patients show no signs of oxidative damage (Góth and Páy, 1996).

Another group of individuals more sensitive to hydrogen peroxide exposure is persons with G6PD deficiency. G6PD deficiency is a genetic disorder of erythrocytes (over 300 variants have been identified) in which the inability of affected cells to maintain NAD(P)H levels sufficient for the reduction of oxidised glutathione results in inadequate detoxification of hydrogen peroxide through glutathione peroxidise (*Gaskin et al., 2001, Tsai et al., 1998*). It is estimated that about 400 million people throughout the world are deficient in G6PD. The frequency in G6PD deficiency in Europe is about 0.1%.

Industry claimed that due to the low levels of hydrogen peroxide in saliva during use of tooth whitening products and conversion of exogenous hydrogen peroxide to water and oxygen, hydrogen peroxide would not be expected to persist long enough in the body to reach G6PD deficient erythrocytes to precipitate an oxidative response.

A third group of individuals that might be more sensitive to hydrogen peroxide exposure is persons with xerostomia, or dry mouth, which occurs when the salivary glands are hypoactive. This may affect the degradation of hydrogen peroxide. However, Marshall et al. (2001) found no difference in the clearance of peroxide from the oral cavity when comparing adults with normal salivary flow and adult with diminished salivary flow (Sjorgren's syndrome). In an Industry sponsored clinical study (2000159), subjects with artificially induced xerostomia (via use of a rubber dental dam) experienced no adverse events after 10 days use of 6% hydrogen peroxide gel strips.

### 3.3.10. Photo-induced toxicity

#### 3.3.10.1. Phototoxicity / photoirritation and photosensitisation

No data submitted

#### 3.3.10.2. Phototoxicity / photomutagenicity / photoclastogenicity

No data submitted

### 3.3.11. Human data

***Toothpastes and mouth-rinses***

*Most of the information below is from the Opinion on hydrogen peroxide and hydrogen peroxide releasing substances used in oral care products (SCCNFP/0158/99).*

#### 3.3.11.1. Rate of degradation

Two *in vitro* studies have shown that hydrogen peroxide in toothpaste formulations is rapidly decomposed in human saliva. In each study, saliva samples were collected from 12 adult subjects. In one study (Sharma [Report], 1996) the saliva was mixed with 1.5 g of a 3% hydrogen peroxide gel (1:1 slurry in water) and the test paste (formulated with baking soda). In the other study (Marshall and Gragg [Report], 1997) toothpastes were prepared that contained 3% hydrogen peroxide and 1100 ppm fluoride from either sodium fluoride or monofluoro-phosphate or stannous fluoride. In both studies the released oxygen was captured and quantified. The majority of the hydrogen peroxide was converted to oxygen in 5 minutes (Sharma [Report], 1996; Marshall and Gragg [Report], 1997).

These findings have been confirmed *in vivo* in the oral cavity of healthy adult humans. Twelve subjects brushed their teeth with 1 g of a toothpaste gel phase containing 0.3% hydrogen peroxide or 3% hydrogen peroxide and 1100 ppm fluoride from sodium fluoride (30.25 mg hydrogen peroxide delivered). The saliva was collected immediately after brushing and 30, 60, 180, 360 and 540 seconds after brushing. The samples were analysed for hydrogen peroxide content. For the 3% hydrogen peroxide treatment, 70% of the delivered peroxide decomposed during brushing. Both in the absence and presence of fluoride, less than 20% of the administered hydrogen peroxide remained after 1 minute. The authors concluded that fluoride does not interfere with the degradation of hydrogen peroxide (Marshall and Gragg [Report], 1997).

Since fluoride salts are common ingredients in dental products consideration has been given to their potential to inhibit the degradation of hydrogen peroxide in the oral cavity. The effect of sodium fluoride (NaF), sodium monofluorophosphate (MFP) and stannous fluoride ($SnF_2$) on the rate of decomposition of 3% $H_2O_2$ was tested *in vitro* at 37°C with human saliva by monitoring the release of oxygen gas. The profiles of oxygen release in the presence of the fluoride salts did not differ from the profile for 3% $H_2O_2$ alone (Marshall and Gragg [Report], 1997).

Marshall et al. (2001) determined the clearance of peroxide from the oral cavity after 1 minute brushing with a 3% hydrogen peroxide dentifrice. Seventy percent of the hydrogen peroxide decomposed during the minute of brushing for infants (3-4 years), juveniles (7-12 years), adults with normal salivary flow and adults with diminished salivary flow (Sjorgren's syndrome).

An i*n vitro* study was conducted to determine if sodium bicarbonate would interfere with $H_2O_2$ decomposition (Sharma [Report], 1996). When 3% hydrogen peroxide gel mixed 1:1 with water was held in human saliva for up to 12 minutes at 37°C, determination of oxygen generation indicated that the hydrogen peroxide was rapidly decomposed. The rate of decomposition was not affected by the presence of 5% $NaHCO_3$ on its own or in a fluoride toothpaste formulation.

#### 3.3.11.2. Exposure

According to *SCCP (2007)* the total amount of toothpaste ingested per application is 0.24 g and the frequency of application per day is 2. Thus, the exposure per day will be 0.48 g. Assuming 0.1% hydrogen peroxide, the amount of hydrogen peroxide ingested per day will be 0.48 mg. With a body weight of 60 kg this will represent (0.48/60) 0.008 mg/kg bw/d.

According to *SCCP (2007)* the total amount of mouth-rinses ingested per application is 1.0 g and the frequency of application per day is 3. Thus, the exposure per day will be 3.0 g. Assuming 0.1% hydrogen peroxide, the amount of hydrogen peroxide ingested per day will be 3.0 mg. With a body weight of 60 kg this will represent (3.0/60) 0.05 mg/kg bw/d.

#### 3.3.11.3. Clinical studies and post-marketing experience with toothpastes containing hydrogen peroxide

Two clinical studies have been conducted to evaluate the change in microbiological flora associated with repeated use, twice daily for six months, of hydrogen peroxide/sodium bicarbonate toothpastes (0.75% $H_2O_2$ with 5% $NaHCO_3$ and 1.5% $H_2O_2$ with 10% $NaHCO_3$). The results showed a low level of yeast recovery throughout the studies, including the final 3 months when all subjects were using the control product (sodium fluoride toothpaste). Evaluation of the gingival status of the subjects revealed no increase in bleeding points and there were no trends in alteration of shade of teeth during the study. Finally, no soft tissue changes attributable to use of any of the products were observed (Fischman et al., 1992, [Abstract], 1992 b).

Ten additional studies are reported in which toothpaste formulations containing hydrogen peroxide up to 1.5% were tested in humans (Table 3.7). In one study the subjects had undergone surgery (Dentino et al., 1995), in the other studies all were healthy subjects. In controlled studies, the incidence of reporting unwanted effects was similar between subjects using a hydrogen peroxide formulation and those in the control groups.

**Table 3.7:** *Summary of clinical studies using hydrogen peroxide toothpastes*

| Formulation | n | Dosage | Safety findings |
|---|---|---|---|
| 0.5% Calcium peroxide toothpaste | 25 subjects | applied 4 times a day for 10 days, then 14 days rest and then applied twice on day 25 | No adverse events in treatment group* |
| 0.5% Calcium peroxide toothpaste | 232 subjects | daily brushing for 7 weeks | Examination at 2, 5 and 7 weeks showed no treatment-related effects* |
| Sodium percarbonate toothpaste (0.75% H2O2) | 74 subjects | 6 months | Transient angular cheilitis in one subject* |
| toothpaste (0.75% H2O2) | 100 subjects | 3 months | No product-related findings* |
| 0.75% H2O2 with 5% NaHCO3 toothpaste | 25 patients | twice daily brushing for 28 days after maxillary flap Gingival surgery (bilateral- double-blind cross over study) | No unwanted effects reported. 75% of patients preferred test product to control (Dentino et al., 1995) |

| Formulation | n | Dosage | Safety findings |
|---|---|---|---|
| 0.75% H2O2 with 5% NaHCO3 toothpaste | 62 subjects + 21 controls | twice daily brushing for 6 months | Oral lesions reported in 3 treated patients and 4 controls (Fischman et al., 1992) |
| 0.75% H2O2 toothpaste | 71 subjects | 3 weeks | An adverse effect was reported by one subject in the treatment group and one control* |
| 0.75% and 1.5% H2O2 toothpaste | 293 subjects | 3 weeks | 24 reports of oral lesions in the active treatment groups; 46 reports in the control group* 5 reports in each of the treated and control groups* No soft tissue changes associated with either test or control product (Fischman et al. [Abstract], 1992b) |
| 0.75% H2O2 toothpaste | 279 subjects | ? | |
| 1.5% H2O2 with 10% NaHCO3 toothpaste | 60 subjects + 20 controls | twice daily brushing for 6 months | |

*unpublished company data

Other clinical trials up to 6 months in length have been conducted with toothpastes containing up to 3% hydrogen peroxide. The trials, along with reported adverse events are summarised in Table 3.8. In none of the trials have adverse events been reported which were related to use of toothpaste with hydrogen peroxide.

**Table 3.8:** *Adverse events in clinical trials with toothpastes containing up to 3% hydrogen peroxide* (unpublished company data)

| Duration | No. subjects/cell | Level of H2O2 in toothpastes (%) | Adverse events |
|---|---|---|---|
| 6 months | 100 | 3, 0.75, 0 | No product related adverse events |
| 6 months | 65 | 3, 0.75, 0 | 1 subject using the 0% peroxide toothpaste suffered tooth sensitivity for one week |
| 21 days | 40 | 1.5, 0 | No product related adverse events |
| 21 days | 34 | 1.5, 0 | No product related adverse events |
| 48 hours | 15 | 1.5, 0.75 | No product related adverse events |
| 21 days | 50 | 3, 1.5 | No product related adverse events |
| 1 month | 29 | 1.5 | No product related adverse events |
| 2 months | 17 | 1.5, 0 | No product related adverse events |

Toothpastes which contain 0.75% $H_2O_2$ are available in USA. In the 3 years between 1991 and 1994, 40.6 million units of these toothpastes were sold and over the same period the Cosmetic, Toiletry and Fragrance Association (CTFA, 1994) received 421 reports of adverse events; this equates to about 1 report for each 100,000 units. The most common reactions reported were oral irritation and burning mouth. Symptoms usually resolved on withdrawing the product. This rate of reporting and the nature of the reports are similar to that for other toothpastes marketed in the USA (COLIPA, 1998).

Since 1995, dual phase toothpastes which consist of a blue gel phase containing 1.5% hydrogen peroxide and a white paste phase with 10% sodium bicarbonate have been available in the USA. The two phases are delivered simultaneously and in equal amounts from a pump dispenser to give a final hydrogen peroxide concentration of 0.75%. The total number of units of this product (0.5, 3.5, 5.2 and 10.4 oz, corresponding to approximately 15, 100, 149 and 300 ml) sold in the period 1995-1997 was 181 millions. During that time, a total of 1223 unfavourable reports were received, of which 977 were considered to be possible adverse reactions (unpublished company data) (COLIPA, 1998). A breakdown of the complaints is given in Table 3.9.

**Table 3.9:** *Unfavourable reports received to marketed toothpaste with 0.75% hydrogen peroxide in the period 1995-1997*(unpublished company data)

| Unfavourable report | Number |
|---|---|
| Cuts/scrapes | 12 [1, 2] |
| Splashes into eyes/face | 31 [1, 2] |
| Swallowed product | 206 [1] |
| Use complaints | 194 [1] |
| Swallowed product with reaction (vomiting/cramps) | 17 |
| "Allergic" reactions | 53 |
| Rashes/hives | 78 |
| Itches | 16 |
| Skin irritations | 75 |
| Mouth irritations | 523 |
| Chapped mouth area/dry skin | 7 |
| Nausea | 18 |
| Stinging | 9 |
| Inhalation/coughing | 1 |
| Use complaints with unfavourable reactions | 180 |

[1]Not included in total reactions
[2]Injury from opening container

3.3.11.4. Clinical studies and post-marketing experience with mouth-rinses containing hydrogen peroxide

Use of 3% hydrogen peroxide 3 to 5 times per day as a mouth-rinse resulted in mucosal irritations in 2 individuals with prior tissue injury. The pre-existing lesions worsened after exposure to hydrogen peroxide (Rees and Orth, 1986). Herrin et al. (1987) have shown that use of 3% hydrogen peroxide with sodium bicarbonate did not cause lesions in healthy individuals. Gingival lesions were seen in patients who used home care solutions employing 5 M sodium chloride in addition to 3% hydrogen peroxide and sodium bicarbonate.

A group of 88 dental students self-administered 6-12.5% hydrogen peroxide. They used it as a mouthwash and dipped their toothbrushes into the solution before brushing their teeth. Application of the hydrogen peroxide was 2-3 times per day for 1-2.5 months. Some gingival changes were noted: 6.4% of the subjects showed "redder" gums, 3.4% showed "paler" gums, and 6.6% developed hyperkeratinised filiform papillae of the tongue (Miller et al., 1938).

When adolescents with fixed appliances (n=30) used a 1.5% hydrogen peroxide solution as a mouth-rinses once every day for 18 months there was no evidence of oral irritation (Boyd, 1989). Similarly there were no reports of adverse events in a study in which 93 subjects rinsed three times a day for 7 days with a 1.5% hydrogen peroxide solution; this study included a control group of 85 subjects (Gomes et al., 1984).

In a clinical study, 58 patients used a mouth-rinse containing 1.5% hydrogen peroxide four times a day for 3 months. The patients were monitored at baseline and after 30, 60 and 90 days including an intraoral examination of soft tissues. There were no side effects associated with the soft tissues and no reports of irritation (Winer et al., 1991).
In a 21-day gingivitis study in humans using a hydrogen peroxide/sodium bicarbonate mouth-rinse, no local adverse reactions were seen which were attributable to the hydrogen peroxide/sodium bicarbonate system (Purdue University [Report], 1990). Summary of human safety data of hydrogen peroxide in mouth-rinses is shown in Table 3.10.

**Table 3.10:** *Summary of human safety data of hydrogen peroxide in mouth-rinses*

| Peroxide formulation | n | Dosage regimen | Safety findings | Reference |
|---|---|---|---|---|
| 1.5% Hydrogen peroxide rinse | 30 subjects | rinsed 4 times a day for 7 days | No treatment-related effects reported | Winer et al., 1991 |
| 1.5% Hydrogen peroxide rinse | 93 test 85 control | rinsed 3 times a day for 7 days | No treatment-related effects reported | Gomes et al., 1984 |
| 1.5% Hydrogen peroxide rinse | 30 children | rinsed once a day for 18 months | No generalized mucosal irritation was noted | Boyd, 1989 |
| 6-12.5% Hydrogen peroxide rinse | 88 dental students | 2-3 times a day for 1-2.5 months | Some gingival changes. 6,.4% showed "redder" gums and 3.4 paler gums. 6.6% developed hyperkeratinised filiform papillae of the tongue | Müller et al, 1938 |
| Hydrogen peroxide/sodium bicarbonate system | (not stated) | 21 days | No adverse effects reported | Purdue University, 1990 |
| 3% Hydrogen peroxide rinse | (not stated | rinsed 3 to 5 times a day | Mucosal irritation in 2 individuals with prior tissue injury | Rees and Orth, 1986 |
| 3% Hydrogen peroxide with sodium bicarbonate | (not stated) | | No adverse effects reported | Herrin et al., 1987 |
| 1.5% Hydrogen peroxide rinse | (not stated) | 18 months | No adverse effects reported | Fischman et al., 1992 |
| 1.5% Hydrogen peroxide rinse | (not stated) | Twice daily for 24 months | Improved gingival heath | Gangler and Staab, 1985 |
| 0.75 or 1.5% Hydrogen peroxide rinse | (not stated) | 4 times daily for 5 weeks | Discoloration of mucosal surfaces | Tombes and Gallucci, 1993 |
| 1.5% Hydrogen peroxide rinse | (not stated) | 2 months | No adverse effects reported | Shibly et al., 1997 |

A 1.5% hydrogen peroxide mouth-rinse has been marketed in the USA since 1985. Between 1985 and 1995, 10 million 8 oz bottles (approximately 240 ml) and 300,000 32 oz (approximately 960 ml) bottles have been sold. Over the same period of time, 39 complaints were received (COLIPA, 1998).

An aqueous based dual phase product which consisted of a blue phase containing 1.5% hydrogen peroxide and a white phase with 10% sodium bicarbonate, has been marketed in the USA since 1995. The two phases are delivered simultaneously and in equal amounts from a dispenser to give a final hydrogen peroxide concentration of 0.75%. The total number of units (2, 10, 20 and 30 oz i.e. 60, 300, 600, 900 ml) of mouth-rinse sold in the period 1995 - 1997 was 24 millions. During that time, a total of 501 unfavourable reports were received of which 192 were considered to be possible adverse reactions (unpublished company data). A breakdown of the complaints is given in Table 3.11 (COLIPA, 1998).

**Table 3.11:** *Unfavourable reports received to marketed mouth-rinse with 0.75% hydrogen peroxide in the period 1995-1997* (unpublished company data)

| Unfavourable report | Number |
|---|---|
| Cuts/scrapes | 12[1, 2] |
| Splashes into eyes/face | 173 [1, 2] |
| Swallowed product | 102 [1] |
| Use complaints | 58 [1] |
| Swallowed product with reaction (vomiting/cramps) | 2 |
| "Allergic" reactions | 4 |
| Rashes/hives | 8 |
| Body reactions | 11 |
| Skin irritations | 12 |
| Mouth irritations | 104 |
| Chapped mouth area/dry skin | 2 |
| Nausea | 12 |
| Stinging | 3 |
| Use complaints with unfavourable reactions | 34 |

[1]Not included in total reactions
[2]Injury from opening container

3.3.11.5 Summary / Comment on human data – Tooth paste and mouth rinses

Several clinical studies with toothpaste and mouth-rinses containing up to 3% hydrogen peroxide have been carried out. All studies seem to have been conducted or sponsored by the manufactures. The duration of the toothpaste studies varied from 48 hours to 6 months. Only three studies with a total of 196 persons had a duration of 6 months. No product related adverse events were mentioned. No treatment-related effects were reported in studies with mouth-rinses containing 1.5% hydrogen peroxide. A 3% hydrogen peroxide solution used as a mouth-rinse 3 to 5 times daily caused mucosal irritation especially if the oral mucosa is already damaged. There is a need for independent long-term studies both with toothpaste and mouth-rinses.

***Tooth whitening products***

3.3.11.6 Exposure

Exposure studies (study no. 2000045 and 2000143) were carried out by Procter & Gamble. Adult subjects (N=12) used either a 5.3% hydrogen peroxide gel strip that delivers 10.6 mg hydrogen peroxide/strip (200 mg of a 5.3% hydrogen peroxide gel; 5, 10, 30 or 60 minute treatments) or 6.0% gel strip. Treatment was on maxillary teeth only. It is concluded that hydrogen peroxide delivered at 6% in tooth whitening products intended for direct application to the teeth, degrades rapidly during wear time. This is indicative of the rapid degradation of hydrogen peroxide that would occur where direct contact with the gingival tissues immediately surrounding the teeth may result during wear time of such tooth whitening products. Salivary hydrogen peroxide levels are low during wear time (<0.02%), thereby demonstrating the minimal oral and systemic exposure that occurs with such tooth whitening formulations. The available peroxide resulting from the cosmetic use 4 strips with 6% hydrogen peroxide will be about 50 mg hydrogen peroxide per day (200 mg of 6% hydrogen peroxide = [200x0.06] x 4 = 48 mg).

**Table 3.12:** *Available hydrogen peroxide in the saliva during the first 30 and 60 minutes (Data from Procter & Gamble Safety study 2000045 [5.3% strips] and 2000143 [6% strips].* **Note:** *The procedure used is only published as a book chapter (Whelton, 1996). The calculations are based on salivary flow of 0.3 ml/min. The numbers in parenthesis represent exposure based on the daily use of 4 strips.*

| Calculation | 5.3% Strip mg 30 min | 6.0% Strip mg | |
|---|---|---|---|
| | | 30 min | 60 min |
| Mean | 0.43 (1.7) | 0.68 (2.7) | 1.20 (4.8) |
| Mean + 2xSD | 1.6 (6.2) | 1.21 (4.8) | 2.55 (10.2) |
| Median | 0.11 (0.44) | 0.62 (2.5) | 0.99 (4.0) |
| Max observation | 1.6 (6.2) | 1.3 (5.4) | 2.86 (11.4) |

If the mean (Table 3.12) is used to calculate exposure this will correspond to (4.8/60) 0.08 mg/kg bw/d while if a maximum exposure of 11.4 mg is used, this will correspond to (11.4/60) 0.19 mg/kg bw/d (The calculations are based on a salivary flow of 0.3 ml/min, while the flow when stimulated is 1.5 – 2.0 ml/min. Since application of the strips most likely will stimulate salivary flow, the numbers given are probably to low).

For comparison, Haywood and Haymann (1989) estimated in an early study that the approximate dose of carbamide peroxide for each application was 90 mg. The advances in technique for preparing custom fitted trays and the improvement in whiteners now allow the use of much less material for each application. A recent estimation indicates that an average amount of commercial whitener used clinically for 10 maxillary teeth (full arch) was 502 mg per application (Li, 1996). The whitener contained 10% carbamide peroxide. It was estimated that about 10% of the applied whitening gel might be consumed during the application (Dahl and Becker, 1995). Therefore, for an individual of 60 kg bodyweight, the exposure to tooth whitener was calculated at [50.2/60] 0.84 mg/kg/day, and the exposure to carbamide peroxide through tooth whitener containing 10% carbamide will be 0.084 mg/kg/day, corresponding to 0.028 mg/kg/day of hydrogen peroxide. Dahl and Becker (1995) point out that overfilling of the tray and excessive biting on the tray are factors that may cause additional release of the bleaching agent. Matis et al. (2002) have concluded that 25% of carbamide peroxide in the tray is swallowed. This will correspond to about 0.07 mg/kg/day.

In the case of the gel strips it has been reported that the user occasionally may swallow the strip. This will result in an exposure of about 12 mg hydrogen peroxide.

The above calculation of daily exposure is claimed to be conservative as it assumed a constant concentration of 10% carbamide peroxide in whitener during the whole application. Several studies have shown that peroxide content decreases with time, particularly significant during the early part of application (ADEPT [Report], 1991, Christensen [Abstract], 1997, Nathoo et al. [Abstract], 1996 Ploeger et al. [Abstract], 1991). In a recent study it was reported that the carbamide peroxide degradation in bleaching trays occurred in an exponential manner and that 10% remained after 10 hours (Gaiao et al. [Abstract], 1998). On the other hand, it may be expected that the exposure is highest in connection with the process of inserting the night guard.

Comparisons of the use of the new strips with the custom fitted trays suggest that the total exposure to hydrogen peroxide is of the same order of magnitude.

The degradation of 10% carbamide peroxide (≈3.6% hydrogen peroxide), worn in a custom-fitted tray, was determined over 10 hours (N=15). The degradation rate in the tray and in the gel on the teeth was rapid for the first hour, and then slowed, with more than 50% loss of active ingredient seen at 4 hours, and more than 85% loss following 10 hours of exposure. The degradation of "grab" sample from the reservoir of tooth no. 8 was slower. On average 56% remained after 4 hours and 23% after 10 hours (Matis et al., 1999).

In an article by Mahony et al. (2003), both maxillary and mandibular teeth were treated with a 5.3% hydrogen peroxide paint-on gel. Peroxide concentrations in the tooth scraping sample after 10, 30 minutes, 1, 2 and 4 hours of daytime wear were 4.56, 3.28, 1.57, 0.51 and 0.14%, respectively. The median peroxide concentration in the saliva at 5, 10 and 20 minutes of daytime wear were 0.001, 0.0001 and 0.0001% respectively. The median peroxide concentrations in the saliva at 30 and 60 minutes were below the limit of detection (0.00007%). Overall, salivary hydrogen peroxide concentration was less than 0.033% measured at any time.

Bleaching strips containing 10% hydrogen peroxide gel were used in a clinical trail involving 16 persons. The median hydrogen peroxide concentration after 5 minutes was 7.3%, 6.4% and 0.7% for strips, teeth and gingival, respectively, declining to 4.6%, 2.9% and 0.1% at 30 minutes. Salivary samples never exceeded a median concentration of 0.014% at any point. Median hydrogen peroxide concentration on strips and teeth remain about 2% over 60 minutes. (In the received summary report, it is written that 14% hydrogen peroxide strips was used) (Walden et al., 2004, report).

Maxillary teeth were treated with 14% hydrogen peroxide strips (100 mg gel load) in a clinical trial involving 15 persons. The concentration was 13.4% on average after sampling 3 strips. The median concentration for the teeth after 10 minutes was 6.9% after 30 minutes 4.2% and 60 minutes wear time 2.9%. Using colorimetric analysis with glycerine normalized analysis, the results after 10 minutes was 11.1%, 30 minutes 8.7% and 60 minutes 8.5%. Thus, there is a considerable difference which increases with time. The highest concentration in saliva was 0.073%, which occurred at 10 minutes time point. Strip hydrogen peroxide level decreased with wear time. After 30 minutes wear time, the median peroxide level on the strip ranged from 3.4 to 11% depending on the analytical method. Tooth hydrogen peroxide level also decreased with wear time. Depending on the analytical method the median peroxide concentration was 10.0 to 12.7% after 5 minutes of wear time and the levels ranged from 4.2 to 8.7% after 30 min. Median salivary peroxide level did not exceed 0.073% for any time point in the study (Report 2003 009).

Slezak et al. (2002) determined the concentration of hydrogen peroxide in saliva after application of a 6.5% hydrogen peroxide paint-on gel. The concentrations were 0.03%, 0.0042% and 0.0001% at 1, 5 and 15 minutes, respectively.

A study involving 17 persons was carried out with 6.5% hydrogen peroxide strips (0.2 g gel load, 13 mg hydrogen peroxide/strip) or 14% hydrogen peroxide strips (0.1 g gel load, 14 mg hydrogen peroxide/strip). The median peroxide concentrations are shown on Table 3.13. It is pointed out that for pair wise comparisons between treatment, there were no significant differences between the strips hydrogen peroxide level at 60 min and for gingival and saliva data for any time point and for area under the curve (Report 2003 046).

**Table 3.13:** *Concentration of hydrogen peroxide in the strip, at the teeth, at the gingiva, and in the saliva during the first 60 minutes (Data from study 2003 046[6.5% and 14% strips])*

| Measurement site | | 0 min (%) | 5 min (%) | 10 min (%) | 30 min (%) | 60 min (%) |
|---|---|---|---|---|---|---|
| Strip | 6.5% | 7.0 | 5.5 | 5.6 | 4.4 | 3.0 |
| | 14% | 14.5 | 10 | 9.2 | 6.2 | 3.0 |
| Teeth | 6.5% | | 4.4 | 3.8 | 2.6 | 1.7 |
| | 14% | | 7.4 | 6.7 | 4.4 | 2.4 |
| Gingiva | 6.5 | | 0.60 | 0.16 | 0.17 | 0.08 |
| | 14% | | 0.36 | 0.18 | 0.12 | 0.06 |
| Salivary | 6.5% | | 0.007 | 0.006 | 0.005 | 0.001 |
| | 14% | | 0.011 | 0.013 | 0.009 | 0.002 |

The use of 5.3% or 6.5% hydrogen peroxide paint-on gel is presented in a clinical study involving 17 persons. When 6.5% paint-on gel is used, median concentrations after 2 and 5 minutes when retractor is used was 8.6% and 9.5%, respectively. In another study with no retractors, the concentration 0.5 minute after application was 10.6%. This increase after application is probably due to the rapid loss of alcohol in the gel (Report 2003 043).

In a study where 6.5% hydrogen peroxide paint-on gel and 6% hydrogen peroxide strip were compared, it was concluded that on the average the hydrogen peroxide concentration on teeth and in saliva was statistically higher for strips compared to paint-on gel both at 5 and 30 minutes time point, while the concentration of paint-on appear to peak at 30 seconds application, the hydrogen peroxide concentration rapidly declined to less than 0.6% at 2% as the paint-on products exposed to saliva, salivary hydrogen peroxide peaked at 0.5 minutes (0.034%) and declined rapidly (Report 2002 126).

The amount of peroxides released into saliva was related to the bleaching system and only partially influenced by the individual salivary flow rate. Bleaching with Vivastyle (10% carbamide peroxide, tray charged with 225 mg) led to lower release of peroxides into saliva compared to Whitestrips (5% $H_2O_2$) (Vivastyle: 0.8 $\pm$ 0.17 mg; Whitestrips: 1.5 $\pm$ 0.84 mg). The peak exposures of hydrogen peroxide in the saliva were 0.06% with Vivastyle and 0.1% with Whitestrips. This will correspond to about 5 $\mu g/cm^2$. Salivary flow rate was not correlated to release of peroxides from the bleaching products (Hanning et al., 2003).
*Hannig et al. (2006)* compared the hydrogen peroxide recovery in saliva during use of different home bleaching products in smokers and non-smokers. Peroxide recovery was evaluated with respect to the safe level reported in the literature. Four different bleaching regimes were used by 10 smokers and 10 non-smokers: Whitestrips, Vivastyle (tray-based) and two paint-on products (Crest Night Effects, Colgate Simply White). Whole saliva was collected and total amount of peroxide (mg) released during the 60 min bleaching period was determined: Colgate Simply White: 2.67 $\pm$ 0.88 (non-smokers); 2.66 $\pm$ 1.17 (smokers); Crest Night Effects: 0.23 $\pm$ 0.13 (non-smokers); 0.25 $\pm$ 0.16 (smokers); Vivastyle: 2.47 $\pm$ 0.82 (non-smokers); 2.44 $\pm$ 1.31(smokers); Whitestrips: 1.39 $\pm$ 0.62 (non-smokers); 2.02 $\pm$ 1.86 (smokers). In terms of amount of peroxide per kg body weight the bleaching systems led to a single exposure of 0.046 mg/kg bw. The authors concluded that smoking did not affect the degrading of hydrogen peroxide from bleaching products. Significantly lower amounts of peroxide were detected in saliva during application of the paint-on product Crest Nights Effects compared with the other bleaching systems.

### 3.3.11.7. Summary / Comment on exposure

It is difficult to assess the exposure, which may vary with the product and the level of active material (hydrogen peroxide) used.

In a study were salivary hydrogen peroxide was determined using a 6% hydrogen peroxide strip, assuming a salivary flow of 0.3 ml/min, the mean calculated exposure from 4 strips per day was 0.08 mg/kg bw/d. Using the maximum measured hydrogen peroxide amount, the exposure would be 0.19 mg/kg bw/d. (If mean + 2SD is used the exposure will be (10.2/60) 0.17 mg/kg bw/d. It should be noted that this might be an underestimation of exposure, since application of the strips most likely will stimulate salivary flow, up to 2.0 ml/min.
The total amount of peroxide (mg) released during a 60 min bleaching period was determined from whole saliva collections with 4 different bleaching regimes (two tray-based [Whitestrips, Vivastyle] (tray-based) and two paint on products [Crest Night Effects, Colgate Simply White]). Amount of hydrogen peroxide released; Whitestrips: 1.39 $\pm$ 0.62 mg, Vivastyle: 2.47 $\pm$ 0.82 mg, Crest Night Effects: 0.23 $\pm$ 0.13 mg, and Colgate Simply White: 2.67 $\pm$ 0.88 mg. In terms of amount of peroxide per kg body weight the bleaching systems led to a single exposure of maximum 0.046 mg/kg bw of hydrogen peroxide (Colgate Simply White). Smoking did not affect the degrading of hydrogen peroxide from bleaching products.

The concentration of hydrogen peroxide in contact with the teeth will be close to the concentration in the bleaching product. The gingival concentration of hydrogen peroxide has been reported to be about 0.7% and 0.6% 5 minutes after application of a strip containing 10% and 6.5% hydrogen peroxide. The level was reduced to 0.1% and 0.2%, respectively after 30 min.

The maximum concentration of hydrogen peroxide in the saliva after teeth bleaching has been reported to be 0.1% corresponding to about 5 $\mu g/cm^2$. The saliva concentration seems to be similar with a 200 mg strip containing 6.5% hydrogen peroxide and a 100 mg strip containing 14% hydrogen peroxide.

For gel strips it has been reported users occasionally may swallow the strip, resulting in an exposure of about 12 mg hydrogen peroxide (0.2 mg/kg bw). When trays are used, overfilling of the tray and excessive biting on the tray are factors that may cause additional release of the bleaching agent.

From the exposure studies, the systemic absorption of hydrogen peroxide is estimated to be in the range from 0.03 mg/kg bw/d to 0.2 mg/kg bw/d. Consequently, an exposure from tooth whitening products with 6% hydrogen peroxide of 0.2 mg/kg bw/d may be used in safety calculation.

### 3.3.11.8. Clinical safety data

In a survey by Clinical Research Associates, 91% of 8,143 dentists stated that they had used vital tooth bleaching, 79% reported success, while 12% were not satisfied with the concept. Side effects reported by the respondents included the following: 62.2% noted tooth hypersensitivity 10.7 % of the time; 45.9% reported soft-tissue irritation 5.6% of the time; 2.1% noted systemic effects 0.2% of the time; and 18.8% reported no side effects (Christensen, 1997).

The most commonly observed clinical effects of treatments with tooth whiteners include mild tooth hypersensitivity to temperature changes and irritation of oral mucosa in some

patients (Li et al. [Abstract], 1996, Haywood, 1993, 1997). Some patients have also reported burning palate, throat and gingiva (Howard, 1992). Tooth hypersensitivity often occurs during the early stage of bleaching treatment, and it is usually transient. The tray rather than the tooth whitening materials may cause the mucosal irritation.

Industry has reported several studies concerning the use of peroxide (2.7-7% hydrogen peroxide) containing tooth whitening products for less than 6 months, resulting in the same adverse events (oral soft tissue irritation and tooth sensitivity) observed in two week studies. The majority of the adverse events were mild and all had resolved within 3 days after the products use was discontinued. No adverse events resolution related to treatment was required. There was a trend toward a slight increase in adverse event incidence with increasing hydrogen peroxide concentration. Oral soft tissue irritation or oral hard tissue adverse incidence in groups using hydrogen peroxide products was not significantly different compared to the concurrent placebo in any study. In only 2 of 14 studies, the total adverse events incidence was statistically significantly greater in subjects using hydrogen peroxide compared to the concurrent placebo groups. Even 6 months continuous use of either a strip or a custom tray peroxide product caused the same mild, transient adverse events (tooth sensitivity and oral soft tissue irritation) as those observed after 14 or 28 days of product use.

A company producing teeth bleaching strips has reported that some of the users of the gel strips have swallowed a strip. In several of these cases, consumers reported minor gastrointestinal symptoms.

The majority of the published peroxide based teeth whitening studies with carbamide peroxide are done with a type of product only available via the dental office. With this system, a carbamide peroxide gel is delivered in a custom-fitted mouthguard, designed to cover either the upper or lower dentition. The filled bleaching tray is worn at home from 2-3 hours for daytime exposure to 8-10 hours for overnight exposure. Treatment is generally daily, and ranges in duration from one week to six months, or until the patient is satisfied with the results achieved. Ten percent carbamide peroxide is a commonly used gel concentration and is equivalent to 3.6% hydrogen peroxide.

*Up to two weeks:* Matis et al. (1998) reported 79% incidence of gingival "sensitivity" and 55% incidence of tooth sensitivity during a 2-weeks exposure to 10% carbamide peroxide. Kowitz et al. (1994) reported 1% of patients discontinuing use of 10% carbamide peroxide due to tooth sensitivity. No other adverse events were reported during this 2-weeks exposure. In both studies, adverse effects returned to normal following the bleaching period. Nathoo et al. (1994) reported no adverse effects of 2-weeks bleaching with 10% carbamide peroxide.

Forty-four subjects were divided into two groups and used either 6% strips or a 3.3% hydrogen peroxide gel in a custom tray. Only the maxillary arch was treated twice daily for 30 minutes over a 14-day treatment period. Thirty-three percent of subjects using the 6% strips had oral soft tissue adverse events and 19% had tooth sensitivity. The numbers in the groups using 3.3% hydrogen peroxide tray system were 23% and 5%, respectively. All of the adverse effect in this study resolved during the study or within several days after product use has been discontinued (Report 2001 111, not received).

The safety and efficacy of three strip-based whitening products containing 6.0 to 8.2% hydrogen peroxide was studied (*Study no 05L10104 HTR 03-122638 Hilltop Research, inc, 2004*). The primary endpoint was the change in tooth shade. Test groups (N=33-35 individuals per group) applied the whitening strips daily as directed. Self reported tooth sensitivity (max. 6% at any time point) and oral irritation (max 12% at any timepoint) were similar for the different whitening products.

*Up to one month:* In a 3-weeks exposure to 10% carbamide peroxide, 95 of the subjects reported tooth sensitivity and 32% reported minor oral discomfort (Reinhardt et al., 1993). Treatment with 10% carbamide peroxide for 4 weeks resulted in no changes in pulp sensitivity or pulpal response, as measured by electric pulp testing, although 14% of the subjects dropped from the study because of tooth sensitivity (Schulte et al., 1994). No changes in pulp sensitivity during 4 weeks exposure to 10% carbamide peroxide film-forming gel were noted. None of the subjects reported oral soft tissue irritation (Kozlovsky et al., 1996).

Nachnani ([Report] 1997) reported that there were no statistically significant difference between the placebo group and the group using bleaching gel at baseline and day 14 and between baseline and 6 months for measurements of pulpal vitality, gingival index, soft tissue evaluation and attached gingiva. Similar results were also reported in a second report (Leonard [Report], 1997). No differences in gingival index scores were detected before, during, or after the use of a whitener containing 10% carbamide peroxide for up to 7 hours daily for 28 days (Schulte et al, 1993).

*Beyond one month:* A whitening product with 10% carbamide peroxide was used for *5 weeks* on 5 women smokers and 6 women who were not smokers. The authors found with the use of biopsies an increase in the thickness of the epithelium producing an increase in cellular proliferation in the basement and parabasal membranes of the gingival epithelium. The authors pointed out that it is not possible to conclude that 10% carbamide peroxide is carcinogenic in clinical situations, but in the present study, it was possible to observe that it alters cellular proliferation and consequently, it could act as a tumour promoter (da Costa Filho et al., 2002).

A 38% incidence of adverse events (tooth sensitivity) occurred in a 2 months study with 10% carbamide peroxide gel; symptoms resolved during treatment or immediately following treatment (Migliore et al., 1991). In a review article by Haywood et al. (1997) several longer-term studies with patients using 10% carbamide peroxide for 6 weeks up to 6 months were reported. Adverse events (tooth sensitivity and gingival irritation) were experienced by 67% of the clinical subjects; symptoms were gone 24 hours post-treatment. Leonard et al (1999) reported an 80% incidence of adverse events in a 6 month study with a 10% carbamide peroxide gel; resolution of symptoms was not reported.

The safety of *three months* use of strips was evaluated. This product is designed as a one-week use and the present conditions represent a twelve times overuse. Forty subjects were divided into two groups and were assigned to either 6% hydrogen peroxide strips or 9.5% hydrogen peroxide strips. Subjects used their product on the maxillary teeth for 30 minutes twice a day for 3 months. For the 6% hydrogen peroxide strips, 6% of subjects had oral soft tissue adverse effects and 44% reported tooth sensitivity. For the 9.5% hydrogen peroxide strips, 6% of subjects had oral soft tissue adverse effects and 59% reported tooth sensitivity. One severe tooth sensitivity adverse effect was reported with the 9.5% hydrogen peroxide strips. All of the adverse effects resolved quickly when product use was discontinued (Report 2002 063).

An *in vivo* study on the effect of carbamide peroxide on enamel was carried out. The action on the morphology of the enamel surface of two whitening products with 10% carbamide peroxide that are on the market was studied (Colgate Platinium and Starbrite). 24 subjects divided into two groups used the products for two weeks. Immediately after the treatment, porosity was increased in the Colgate group whilst erosive alterations were observed in the Starbrite group. After *three months,* the situation was as it was before treatment (Turkum et al., 2002).

A study involving 13 adults with teeth stained by tetracycline ingestion and treated with tooth bleaching agent nightly for *six months* is reported (Haywood and Leonard [Abstract],

1996). Average treatment time was 958 hours (ranging from 568 to 1,322 hours). Tooth hypersensitivity or gingival irritation occurred, but was managed by reduction in treatment time per application, less frequent application, or interruption of treatment. None of the teeth had required endodontic therapy or crowns, nor had any patient experienced gingival sensitivity or tooth hypersensitivity since completion of the treatment.

In a study, 70 subjects (35 controls and 35 using a 10% carbamide peroxide in anhydrous glycerol as an oral hygiene substance) were followed for *up to 3 years*. No evidence of adverse effect on oral tissues was observed (Fogel and MaGill, 1971). In another study where two tooth bleaching agents containing 10% carbamide peroxide were used (the mean treatment time was 302.5 hours), the main adverse effects were tooth hypersensitivity (52%) and gingival irritation (31%). Either or both occurred in 66% of the patients. The adverse effects were transient, with an average duration of 4-7 days. At 18 months (range 14-25 months) after the treatment, no side effects had re-occurred or continued (Haywood et al., 1994). However, in a study involving 40 patients which is probably an update, four of the patients reported tooth hypersensitivity at 7 years while none had reported tooth hypersensitivity at 1.5 and 3 years. Three of these had also reported tooth hypersensitivity prior to the initial treatments. No patient reported having a crown or restoration on any tooth whitened because of fracture, nor did anyone report having a root canal on any treated tooth. It is concluded that side effects occur during treatment, but not afterwards and that there are no significant long-term side effects up to 3 years associated with the use of two tooth bleaching agents containing 10% carbamide peroxide (Leonard, 1998).

It is stated in the dossier that a number of investigators reported that the use of 10% carbamide peroxide in anhydrous glycerol was effective in reducing risk of gingivitis (Zinner et al, 1978) and dental caries (Fogel and MaGill, 1971) and improving oral hygiene (Tartakow et al, 1978). Its use for four times daily up to 3 years did not have any adverse effects on gingival tissues or any evidence of other side effects.

*Juvenile study:* In twenty-eight day study, 9.5% hydrogen peroxide strips were used by adolescence (12-18 years). The product is designed as one-week kit, but the study represents a two-times overuse. Subjects either used 9.5% hydrogen peroxide strips (30 min, twice a day) or a 3.3% hydrogen peroxide gel in a custom tray (8 hours at night). Subject used their assigned product on the maxillary arch only for two weeks followed by their mandibular arch only for two weeks. In the 9.5% hydrogen peroxide strip group, 13% had oral soft tissue adverse effects and 18% reported tooth sensitivity. There were no oral soft tissue adverse effects in the 3.3% hydrogen peroxide tray group and 42% of the subjects reported tooth sensitivity. All of the adverse effects resolved quickly when product use was discontinued (Report 2003 016).

#### 3.3.11.9 Summary / Comment on clinical safety data

The cosmetic industry and their organisations have pointed out that over 100 published and unpublished clinical studies, comprising approximately 4000 subjects in total are available. In addition, there exists a 7-year follow-up study on a small group of tooth whitening products users. It should be noted that only 9 of the 15 persons in the long-term study agreed to clinical examination. Six studies, all with less than 100 people, had up to 6 months follow-up. The majority of the studies seemed to be less than 1.5 month and involve less than 150 persons. Only one 28-day study has been reported with adolescents (12 – 18 years old). For a case-reference study to detect a doubling of the risk for an adverse effect that occurs at a level of 1:1000 in the reference group, the study group must have at least 1000 people. The majority of the studies were judged to be at high risk of bias and were either sponsored or conducted by the manufacturers. Thus, there is a need of good clinical studies of the use of tooth whitening products as well as long-term clinical data and epidemiological studies that assess the possible adverse effects of tooth whitening products within the oral cavity.

In a large survey of dentists, 91% of 8,143 dentists stated that they had used vital tooth bleaching,. Side effects reported by the respondents included the following: 62.2% noted tooth hypersensitivity, 45.9% reported soft-tissue irritation, 2.1% noted systemic effects, and 18.8% reported no side effects.

The most commonly observed clinical effects of treatments with tooth whiteners include mild tooth hypersensitivity to temperature changes and irritation of oral mucosa. Some patients have also reported burning palate, throat and gingiva. Tooth hypersensitivity often occurs during the early stage of bleaching treatment, and it is usually transient.

According to industry, market experience indicates that hydrogen peroxide tooth whitening products are well tolerated by consumers, with an adverse event incidence rate of 0.1%. The top five complaints received by consumers have been mouth irritation, oral miscellaneous, tooth hypersensitivity, gastrointestinal, and stained teeth. Oral cavity related effects represent the majority of health effects reported, with 58% of symptoms reported being tooth sensitivity and 56% of symptoms reported being oral soft tissue irritation. Whitening products that contain peroxide are known to have the potential to produce oral irritation and tooth hypersensitivity. These effects have usually been transient in nature and resolved shortly after cessation of product use.

### 3.3.12. Special investigations

Industry stated in submission III that the reactivity of peroxides is limited to endogenous and exogenous sources of colour – including dietary stains and possibly non-functional matrix components of the teeth. It is claimed that bleaching per se, even with concentration of up 16% hydrogen peroxide under exaggerated use conditions (up to 6 weeks *in vitro*), does not damage either enamel, coronal dentin (subsurface to bleaching) or root dentin and that bleaching did not disperse or dissolve smear layers of exposed root dentin. It is claimed that current bleaching systems do not adversely affect tooth vitality, since pulp concentrations of peroxide do not reach levels needed to produce damage. Bleaches do not significantly damage restorations, although restoring teeth should be avoided immediately after bleaching due to a transient reduction in bond strength. *In vitro* studies, under exaggerated conditions of use, have demonstrated release of small amounts of mercury from amalgams at levels, which are well within the limits for mercury exposure in the guidelines set out by the WHO.

***Enamel and Dentine Surface Morphology and Chemistry***

Scanning electron microscopy (SEM) has been used for qualitatively analysing the surface morphology of enamel and dentine specimens following bleaching. In addition profilometry has been used to measure the surface roughness.

Some authors have reported alterations of enamel surfaces, including shallow depression, increased porosity and slight erosion, associated with whitening treatments (Bitter, 1992; Bitter and Sander, 1993; Josey et al., 1996). In one study with two bleaching gels containing 16% and 35% carbamide peroxide, the authors concluded that the results indicated a need to warn patients of the potential for enamel alteration and its detrimental effect on tooth structure even if the long-term consequences have yet to be conclusively determined (Bitter 1998). It should be noted that studies have demonstrated that also soft drinks (e.g., Coca-Cola, Pepsi Cola) and fruit juices cause demineralisation and alteration of enamel (Grobler et al., 1990; Grando et al., 1996) which are comparable to those reported for whitening agents (McCracken and Haywood, 1996).

Shannon et al. (1993) subjected enamel slabs to different bleaching agents containing 10% carbamide peroxide for 15 hours a day for 2- and 4-week periods and evaluated by scanning electron microscopy. During the remaining 9 hours, the slabs were exposed to

human saliva *in vivo*. Significant surface alterations in enamel topography were observed for slabs treated with the bleaching solutions for 4 weeks. Cubbon and Ore (1991) and Hammel (1998) have reported two clinical cases of serious adverse effects on enamel associated with whitening agents, both of which involved the use of "over-the-counter" products.

Attin et al. (1997) assessed effects of bleaching on enamel concurrent with fluoride remineralization. While bleaching produced a slight surface softening in their protocol, the group found that topical fluoride reversed this effect, promoting surface hardening through remineralization. *Rothuijzen et al. [abstract](to be published)* found that *in vitro* bleaching without intermittent remineralisation periods VivaStyle (10% carbamide peroxide; pH 5.2) rendered enamel volunerable for subsequent demineralization, while bleaching with Opalescence (10% carbamide peroxide + 0.11% fluoride; pH 6.7) had a protective effect.

Numerous studies have indicated neglible changes in enamel surface texture associated with peroxide bleaching (McGuckin et al., 1992). When changes are observed, they are for the most part minor, involving the formation of shallow depressions or increased porosities. These are likely to be a side effect of the bleaching matrices. These changes are expected to be normalized through later prophylaxis or through salivary remineralization.

The majority of studies confirming the safety of bleaching systems are contrasted with a few investigations that have shown surface degradative changes associated with bleaching processes. Rotstein et al. (1996) reported that application of 35% carbamide peroxide produced etching and demineralisation on dental enamel surfaces and in subsurface areas.

The majority of the more recent studies that have used scanning electron microscopy or profilometry during the last years showed no significant changes in enamel surface morphology following bleaching even with one of the highest concentrations of hydrogen peroxide (35%) *(Sulieman et al., 2004).* Similarly, the lower levels of 6.5% hydrogen peroxide *(Duschner et al., 2006)* and 6.0% hydrogen peroxide *(Duschner et al., 2006; Joiner et al., 2004; Nucci et al., 2004),* and 10% carbamide peroxide *(Nucci et al., 2004; Justino et al.,[abstract] 2004)* were also shown to have no significant effects on enamel surface morphology following simulated 2 weeks product usages. This is contrasted with studies by *Pinto et al. (2004), Cavalli et al. (2004a)* and *Yeh et al. (2005)* who observed some changes in enamel morphology following bleaching with hydrogen peroxide or carbamide peroxide.

The differences between the positive and null effects on enamel may be due to differences in the *in vitro* protocols used and this is reflected in their differences with respect to replicating the *in vivo* environment. For example, *Yeh et al. (2005)* stored their samples between bleaching sessions in distilled water. *Pinto et al. (2004)* and *Cavalli et al. (2004a)* used artificial saliva consisting only of inorganic calcium and phosphate components, and were devoid of any organic components which could have the potential of forming a protective salivary pellicle. In the case of three studies which showed no effect of bleaching products on enamel surface morphology, *(Duschner et al. 2006; Joiner et al. 2004; Justino et al. [abstract] 2004)* human whole saliva was used as a key part of replicating the *in vivo* situation. Indeed, *Justino et al. [abstract] (2004)* demonstrated that any adverse effects evident for *in vitro* bleached and stored in water enamel specimens were not seen for similarly treated specimens placed on an intra-oral device and worn in the mouth.

In terms of changes in human enamel surface chemical composition, no differences were found between enamel treated with 30% hydrogen peroxide (120 hr treatment), 10% carbamide peroxide (6 h/day for 14 days) or water controls as measured by Raman spectroscopy *(Park et al. 2004; Goo et al 2004)*. Similar results were obtained for electron spectroscopy for chemical analysis (ESCA) techniques used on human enamel treated with

either 10% carbamide peroxide, 7% hydrogen peroxide or 12% hydrogen peroxide (7 h/d for 14 days) *(Pugh et al. 2005)*.

COLIPA concluded that the majority of studies indicate that hydrogen peroxide and carbamide peroxide containing products have no significant deleterious effects on enamel and dentine surface morphology and that the contrasting studies that do show an effect, in general, have some limitations in the *in vitro* methodologies used which do not reflect the *in vivo* situation accurately.

***Enamel and Dentine Surface Microhardness***

Surface microhardness (SMH) measurement has been a frequently used technique for evaluating the effects of peroxide and bleaching products on enamel and dentine.

There are numerous published *in vitro* reports in the literature detailing the detrimental effects or lack of effects of peroxide-containing tooth whitening products on enamel microhardness (Seghi and Denry, 1992; Murchison et al., 1992), enamel resistance to abrasion (Seghi and Denry, 1992), dentin microhardness (Nathoo et al., 1994; Pecora et al., 1994), dentin roughening (Zalkind et al., 1996; Atrushkevich and Vasiukova, 1996), and restoration microhardness (Bailey and Swift, 1992; Nathoo et al., 1994). Results are dependent on the methodology used and the materials or products tested.

Several studies also reported minimal or no effects of whitening agents containing 10% carbamide peroxide on microhardness and mineral content of human enamel surfaces (Shannon et al., 1993; McCracken and Haywood, 1995, 1996; Nathoo et al., 1994; Murchison et al., 1992).

Crest Whitestrips gel containing up to 6.5% hydrogen peroxide was applied for up to 70 hours bleaching (five kits). Human tooth enamel specimens were cycled through a daily regime including salivary immersions and treatment with commercial tooth whitening gels containing hydrogen peroxide or carbamide peroxide. Following *in vitro* laboratory cycling, the teeth were cross-sectioned and remounted for observation of microhardness and ultrastructural characteristics in subsurface regions. It was concluded that the peroxide bleaching gels produced no changes in subsurface enamel and dentin ultrastructure or architecture. *Tooth preparation* - Human teeth were collected by dentists and periodontists in the course of their typical practice in the Cincinnati region. These teeth were collected as part of a longstanding Procter and Gamble program of tooth collection and preservation for their laboratory requirements. The results provided support for the clinical experience that vital tooth bleaching produces no effects on the structure or function of teeth (White et al., 2004a).

A 10% carbamide peroxide bleaching agent was evaluated against a placebo agent. Two hundred and forty dental fragments were randomly fixed on the vestibular surface of the first superior molars and second superior premolars of 30 volunteers. The results suggest that treatment with 10% carbamide peroxide bleaching materials for three weeks alters the enamel microhardness, although it does not seem to alter the dentin microhardness (Basting et al. 2001).

An *in vitro* study aimed to evaluate the effect of bleaching agents on dentin microhardness during and after bleaching was performed. Specimens were randomly assigned to seven groups using different bleaching agents as well as a placebo agent. The 42-day whitening treatment consisted of daily application of the agents to the dentin surface for 8 hours, followed by immersion in artificial saliva for 16 hours. After the bleaching treatment, specimens were kept immersed in artificial saliva for 14 days. Microhardness was measured at baseline as well as different times during bleaching and during the post-treatment period. It is concluded that throughout the bleaching treatment, depending on the agent applied, dentin showed a transitory decrease in microhardness values. In the post-treatment period,

artificial saliva presented a remineralizing effect on the bleached surfaces (de Freitas et al. 2004).

Research has been carried out supporting the hard tissue safety of bleaching processes associated with strip bleaching gels (White et al., 2000). Studies included the assessment of strip gels containing different peroxide concentrations ranging from 5.3 to 16% hydrogen peroxide. Studies also included the application of gels for time periods up to 5x recommended consumer use. In a novel *in vitro* cycling protocol, bleach activity was first confirmed with image analysis colorimetry of enamel and dentin surfaces. Surface microhardness and texture assessments were complemented with analyses on cross sections samples. Hardness evaluations were then further complemented with ultrastructural observations realized through application of 3D confocal laser scanning microscopy image reconstructions, carried out on naturally wet specimens. Results illustrate the safety of the 6% hydrogen peroxide bleaching gels to both topically treated enamel and dentin and to surface regions of these specimens for all concentrations of hydrogen peroxide and all exposure regimens.

*Sulieman et al. (2004)* and *Park et al. (2004)* showed that 35% hydrogen peroxide treatment for 30 mins and 30% hydrogen peroxide treatment for 120 hours, both on human enamel, showed no significant reduction of SMH. Cycling experiments on enamel with 6-9.5% hydrogen peroxide where treatments were 30 mins, twice per day for 14 days simulated use *(Teixeira et al. 2004)*, 6% and 6.5% hydrogen peroxide for 30 mins twice/day for 28 days *(Duschner, 2006)*, 12% hydrogen peroxide for 7 h/day for 14 days *(Pugh et al., 2005)*, 6% hydrogen peroxide for 20 mins, twice/day for 14 days *(Joiner et al., 2004)* all showed no reduction in SMH. A similar conclusion was obtained for similar simulated use cycling experiments when 10% carbamide peroxide *(Justino et al., 2004; Unlu et al., 2004; Pugh et al., 2005; Leonard et al., 2005)*, 11% carbamide peroxide *(Wong et al.,[abstract] 2006)* and 15% carbamide peroxide *(Unlu et al., 2004)* were tested. *Leonard et al.(2005),* however, point out that when evaluating enamel microhardness, consumer available paint-on bleaching solutions may adversely affect enamel microhardness compared to a control and 10% carbamide peroxide dentist-prescribed, home-applied bleaching product.

*Lewinstein et al. (2004)* observed a reduction in SMH following 35% hydrogen peroxide or 35% carbamide peroxide treatments on human enamel which was reversed when treated with a 0.05% fluoride solution. Similarly, *Basting et al.[abstract] (2005)* also observed a slight reduction in SMH of human enamel following 8 h/day for 42 days of 10% carbamide peroxide treatments. In an *in situ* type study, *Rodrigues et al. (2005)* noted a slight reduction in SMH following in office 37% carbamide peroxide treatment (30 minutes x 2 on 3 days) plus at home use of 10% carbamide peroxide (6 h for 21 days). However, this was not significantly different from an equivalent series of placebo treatments and the authors considered the observed SMH reductions as clinically insignificant.

A reduction in enamel SMH was observed by *Hairul Nizam et al (2005)* following 24 h treatment with 30% hydrogen peroxide solution. Also two other studies observed a reduction in enamel SMH following bleaching with up to 35% hydrogen peroxide or 35% carbamide peroxide *(Pinto et al., 2004; Attin et al., 2004)*. COLIPA points out that the conflicting data may be due to differences of the *in vitro* methods used. In particular, the two last studies used artificial saliva containing no organic components which could have formed a protective layer, there were no fluoride treatments to aid remineralisation and the study by *Attin et al (2004)* used bovine enamel which is known to have a three-fold faster rate of lesion progression compared to human enamel (see also *Attin, 2006)*.

For dentine, no significant changes in SMH were reported in experiments involving 35% hydrogen peroxide for 30 minutes *(Sulieman et al 2004)* and 10% or 15% carbamide peroxide treatments for up to 28 hours *(Unlu et al., 2004)*, or in cycling experiments using,

6% or 6.5% hydrogen peroxide treatments for 30 minutes twice/day for 28 days *(Duschner et al., 2006)* or 6% hydrogen peroxide for 20 minutes twice/day for 14 days *(Joiner et al., 2004)*.

A transitory decrease in dentine SMH has been observed in some studies but recovered following a remineralisation period *(Freitas et al., 2004a, 2004b)* or 0.05% fluoride solution treatment *(Lewinstein et al., 2004). Arcari et al. (2005)* reported small reductions in dentine SMH (5.4%). A significant reduction in dentine SMH was observed for one 10% carbamide peroxide product *(Basting et al., [abstract] 2005)*. In addition, the study by *Hairul Nizam et al. (2005)* showed a reduction in dentine SMH.

COLIPA claimed that overall the majority of studies indicate that hydrogen peroxide and carbamide peroxide containing products have no significant deleterious effects on enamel and dentine SMH, even if one of the highest levels of hydrogen peroxide is used. The few contrasting studies that do show an effect, in general, have some limitations in the *in vitro* methodologies used which do not reflect the *in vivo* situation accurately. Indeed, some studies demonstrated a transitory reduction in SMH which were recovered following a remineralisation period.

***Subsurface Enamel and Dentine***

Since hydrogen peroxide will diffuse through enamel towards the enamel-dentine junction, some studies have investigated the effects of bleach agents on subsurface enamel and dentine. This is typically accomplished by bleaching whole teeth or fragments and then cutting and polishing the specimens to reveal the internal subsurface enamel and dentine areas, followed by micro-hardness measurements.

Using the above approach, *Teixeira et al. (2004)* found no reductions in enamel subsurface microhardness following treatments with 6%-9.5% hydrogen peroxide (30 minutes x 2/day) or 10% carbamide peroxide (6 h/day) for 14 days in total. Similar results were found for both subsurface enamel and dentine following 6% hydrogen peroxide for 20 minutes x 2/day, for 14 days *(Joiner and Thakker 2004)* or for 14 hours and 70 hours total bleaching time with 5.3% hydrogen peroxide *(White et al., 2004)*. In contrast, the study by *Attin et al. (2005)* showed some reduction in subsurface enamel but not subsurface dentine following bleaching protocols with up to 35% hydrogen peroxide or 35% carbamide peroxide. Again this contrast may be due to differences in the methodology.

An alternative approach to investigating the effects of bleaching on subsurface enamel, dentine and the enamel-dentine junction is to use confocal laser scanning microscopy which enables their ultrastructure to be investigated. Studies on bleached tooth specimens have demonstrated no changes in enamel and dentine ultrastructure *(White et al., 2004; Duschner et al., 2006)*. On the other hand, *Markovic et al. (2007)* exposed teeth *in vitro* to either 10% or 16% carbamide peroxide for 4 hours per 7 days. The statistical analysis showed significantly higher microroughness for both groups of carbamide peroxide exposed enamel surfaces.

The ultimate tensile strength of subsurface enamel following treatment with up to 35% hydrogen peroxide and 37% carbamide peroxide *(Silva et al.,[abstract] 2005)* and 10 – 20% carbamide peroxide *(Cavalli et al., 2004b)* has been shown to be reduced compared to non bleached controls. *Cavalli et al. (2004b)* point out that the effects of bleaching agents on the mechanical properties of enamel have not been extensively studied. Although it is quite difficult to clinically associate enamel cracking or fractures with previous bleaching treatments, there is increasing evidence that enamel structural changes may occur due to exposure to such substances that may ultimately compromise its strength. There study showed that the ultimate tensile strength of enamel was significantly reduced when a routinely used bleaching regimen was followed and that the clinical implications must be further investigated.

*Tam et al. (2007)* have studied the effects of *in vitro* prolonged tooth bleaching on the fracture toughness of human dentin. Dentin from recently extracted molar teeth was directly or indirectly treated to simulate a prolonged at-home (10% carbamide peroxide or 3% hydrogen peroxide, 6 hours/day, 5 days/week for 8 weeks) or in-office (30% hydrogen peroxide, 1 hour/week for 8 weeks) bleaching regimen ($N$ = 8/group). For direct bleach application, the treatment materials were applied onto dentin that was already prepared as compact tension specimens. For indirect bleach application, bleach was applied to the enamel of intact teeth prior to specimen preparation. There was a significant decrease in dentin fracture toughness after 8 weeks of *direct* bleach treatment. There were no significant differences between the bleach and control groups after 8 weeks of *indirect* bleach treatment ($p$ = 0.19). The authors conclude that caution should be considered when using bleach for prolonged treatment times in clinical cases where there is dentin exposure such as occlusal attrition or gingival recession.

COLIPA claimed that the majority of relevant *in vitro* studies indicate that hydrogen peroxide and carbamide peroxide containing products have no significant deleterious effects on subsurface enamel and dentine microhardness or ultrastructure. Only one *in vitro* study showed a decrease in the subsurface microhardness of enamel.

***Effects of Acid Challenges and Abrasion on Bleached Enamel/Dentine***

*Sulieman et al. (2004)* found that pre-bleaching human enamel and dentine with 35% hydrogen peroxide for 30 minutes had no subsequent deleterious effect on enamel and dentine loss caused by citric acid erosive challenges or brushing with toothpaste, as measured by profilometry. Similarly, bleaching human enamel and dentine with 10%-22% carbamide peroxide for 2 h x 20 treatments did not increase their susceptibility to acid erosion or caries lesion formation as measured by quantitative light-induced fluorescence and transverse microradiography *(Pretty et al., 2005)*.

*Cia Worschech et al. (2006)* studied how tooth bleaching abrasive dentifrices might change the outer superficial enamel. Human enamel slabs were exposed *in vitro* to a 10% carbamide peroxide bleaching agent at different times and submitted to different superficial cleaning treatments. Bleaching was performed on the enamel surface for six hours daily. After that, each slab received a cleaning surface treatment and was stored in artificial saliva. The study showed that the sole use of 10% carbamide peroxide did not alter the enamel surface roughness, but the cleaning treatments that employed the use of brushing with abrasive dentifrices resulted in a significant increase of enamel surface roughness.
The study by *Wiegand et al. (2004)* showed that bleaching with 35% or 38% hydrogen peroxide (15 min x 2/d, for 4 d) or 35% carbamide peroxide (1 h on 4 d) gave no significant increase in enamel wear caused by brushing with toothpaste. In the same study, they did show a significant increase in enamel wear following treatment protocols with 5.3% hydrogen peroxide, 10% carbamide peroxide and 15% carbamide peroxide. The authors conclude that bleaching treatment may result in increased tooth brushing abrasion. Acidic agents or long duration of bleaching seem to lead to an increased susceptibility to enamel loss by tooth brushing abrasion.

COLIPA concluded that *in vitro* studies indicate that hydrogen peroxide and carbamide peroxide containing products have no significant clinically relevant effects on subsequent enamel and dentine loss caused by acidic erosive challenges, toothpaste abrasion or caries lesion formation.

***Effects on Restorative Materials***

While dental enamel is the focus of peroxide whitening reactions, dental restorative materials can be visualized as a collateral substrate for bleaching effects. With respect to bleach effects on restoration colour, research supports the conclusion that restorative materials are generally unaffected by peroxide bleaching procedures (Swift, 1997, 1998).

However, composite restorations may lighten a very small amount during bleaching, but this is detectable by colorimeter measurements only.

The effects of peroxide bleaching on restoration surface texture and chemistry are strongly dependent upon restoration type (Swift, 1998). Thus, porcelain or other ceramic restoratives as well as dental gold appear generally unaffected by bleaching procedures. Composite restorations, on the whole would seem to be more reactive to bleach effects, but these still may include only minor etching or softening depending upon treatment conditions. In studies of strip bleaching gels, glass ionomers were largely unaffected (Schenk-Meuster et al., 2002, Nathoo et al., 1994).

The most noteworthy chemical interactions that have been reported with bleaching procedures include cements and amalgam restorations. Zinc phosphate cement has been previously observed to be completely solubilized by a carbamide peroxide bleaching gel (Christensen et al., 1991). Dental amalgams show signs of oxidative reactivity with bleaching gels with minor localized spotting and colour changes observed on amalgam surfaces (Schenk-Meuster et al., 2002). *In vitro* studies, under exaggerated conditions of use, have demonstrated release of very small amounts of mercury from amalgams, which are at levels well within the limits for mercury exposure in the guidelines of WHO (Hummert et al., 1993, Rotstein et al., 1997).

***Uptake of Bleaches and Transport to Dental Pulp***

It is well established that a common adverse effect of vital tooth bleaching is dentinal hypersensitivity. As discussed previously, uptake studies have confirmed that peroxide is taken up into dental pulp from 30 – 35% peroxide in-office treatments and 6% peroxide consumer bleaching systems. Several studies have examined the effects of vital bleaching on pulp histology. These studies involve the use of vital teeth scheduled for orthodontic extraction that are then exposed to bleach or control treatments prior extraction, fixation and assessments. Researchers have observed that vital tooth bleaching produces histological evidence of minor inflammation of superficial layers of pulp adjacent to the pulp-dentin junction (Robertson and Melfi, 1980). It is noteworthy that the minor inflammatory response of the pulp to the introduction of bleaching seems to be concurrent with the pain response expressed by consumers having increased hypersensitivity.

In two studies, extracted human teeth were sectioned above the cemento-enamel junction and oriented so that an enamel surface was immersed in a solution of hydrogen peroxide (Bowles and Ugwuneri, 1987) or a gel of hydrogen peroxide or carbamide peroxide (Cooper et al., 1992). After exposure, acetate buffer that had been placed in the pulp cavity was subjected to a peroxidase-based assay to measure the extent of peroxide penetration through enamel and dentin. Peroxide was detected in the pulp cavity as early as 15 minutes following exposure of enamel to 1, 10 or 30% hydrogen peroxide, and the amounts detected showed a significant dose relationship (Bowles and Ugwuneri, 1987). Carbamide peroxide appears to result in less penetration than the equivalent amount of hydrogen peroxide. Exposure to a 15% carbamide peroxide gel (equivalent to 5.3% hydrogen peroxide) resulted in a mean pulp cavity concentration of peroxide that was less than half that caused by exposure to gelled 5% hydrogen peroxide (Cooper et al., 1992).

Laboratory research has demonstrated that hydrogen peroxide is readily transported through tooth enamel into dentin and pulp and it is reported that significant amounts of hydrogen peroxide diffuse through dentin after application of carbamide peroxide and hydrogen peroxide-based bleaching agents (Hanks et al., 1993). Despite this uptake, the development of pulpal damage associated with vital tooth bleaching is remarkably low. It is pointed out that peroxide concentration from 14% hydrogen gel was far below levels required for the initiation of significant enzyme inhibition (White et al., 2004b).

In a study of Slezak et al. (2002) the pulp penetration was studied with 6.5% hydrogen peroxide and 9% hydrogen peroxide paint-on gel. It was claimed that pulpal penetration over two 30 minutes applications of peroxide under *in vitro* conditions produced a level of approximately 1000 times lower than the amount of peroxide required to inhibit pulpal enzymes. The levels were also well below concentrations shown to result in no damage to the pulp tissue.

#### 3.3.12.1 Summary / Comments on special investigations

*Enamel and Dentine Surface Morphology and Chemistry.* The majority of studies indicate that hydrogen peroxide and carbamide peroxide containing products have no significant deleterious effects on enamel and dentine surface morphology. However, there are some limitations in the *in vitro* methodologies used which do not reflect the *in vivo* situation accurately.

*Enamel and Dentine Surface Microhardness.* The majority of studies indicate that hydrogen peroxide and carbamide peroxide containing products have no significant deleterious effects on enamel and dentine surface microhardness. Some studies demonstrated a transitory reduction in dentin surface microhardness which were recovered following a remineralisation period.

*Effects of Acid Challenges and Abrasion on Bleached Enamel/Dentine. In vitro* studies indicate that hydrogen peroxide and carbamide peroxide containing products have no significant clinically relevant effects on subsequent enamel and dentine loss caused by acidic erosive challenges, toothpaste abrasion or caries lesion formation.

*Effects on Restorative Materials.* Research supports the conclusion that restorative materials are generally unaffected by peroxide bleaching procedures. However, composite restorations may lighten a very small amount during bleaching, but this is detectable by colorimeter measurements only.

The effects of peroxide bleaching on restoration surface texture and chemistry are strongly dependent on restoration type. Porcelain or other ceramic restoratives as well as dental gold appear generally unaffected by bleaching procedures. Composite restorations seem to be more reactive to bleach effects, mainly minor etching or softening depending in relation to treatment.

The most noteworthy chemical interactions that have been reported with bleaching procedures on cements and amalgam restorations. Zinc phosphate cement has been previously observed to be completely solubilized by a carbamide peroxide bleaching gel. *In vitro* studies have demonstrated release of very small amounts of mercury from amalgams.

*Uptake of Bleaches and Transport to Dental Pulp.* Uptake studies have confirmed that peroxide is taken up into dental pulp from 30 – 35% peroxide in-office treatments and 6% peroxide consumer bleaching systems. It has been found that vital tooth bleaching produces histological evidence of minor inflammation of superficial layers of pulp adjacent to the pulp-dentin junction. The minor inflammatory response of the pulp to the introduction of bleaching seems to be concurrent with the pain response expressed by consumers having increased hypersensitivity.

It is claimed that pulpal penetration under *in vitro* conditions produced a level much lower than the amount of peroxide required to inhibit pulpal enzymes. Carbamide peroxide appears to result in less penetration to the pulp than the equivalent amount of hydrogen peroxide.

### 3.3.13. Safety evaluation (including calculation of the MoS)

#### 3.3.13.1. Calculation of MoS

SCCP is of the opinion that the risk of systemic effects is low. This is supported by the fact that hydrogen peroxide which may enter into the bloodstream is rapidly metabolised. However, adverse effects have been observed in repeated dose studies in animals, allowing calculation of a MoS for systemic toxicity. Irritating effects both due to a direct effect in the oral cavity and in the gastrointestinal system after swallowing are of concern.

***Toothpastes and mouth-rinses***

**Repeated dose toxicity**

A NOAEL of *20 mg/kg bw/day of hydrogen peroxide* has been obtained from a 100 days rat gavage study based on a significantly reduced plasma catalase level at higher dose levels

Estimated daily exposure *(SCCP, 2007)*:
Toothpaste: 480 mg/day
Mouth-rinses: 3000 mg/day

*Amount of hydrogen peroxide at 0.1% hydrogen peroxide*
Toothpaste: 0.48 mg/day, Systemic exposure (0.48/60) 0.008 mg/kg bw/d
**MOS = (20/0.008) 2500**

Mouth-rinse: 3.0 mg/d, Systemic exposure (3.0/60) 0.05 mg/kg bw/d
**MOS = (20/0.05) 400**

**Conclusion**
**The calculated MOS for repeated dose toxicity is considered to give sufficient protection in relation to the use of 0.1% hydrogen peroxide in its free form or when released when used in oral hygiene products.**

***Tooth whitening products***

Safety calculation of tooth whitening products due to hydrogen peroxide may be performed by MOS calculations based on the systemic exposure or by comparing the exposure in $\mu g/cm^2$ caused by tooth whitening products with that causing an adverse effect.

**MOS calculation**
Estimated daily exposure from tooth whitening products containing 6% hydrogen peroxide is estimated to **0.2 mg/kg bw/d** (see discussion)

**Repeated dose toxicity**
A NOAEL of *20 mg/kg bw/day of hydrogen peroxide* has been obtained from a 100 days rat gavage study based on a significantly reduced plasma catalase level at higher dose levels

**MOS = (20/0.2) 100**

**Conclusion**
**The calculated MOS for repeated dose toxicity is on the borderline of that considered to give sufficient protection in relation to the use of 6% hydrogen**

**peroxide in its free form or when released when used in tooth whitening products. For hydrogen peroxide concentrations above 6% the MOS will be below 100, and thus not considered safe.**

### 3.3.13.2. Regulations

IARC has concluded that there is *"limited"* evidence of carcinogenicity of hydrogen peroxide in experimental animals (IARC 1999).

No labelling is required in EU for hydrogen peroxide solutions of less than 5%. Solutions containing above 5% hydrogen peroxide are labelled harmful. In addition solutions containing: 5 – 8% hydrogen peroxide are labelled "Irritating to eyes" (R36), more than 8% are labelled "Harmful if swallowed" and "Risk of serious damage to eyes" (R22-41), more than 35% have an additional label "Irritating to respiratory system and skin", higher concentrations are in addition labelled "Cases burn".

According to Annex III of the Cosmetic Products Directive, oral hygiene products must not contain more than 0.1% hydrogen peroxide.

### 3.3.14. Discussion

*Chemistry*
Possible impurities in hydrogen peroxide and carbamide peroxide as well as the hydrogen peroxide releasing substances discussed in the appendix (sodium perborate, sodium percarbonate and peroxymonosulphate) are not known. No information on the stability of hydrogen peroxide and hydrogen peroxide releasing substances in oral hygiene products and tooth whitening products was submitted.

*Acute toxicity of hydrogen peroxide*
The oral and dermal LD50 in rats is higher than 600 mg/kg bw. The dermal LD50 in rabbits is 630 mg/kg bw.

A 16-month-old boy (body weight 11.6 kg) died after ingestion of about 600 mg/kg bw.

*Irritation and corrosivity*
Skin irritation in rabbits following 4 hour exposure to 10% hydrogen peroxide was slight. A 5% solution of hydrogen peroxide was slightly irritating to the eye while a 10% solution was highly irritating. The threshold of detection for irritation was about 0.1% when hydrogen peroxide was administered as drops directly to the human eye.

Stomach gavage of 15 mg/kg bw of carbamide peroxide (5.4 mg/kg bw of hydrogen peroxide) produced ulceration of gastric mucosa in rats observed after 1 hour; the lesions appeared to be healing after 24 hours. No effects were observed with 5 mg/kg bw of carbamide peroxide (1.8 mg/kg bw of hydrogen peroxide) (Dahl and Becher, 1995).

Solutions containing more than 5 % hydrogen peroxide are in EU labelled harmful due to eye and skin irritation.

*Skin sensitisation*
Hydrogen peroxide is not considered to cause skin sensitisation.

*Dermal / percutaneous absorption*
Biological membranes are highly permeable to hydrogen peroxide. Thus, hydrogen peroxide is expected to be readily taken up by the cells constituting the absorption surfaces, but at the same time it is effectively metabolised, and it is uncertain to what extent the unchanged

substance may enter into blood circulation. Moreover, the red blood cells have an immense metabolic capacity to degrade hydrogen peroxide.

*Repeated dose toxicity*
In a 90 day study in mice with hydrogen peroxide in the drinking water, a NOAEL of 100 ppm was found based on dose-related reductions in food and water consumption, and on the observation of duodenal mucosal hyperplasia. This corresponds to 26 and 37 mg/kg bw/day for males and females, respectively. In a 100 days rat gavage study a NOAEL of 20 mg/kg bw/day was found based a significantly reduced plasma catalase level at higher dose levels.

A NOAEL of 20 mg/kg bw/day of hydrogen peroxide may be used for calculation of MOS for repeated dose toxicity.

*Mutagenicity / Genotoxicity*
Hydrogen peroxide is a mutagenic and genotoxic in a variety of *in vitro* test systems. The responses observed were modified by the presence of degrading enzymes (catalase), the extent of formation of hydroxyl radicals by Fenton reaction, and the cells repair abilities.

The available studies are not in support of a significant genotoxicity/mutagenicity for hydrogen peroxide under *in vivo* conditions. Further studies of genotoxicity and mutagenicity tissues in direct contact with hydrogen peroxide are needed. Mechanistic studies suggest that cells are adapted to repair DNA damage caused by oxidants; on the other hand there is some evidence that hydrogen peroxide may inhibit the repair of DNA lesions inflicted by other types of reactive chemicals.

*Carcinogenicity*
A drinking water study in mice showed that hydrogen peroxide caused duodenal hyperplasia at a high frequency and localised duodenal carcinomas at a low frequency. A subsequent study with different strains of mice showed a strong negative correlation between incidence of duodenal tumours and catalase activity in duodenal mucosa. In one study with rats a high incidence of forestomach papillomas were found after receiving 1% hydrogen peroxide in the drinking water. While humans do not have a forestomach, they do have comparable squamous epithelium tissues in the oral cavity and the upper 2-3 part of the oesophagus. Thus, in principle, carcinogens targeting the forestomach squamous epithelium rodents are relevant for humans. Also, the target tissues for carcinogens may differ between experimental animals and humans and a forestomach carcinogen in rodent may target a different tissue in humans. Some tumour promotion studies indicate that hydrogen peroxide may act as a promoter.

Hydrogen peroxide has a weak potential to induce local carcinogenic effects. The mechanism is unclear, but a genotoxic mechanism cannot be excluded. As regard to tumour promotion, several mechanisms (direct genotoxicity, impairment of DNA repair, and chronic inflammation) might be operative.

*Reproductive toxicity*
No appropriate animal studies were available for a complete evaluation of reproductive and developmental toxicity. Limited studies with mice and rats exposed to hydrogen peroxide in drinking water suggested no grave disturbances on the male or female reproductive functions.

*Toxicokinetics*
Hydrogen peroxide is a normal metabolite in the aerobic cells. It is produced from superoxide anion spontaneously or as a result of the activity of superoxide dismutase (SOD). Hydrogen peroxide occurs under most conditions at submicromolar concentrations in the organism. Because hydrogen peroxide reacts slowly with organic substrates, it can

diffuse considerable distances in biological systems. There are two main hydrogen peroxide metabolising enzymes, catalase and glutathione peroxidase which control the hydrogen peroxide concentration. Catalase deals with large amounts of $H_2O_2$ that may be generated in peroxisomes. Glutathione peroxidase (GSH peroxidase) metabolises $H_2O_2$ in both the cytosolic and mitochondrial compartments. Significant amounts of topically applied hydrogen peroxide can penetrate the epidermis or mucous membranes followed by rapid spontaneous or enzyme-catalysed decomposition to oxygen and water in the underlying tissue. The local spontaneous or enzymatic-catalysed breakdown prevents it to enter the general circulation and thus its systemic distribution.

In the presence of traces of transition metal ions, superoxide anion and hydrogen peroxide undergo the so-called iron-catalyzed Haber-Weiss reaction which results in OH• formation. The hydroxyl radical is highly reactive and oxidises all organic chemicals, including biomolecules, when present in very close proximity to the place where the hydroxyl radical is formed. Superoxide and $H_2O_2$ are less reactive and can diffuse away from their site of formation, leading to OH• generation whenever they meet a "spare" transition metal ion.

Acatalasemic individuals are more susceptible to hydrogen peroxide exposure because of a hereditary disorder in their hydrogen peroxide metabolising enzymes, i.e. the blood catalase activity level is below normal (hypocatalasemia). Acatalesemia is a rare (frequency 0.2-0.4%) genetic defect occurring particularly in the Orient. Another group of individuals more sensitive to hydrogen peroxide exposure is those with G6PD deficiency. It is estimated that about 400 million people throughout the world are deficient in G6PD. The frequency in G6PD deficiency in Europe is about 0.1%. Industry claimed that due to the low levels of hydrogen peroxide in saliva during use of tooth whitening products and conversion of exogenous hydrogen peroxide to water and oxygen, hydrogen peroxide would not be expected to persist long enough in the body to reach G6PD deficient erythrocytes and to provoke an oxidative response.

***Studies on specific product types***

*Toothpastes and mouth-rinses*

Exposure

According to SCCP (2007) the total amount of toothpaste ingested per day is 480 mg. Assuming 0.1% hydrogen peroxide, the amount of hydrogen peroxide ingested per day will be *0.008 mg/kg bw/d from toothpaste*. The amount of mouth-rinses ingested is 3 g per day. Assuming 0.1% hydrogen peroxide, the amount of hydrogen peroxide ingested per day will be *0.05 mg/kg bw/d from mouth-rinses*.

Mucous membrane irritation

Ulceration of gastric mucosa in rats was observed 1 hour after gavage with a dose corresponding to 5.4 mg/kg bw of hydrogen peroxide while no effects were found at 1.8 mg/kg bw of hydrogen peroxide. This is (1.8/0.008) 225 times higher than the dose expected using toothpaste containing 0.1% hydrogen peroxide, but only (1.8/0.05) 36 times higher than the dose expected using a mouth-rinse containing 0.1% hydrogen peroxide.

Clinical safety data

Several clinical studies with toothpaste containing up to 3% hydrogen peroxide have been carried out. All studies were conducted by the manufactures. The duration of the studies varied from 48 hours to 6 months. Only three studies with a total of 196 persons had a duration of 6 months. No product related adverse events were mentioned.

For mouth-rinses, no treatment-related effects were reported in several studies with products containing 1.5% hydrogen peroxide. In a human study where a mouth-rinse containing 3% hydrogen peroxide was used 3 to 5 times per day, mucosal irritation was found in 2 individuals with prior tissue injury. The pre-existing lesions worsened after exposure to hydrogen peroxide. There is a need for independent long-term studies with both toothpaste and mouth-rinses.

*Tooth whitening products*

Exposure

It is difficult to assess the exposure, which may vary with the product and the level of active material (hydrogen peroxide) used.

In a study were salivary hydrogen peroxide was determined using a 6% hydrogen peroxide strip, assuming a salivary flow of 0.3 ml/min, the calculated exposure from 4 strips a day was 0.08 mg/kg bw/d. If mean + 2SD is used the exposure will be 0.17 mg/kg bw/d. It should be noted that the numbers may be minimum numbers since application of the strips most likely will stimulate salivary flow, which may be as high as 2.0 ml/min. Thus, the exposure may be underestimated.

The total amount of peroxide (mg) released during a 60 min bleaching period was determined from whole saliva collections with 4 different bleaching regimes (two tray-based [Whitestrips, Vivastyle] (tray-based) and two paint on products [Crest Night Effects, Colgate Simply White]). Amount of hydrogen peroxide released; Whitestrips: 1.39 $\pm$ 0.62 mg, Vivastyle: 2.47 $\pm$ 0.82 mg, Crest Night Effects: 0.23 $\pm$ 0.13 mg, and Colgate Simply White: 2.67 $\pm$ 0.88 mg. In terms of amount of peroxide per kg body weight the bleaching systems led to a single exposure of maximum 0.046 mg/kg bw of hydrogen peroxide (Colgate Simply White). Smoking did not affect the degrading of hydrogen peroxide from bleaching products.

The concentration of hydrogen peroxide in contact with the teeth will be close to the concentration in the bleaching product. The gingival concentration of hydrogen peroxide has been reported to be about 0.7% and 0.6% 5 minutes after application of a strip containing 10% and 6.5% hydrogen peroxide. The level was reduced to 0.1% and 0.2%, respectively after 30 min.

The maximum concentration of hydrogen peroxide in the saliva after teeth bleaching has been reported to be 0.1% corresponding to about 5 $\mu g/cm^2$. The saliva concentration seems to be similar with a 200 mg strip containing 6.5% hydrogen peroxide and a 100 mg strip containing 14% hydrogen peroxide.

For gel strips it has been reported users occasionally may swallow the strip, resulting in an exposure of about 12 mg hydrogen peroxide (0.2 mg/kg bw). When trays are used, overfilling of the tray and excessive biting on the tray are factors that may cause additional release of the bleaching agent.

From the exposure studies the systemic absorption of hydrogen peroxide is estimated to be in the range from 0.03 mg/kg bw/d to 0.2 mg/kg bw/d. Consequently, *an exposure from tooth whitening products with 6% hydrogen peroxide of 0.2 mg/kg bw/d may be used in safety calculation.*

Mucous membrane irritation

Ulceration of gastric mucosa in rats was observed 1 hour after gavage with a dose corresponding to 5.4 mg/kg bw of hydrogen peroxide while no effects were found at 1.8 mg/kg bw of hydrogen peroxide. This is only (1.8/0.2) 9 times higher than the dose

expected using tooth whitening products containing 6.0% hydrogen peroxide. In cases where gel strips were swallowed accidentally, gastric symptoms have been reported.

Carcinogenicity

A tumour frequency of 5% was found in Sencar mice after skin painting with the initiator DMBA followed by skin painting with hydrogen peroxide at a concentration corresponding to *470 μg/cm²day* 2 times a week for 25 weeks. Since the maximum concentration of hydrogen peroxide in the saliva after teeth bleaching has been reported to be about *5 μg/cm²*, the amount by $cm^2$ in users of tooth whitening products, who may also be exposed to carcinogenic polycyclic hydrocarbons from smoking, is only about (470/5) 94 times higher than the amount giving 5% tumour in mice.

Clinical safety data

The cosmetic industry and their organisations have pointed out that over 100 published and unpublished clinical studies, comprising approximately 4000 subjects in total, are available. In addition, there exists a 7.5-year follow-up study on a small group of tooth whitening products users. It should be noted that only 9 of the 15 persons in the long-term study agreed to clinical examination. Six studies, all with less than 100 people, had up to 6 months follow-up. The majority of the studies seemed to have lasted less than 1.5 month and involve less than 150 persons. Only one 28-day study has been reported with adolescents (12 – 18 years old). For a case-reference study to detect a doubling of the risk for an adverse effect that occurs at a level of 1:1000 in the reference group, the study group must have at least 1000 people. The majority of the studies were judged to be at high risk of bias and were either sponsored or conducted by the manufacturers. Thus, there is a need of independent and well conducted clinical studies during the use of tooth whitening products as well as long-term clinical data and epidemiological studies that assess the possible adverse effects of tooth whitening products within the oral cavity.

In a large survey of dentists, 91% of 8,143 dentists stated that they had used vital tooth bleaching. Side effects reported by the respondents included the following: 62.2% noted tooth hypersensitivity, 45.9% reported soft-tissue irritation, 2.1% noted systemic effects, and 18.8% reported no side effects.

Tooth sensitivity is a common side-effect of external tooth bleaching. Data from various studies of tooth bleaching revealed that up to 65% of the patients reported increased tooth sensitivity. Tooth sensitivity normally persists for up to 4 days after the cessation of bleaching treatment, but a longer duration of up to 39 days has been reported. In clinical trials with bleaching with hydrogen or carbamide peroxide in custom-made trays, 25 to 40% of the patients reported gingival irritation during treatment.

The safety of three months use of strips was evaluated. The product was designed as a one-week use and the present conditions represent a twelve times overuse. Forty subjects were divided into two groups and were assigned to either 6% hydrogen peroxide strips or 9.5% hydrogen peroxide strips. Subjects used their product on the maxillary teeth for 30 minutes twice a day for 3 months. For the 6% hydrogen peroxide strips, 6% of subjects had oral soft tissue adverse effects and 44% reported tooth sensitivity. For the 9.5% hydrogen peroxide strips, 6% of subjects had oral soft tissue adverse effects and 59% reported tooth sensitivity. One severe tooth sensitivity adverse effect was reported with the 9.5% hydrogen peroxide strips. All of the adverse effects resolved when product use was discontinued.

Special investigations

*Enamel and Dentine Surface Morphology and Chemistry.* Scanning electron microscopy (SEM) has been used for qualitatively analysing the surface morphology of enamel and dentine specimens following bleaching. In addition profilometry has been used to measure the surface roughness. The majority of these *in vitro* studies indicate that hydrogen

peroxide and carbamide peroxide containing products have no significant deleterious effects on enamel and dentine surface morphology and that the contrasting studies that do show an effect, in general, have some limitations in the *in vitro* methodologies used. It should be noted, however, that two clinical cases of serious adverse effects on enamel associated with whitening agents, both of which involved the use of "over-the-counter" products, have been reported.

*Enamel and Dentine Surface Microhardness.* Surface microhardness (SMH) measurement has been a frequently used technique for evaluating the effects of peroxide and bleaching products on enamel and dentine. There are numerous published *in vitro* reports in the literature detailing the detrimental effects or lack of effects of peroxide-containing tooth whitening products on enamel microhardness, enamel resistance to abrasion, dentin microhardness, dentin roughening, and restoration microhardness. The results are dependent on the methodology used and the materials or products tested. An effect is often observed with artificial saliva containing no organic components or if no fluoride treatments to aid remineralisation is used. Moreover, it has been found that bovine enamel has a three-fold faster rate of lesion progression compared to human enamel. The majority of the more recent studies indicate that hydrogen peroxide and carbamide peroxide containing products have no significant deleterious effects on human enamel and dentine SMH.

*Subsurface Enamel and Dentine.* Since hydrogen peroxide will diffuse through enamel towards the enamel-dentine junction, some studies have investigated the effects of bleach agents on subsurface enamel and dentine. This is typically accomplished by bleaching whole teeth or fragments and then cutting and polishing the specimens to reveal the internal subsurface enamel and dentine areas, followed by microhardness measurements. An alternative approach to investigating the effects of bleaching on subsurface enamel, dentine and the enamel-dentine junction is to use confocal laser scanning microscopy which enables their ultrastructure to be investigated. The majority of relevant *in vitro* studies indicate that hydrogen peroxide and carbamide peroxide containing products have no significant deleterious effects on subsurface enamel and dentine microhardness or ultrastructure. It should be noted, however, that the effects of bleaching agents on the mechanical properties of enamel have not been extensively studied. Although it is difficult to clinically associate enamel cracking or fractures with previous bleaching treatments, there is increasing evidence that enamel structural changes may occur due to exposure to such substances.

*Effects of Acid Challenges and Abrasion on Bleached Enamel/Dentine.* Some studies have reported that pre-bleaching human enamel and dentine with hydrogen peroxide or carbamide peroxide had no subsequent deleterious effect on enamel and dentine loss caused by citric acid erosive challenges or brushing with toothpaste. Other studies indicated that acidic agents or long duration of bleaching may lead to an increased susceptibility to enamel loss by tooth brushing abrasion.

*Effects on Restorative Materials.* Porcelain or other ceramic restoratives as well as dental gold appear generally unaffected by bleaching procedures. Composite restorations, on the whole would seem to be more reactive to bleach effects, but these still may include only minor etching or softening depending upon treatment conditions. In studies of strip bleaching gels, glass ionomers were largely unaffected. Zinc phosphate cement has been observed to be completely solubilized by a carbamide peroxide bleaching gel. Dental amalgams show signs of oxidative reactivity with bleaching gels with minor localized spotting and colour changes observed on amalgam surfaces. *In vitro* studies, under exaggerated conditions of use, have demonstrated release of very small amounts of mercury from amalgams, which are at levels well within the limits for mercury exposure in the guidelines of WHO.

*Uptake of Bleaches and Transport to Dental Pulp.* Hydrogen peroxide is readily transported through tooth enamel into dentin and pulp. Despite this uptake, the development of pulpal

damage associated with vital tooth bleaching is low. It is pointed out that peroxide concentration from 14% hydrogen peroxide gel was far below levels required for the initiation of significant enzyme inhibition. Vital tooth bleaching produces histological evidence of minor inflammation of superficial layers of pulp adjacent to the pulp-dentin junction. The minor inflammatory response of the pulp to the introduction of bleaching seems to be concurrent with the pain response expressed by consumers having increased hypersensitivity.

Bleaching agents can also enter the pulp via leakage from tooth restorations, particularly at the cemento-enamel junction and following thermal stress (Crim, 1992). Histological evaluation of the pulp after vital bleaching with 10 % carbamide peroxide revealed mild inflammatory changes in 4 out of 12 teeth both after 4 days and 14 days treatment, and no changes after 14 days treatment followed by "recovery" phase of 14 days (González-Ochoa 2002).

Catalase activity in the dental pulp is very low and there is virtually no glutathione peroxide activity (Bowles and Burns, 1992). Application of a 3% hydrogen peroxide solution to the dentin of rat incisors caused emphysema and capillary stasis, and slowed down the blood circulation in the underlying pulp. Direct application of hydrogen peroxide to the pulp itself caused permanent damage to the capillary net (Gaengler, 1976). This study describes, however, extreme conditions which would not be expected to be present when hydrogen peroxide is used by humans in oral hygiene products.

*Summary*
The EU regulation states that the hydrogen peroxide content of oral hygiene products should not exceed 0.1%. In the case of toothpastes and mouth-rinses the exposure times are short and a risk assessment has been made on the basis of MOS.

Tooth whitening products are available in many forms, e.g. trays with gel, gel strips, paste-on gel. Moreover, although the majority of the products contain hydrogen peroxide or carbamide peroxide, other chemicals such as sodium percarbonate, sodium perborate, and potassium peroxymonosulphate may be used. The later chemicals are briefly discussed in Appendix, and should be regulated similarly as hydrogen peroxide on the basis of hydrogen peroxide or reactive oxygen products released. Sodium perborate fulfils the criteria of a classification of toxic to reproduction category 2 (R61). Additionally, there is a current proposal that sodium perborate should be so classified (http://ecb.jrc.it/classification-labelling/search-classlab/ (Search Working Database)).

In the case of tooth whitening products the exposure time will be considerable. The safety of tooth whitening products is based both on calculations of MOS as well as consideration of possible acute and long-term effects.

All trials with tooth whitening products were short term and the majorities of the studies were judged to be at high risk of bias and were either sponsored or conducted by the manufacturers. The majority of the studies are performed *in vitro*. Moreover, no *in vivo* studies including multiple use of tooth whitening products are available. Therefore, in order to carry out a robust risk assessment, there is a need for long-term and independent clinical data and long-term epidemiological studies in order to evaluate possible adverse effects within the oral cavity associated with use of tooth whitening products. These studies should include participants representing diverse populations and be performed as described in the SCCP document "A guidance document on epidemiological and clinical studies on tooth whitening products" (SCCP/0974/06).

### 3.3.15. Concerns

Bottenberg (2004) points out: "Tooth bleaching is not just a simple cosmetic operation. It is important to diagnose correctly the cause of the staining or discoloration, to establish a prognosis whether the stain can be removed or not, to give the patient a complete clinical examination in order to establish other oral health problems be they related or not to the discoloration, and to counsel the patient about the best way of treating or not treating this esthetical problem." "Before bleaching is performed, defective restorations should be replaced. All tooth and restoration surfaces must be thoroughly cleaned and adequate oral hygiene instruction are to be given to the patient."

Hydrogen peroxide has well-known potential to cause irritations. The use of hydrogen peroxide in orally applied products might cause irritation of mucous membranes in the oral cavity and in the gastric tract if the product is swallowed accidentally. Mucosal irritation is commonly observed as a side effect in clinical studies and consumer complaints.

Conditions such as pre-existing tissue injury or the concurrent use of alcohol and/or tobacco while using tooth whiteners may also exacerbate their toxic effects. Hydrogen peroxide, even at concentrations as low as 3%, may be especially harmful to oral tissues if they have been previously injured (Rees and Orth, 1986). Therefore, particular care should be taken in administering bleaching agents to patients with gingivitis, periodontal disease, or pre-existing gingival lesions, and to those using alcohol and tobacco (Tipton et al., 1995). This mixed exposure may be of concern since smokers are likely candidates for tooth bleaching.

Ulceration of gastric mucosa was observed in rats 1 hour after gavage with a dose corresponding to 5.4 mg/kg bw of hydrogen peroxide while no effects were found at 1.8 mg/kg bw of hydrogen peroxide. The ratio between the "no effect level" in rats and the dose after use of mouth-rinses containing 0.1% hydrogen peroxide and tooth whitening products containing 6% hydrogen peroxide are low. In relation to this it should be noted that in the case of gel strips, it has been reported that the user occasionally may swallow the strip. This will result in an exposure of about 12 mg hydrogen peroxide. In several of these cases, consumers reported minor gastrointestinal symptoms.

Clinical studies have mostly been designed to assess efficiency of external tooth-bleaching and the risk of adverse effects has not been the main focus in these studies. In the clinical studies published on tooth-bleaching that address adverse effects, the number of participants was small and many studies did not have control groups. This is considered inadequate, since for a case-reference study that should detect a doubling of the risk for an adverse effect occurring at a level of 1:1000 in the reference group, the study group must have at least 1000 people, and for detection of a 10% increase in the risk, more than 10.000 people must be enrolled in the study (Dahl and Pallesen, 2003). Although the first articles on night guard bleaching with hydrogen or carbamide peroxide were published nearly 20 years ago, and the these products have been in the market place for many years in some countries, there have so far been no systematic epidemiological studies to assess possible long term effects. It is also of concern that only one 28-day study has been reported with adolescents (12 – 18 years old). Therefore, a number of potential adverse effects of external tooth-bleaching cannot be properly assessed as this time.

Tobacco use and alcohol abuse are the main risk factors for cancer in the oral cavity (IARC, 1988, 2004, 2007). Since hydrogen peroxide can act as a promoter, it may increase the risk of oral cancer in persons that already have an elevated risk due to tobacco use, alcohol abuse, or genetic predisposition. The risk may further increase with repeated exposure to tooth whiteners. Based on a case report, it has been suggested that users of tooth whiteners should be further studied with regard to oral cancer (Burningham et al., 2004).

All bleaching materials demonstrate diffusion of hydrogen peroxide through dentin. Few investigators have addressed the possible pathophysiological effects on oral and pulpal tissues from long-term treatment. The dental pulp is vulnerable through exposed dentin in patients with gingival retraction, attrition, cervical abrasion, and leaking restorations, and the gingiva may be exposed directly to hydrogen peroxide gels during treatment. Significant amounts of hydrogen peroxide diffuse through dentin after application of carbamide peroxide and hydrogen peroxide-based bleaching agents (Hanks et al., 1993).

The bleaching procedure produces a transient reduction on the bond strengths of enamel and dentin immediately following bleaching. Because of the transient reduction in bond strength, dentists should delay restoration placement for periods of one to two weeks after bleaching to ensure sufficient bond strength and retention on teeth.

Data from laboratory studies documented increased mercury release from dental amalgams exposed to carbamide peroxide solutions for periods ranging from 8 hrs to 14-28 days. The amount mercury released varied with type of amalgam and type of bleaching agent and ranged from 4 to 30 times higher than in saline controls. However, the clinical significance of the loss of mercury from amalgam is unclear. It is concluded that bleaching of teeth containing amalgam restoration should be approached with caution (Swift and Perdigao, 1998).

## 4. CONCLUSION

In response to the questions asked, the SCCP is of the opinion that for practical reasons, the following concentration limits of hydrogen peroxide, when used in oral hygiene and tooth whitening products, should be considered: up to 0.1%, up to 6%[3], and more than 6%. The limit of 0.1% is based on a level at which there is no risk to the consumer from the use of hydrogen peroxide in oral hygiene and tooth whitening products under normal or reasonable foreseeable conditions of use. In toothpastes and mouth rinses, the hydrogen peroxide concentration should not exceed 0.1% (SCCNFP/0158/99). The limit of 6% for tooth whitening products refers to the limit given in the Terms of Reference in relation to the Opinion SCCP/0844/04. It should also be noted that for hydrogen peroxide concentrations above 6%, the MOS will be below 100; therefore, products containing more than 6% hydrogen peroxide are not safe for use by the consumer.

The present opinion refers to the concentration of hydrogen peroxide in its free form or when released (some hydrogen peroxide releasing substances may not be specifically mentioned in this opinion). In the case of the substances discussed in Appendix (sodium percarbonate, sodium perborate, and potassium peroxymonosulphate), it refers to the concentrations of the substances that will result in the same amount of hydrogen peroxide or reactive oxygen species being available as the specified concentrations of hydrogen peroxide above.

Sodium perborate fulfils the criteria of a classification of toxic to reproduction category 2 (R61). Additionally, there is a current proposal that sodium perborate should be so classified (http://ecb.jrc.it/classification-labelling/search-classlab/ (Search Working Database)).

The available data does not permit a distinction between the different tooth whitening products (e.g. tray-based gel, gel strips, paint-on gel) with regards to adverse effects.

[3] Higher concentrations may be used provided that the total amount of hydrogen peroxide is equal or lower than in products using 6% hydrogen peroxide and 0.2 g gel load and that studies have demonstrated that the concentration of hydrogen peroxide in the saliva and on the gingiva is not higher than in products containing 6% hydrogen peroxide.

This opinion only concerns the cosmetic use of oral hygiene and tooth whitening products.

**Oral hygiene and tooth whitening products containing up to 0.1% hydrogen peroxide**

- The use of oral hygiene and tooth whitening products containing up to 0.1% hydrogen peroxide does not pose a risk to the health of the consumer.

**Tooth whitening products containing > 0.1% and ≤ 6% hydrogen peroxide**

- Based on the available data, the SCCP is not in a position to define a level of hydrogen peroxide and a frequency of application that would result in exposure which would be considered safe for the consumer.

- With increasing concentration of hydrogen peroxide and frequency of application there will be an increasing risk associated with the use of these products. It cannot be anticipated what the exposure would be if the products were to be freely and directly available to the consumer.

- Potential risks associated with the use of products containing more than 0.1% and up to 6% hydrogen peroxide may be reduced if:

  a) they are used only after clinical examination to ensure the absence of risk factors identified below or other oral pathology of concern.

    - Particular care in using them should be taken by persons with gingivitis and other periodontal diseases or defective restorations. Conditions such as pre-existing oral tissue injury or concurrent use of tobacco and/or alcohol may exacerbate the possible toxic effects of hydrogen peroxide (see e.g. section 3.3.15).

    - Their use is not recommended prior to or immediately after dental restoration.

  b) exposure to tooth whitening products containing 0.1 to 6% hydrogen peroxide is to be limited in a manner that ensures that the products are used only as intended in terms of frequency and duration of application to avoid reasonably foreseeable misuse.

- There is an absence of good clinical data and long-term epidemiological studies that assess the possible adverse effects within the oral cavity (see SCCP/0974/06). The SCCP recommends that, in consideration of public health, independent long-term safety evaluations should be performed (see SCCP/0974/06).

- In the absence of specific data on the safety of tooth whitening products in children/adolescents, the SCCP is not in a position to assess the potential health risks associated with their use in this population subgroup.

**Tooth whitening products containing > 6 % hydrogen peroxide**

Because of the increasing risks of acute and long-term effects, tooth whitening products containing > 6.0% hydrogen peroxide are not considered safe for use by the consumer.

## 5. MINORITY OPINION

Not applicable

## 6. REFERENCES

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# 7. APPENDIX

## SODIUM PERBORATE

*Most of the chemical and toxicological information is from ECB (2003).*

### 3.1. Chemical and Physical Specifications

| 3.1.1. | Chemical identity |
|---|---|

| 3.1.1.1. | Primary name and/or INCI name |
|---|---|

Sodium perborate

| 3.1.1.2. | Chemical names |
|---|---|

Sodium perborate
Perboric acid, sodium salt
Sodium peroxoborate
Metaborate peroxyhydrate

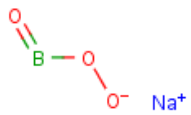
| 3.1.1.3. | Trade names and abbreviations |
|---|---|

Dexol

| 3.1.1.4. | CAS / EINECS/ELINCS number |
|---|---|

| CAS | EINECS | NAME |
|---|---|---|
| 15120-21-5 | 239-172-9 | sodium perborate |
| 11138-47-9 | 234-390-0 | perboric acid, sodium salt |
| 7632-04-4 | 231-556-4 | sodium peroxymetaborate |
| 10332-33-9 | | perboric acid (HBO(O2)) sodium salt, monohydrate |
| 10486-00-7 | | perboric acid (HBO(O2)) sodium salt, tetrahydrate |

| 3.1.1.5. | Structural formula |
|---|---|




| 3.1.1.6. | Empirical formula |
|---|---|

Formula: $BO_3Na$

| 3.1.2. | Physical form |
|---|---|

White, odourless, crystalline powder

| 3.1.3. | Molecular weight |
|---|---|

Molecular weight: 81.80

| 3.1.4. | Purity, composition and substance codes |
|---|---|

Purity: Approximately 95%

| 3.1.5. | Impurities / accompanying contaminants |
|---|---|

Impurities: Borax <2%
Several metals < 200 ppm total metal content

Additives: $MgSO_4 \leq 1.2\%$ stabilizer

| 3.1.6. | Solubility |
|---|---|

Soluble in water (sodium perborate monohydrate, 15-16 g/l; sodium perborate tetrahydrate 23 g/l) giving a solution of hydrogen peroxide and sodium borate

| 3.1.7. | Partition coefficient (Log $P_{ow}$) |
|---|---|

Log $P_{ow}$: /

| 3.1.8. | Additional physical and chemical specifications |
|---|---|

| | |
|---|---|
| Appearance: | Colourless crystals |
| Melting point: | Monohydrate decomposes > 50 °C; Tetrahydrate 60 – 65.5 °C |
| (melting in | its own crystallization water, beginning decomposition |
| Boiling point: | / |
| Density: | Monohydrate, 0.4-0.65; Tetrahydrate 0.65-0.9 |
| Rel. vap. dens.: | / |
| Vapour Press.: | / |

Conversion factor: Monohydrate dose x 0.341 = equivalent dose (hydrogen peroxide)
Tetrahydrate dose x 0.221 = equivalent dose (hydrogen peroxide)

| 3.1.9. | Stability |
|---|---|

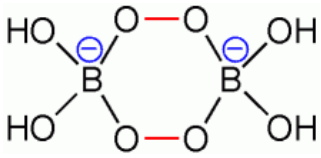
Stable when kept cool and dry, but decomposes with liberation of oxygen in warm moist air.

## 3.2. Function and uses

Main consumer exposure to sodium perborate is due to its use in laundry (15-25%), dishwasher detergents (10-15%) and as stain removers.

The generation of active oxygen in aqueous solutions is the basis for the use of sodium perborate as bleaching component in detergent products and bleaching agents. The purity of the technical products is characterized by their active oxygen content. Pure sodium perborate monohydrate contains a maximum of 16.0% active oxygen, pure tetrahydrate max. 10.4% (calculated from the empirical formula assuming that the peroxo bridges in the molecules are completely degraded with oxygen emerging).

The crystalline reagent is available as a hydrate with the general formula $NaBO_3 \bullet n\ H_2O$ (n: 1-4). The compound itself is a dimer:




$2Na^+$

Sodium perborate is soluble in water and releases hydrogen peroxide. Unlike percarbonate, perborate is not just an addition compound of peroxide, but contains true peroxygen bonds. In dilute solution, an equilibrium exists that still contains peroxoborate anions. These peroxoborate species are able to deliver the hydroperoxide anion at a lower pH than when $H_2O_2$ is used. Sodium perborate monohydrate and tetrahydrate contain theoretically about 34% and 22% hydrogen peroxide, respectively.

Sodium perborate has been used in vital tooth bleaching systems (e.g. Supersmile Whitening Toothpase, Vitint Tooth Gel, Vitint Mouth Contitioner, Vitint Irresistible, Vitint Safe & White Whitening Gel, Vitint System A [for use by dentist only].The concentrations of sodium perborate in the different products are not given.

## 3.3. Toxicological Evaluation

### 3.3.1. Acute toxicity

LD50 in mice 3250 mg/kg after oral administration (Shokuin et al, 1986)

LD50 in mice 1060 mg/kg after oral administration (tetrahydrate) Gigiena (1984)

LD50 in rat 1200 mg/kg after oral administration (tetrahydrate) Gigiena (1984)

The acute dermal toxicity of sodium perborate monohydrate is low in rabbits. After dermal application of 2000 mg sodium perborate monohydrate/kg bw, 9 of 10 animals survived (Interox, 1987a).

The fatal dose of boric acid, sodium borate, or sodium perborate is 0.1-0.5 g/kg in humans (Dreisbach, 1987).

### 3.3.2. Irritation and corrosivity

#### 3.3.2.1. Skin irritation

In some studies with sodium perborate monohydrate after prolonged exposure very mild irritating effects were observed which were not completely reversible in some cases.

Solutions of 10 % sodium perborate tetrahydrate are mildly irritating. There is no information on the irritating potential of more concentrated solutions.

Ref.: ECB (2003)

#### 3.3.2.2. Eye irritation

In all studies according to standard protocols sodium perborate showed severe eye irritating effects, when applied as solid substance to the eyes (Bagley et al., 1994; ICI, 1986a,b; Interox, 1987b; Momma et al., 1986). Moderate corneal opacity, severe iritis and conjunctival effects which consisted of severe redness, moderate chemosis and severe discharge were recorded. The effects were not completely reversible. Rinsing within 30 seconds after application reduced the severity of the effect considerably (Momma et al., 1986). Also with lower concentrations than in standard protocols the effect was weaker (Procter & Gamble, 1965, 1973). The irritating potential of sodium perborate tetrahydrate seems to be lower than for the monohydrate, being consistent with its higher water content (ICI, 1986 a, b)

*Comment*
Sodium perborate caused strong eye irritation in animal studies, the effects being not reversible in most of the animals tested. Although the scores for irritation are not sufficient for classification with R41, due to the irreversible effect, both sodium perborate monohydrate and sodium perborate tetrahydrate are proposed to be classified with R41, "Risk of serious damage to eyes".

Ref.: ECB (2003)

### 3.3.3. Skin sensitisation

Sodium perborate monohydrate was tested in a Bühler test according to OECD guideline 405 test in 10 guinea pigs (5 males, 5 females) applying 0.5 ml/animal (not further specified, probably powder as it is, 6 h/d, occlusive) once every seven days for a total of three applications. 14 days after the last induction the animals were challenged with 0.5ml of a 5% solution in distilled water (maximum non-irritant concentration). 10 untreated animals served as controls. One of 10 test group animals as well as one of 10 control group animals showed a very slight erythema after 24 h. The test substance was regarded to be not skin sensitising in this test (Interox, 1987c).

### 3.3.4. Absorption

#### 3.3.4.1. Dermal / percutaneous

There are no valid quantitative data on the absorption of sodium perborate following dermal exposure. Absorption from the mucous membranes of the mouth seems to be low. Dermal absorption of $H_2O_2$ is negligible. A thorough investigation showed that dermal absorption of other boron compounds is very low. Therefore for the risk assessment dermal absorption of 1% was assumed.

Ref.: ECB (2003)

#### 3.3.4.2. Oral / inhalation

From a study with human volunteers it can be concluded that oral absorption is higher than 30 %. For the risk assessment, oral absorption and the absorption via inhalation of sodium perborate hydrates are assumed to be 100 %.

Ref.: ECB (2003)

### 3.3.5. Repeated dose toxicity

Oral

Experiments in rats with oral application (via gavage) of sodium perborate have been performed with the tetrahydrate (Degussa, 1989, 1000 mg/kg bw/d, 215 mg/ml, 28 days) or not further specified perborate (Dufour, 1971, 1000 mg/kg bw/d, 8 days (100 mg/ml, 200 mg/kg bw/d, 6 days). Targets for toxic effects at a dose level of 1000 mg/kg bw are the stomach, the haematologic system and possibly the testes.

In the 28 day-study from Degussa (1989) acanthosis and hyperkeratosis in the forestomach and hyperplasia of the fundic mucosa were observed after application of 1000 mg/kg bw/d via gavage. No effects on the stomach were found in the study of Dufour et al. (1971) in rats receiving the same dose. But in this study the exposure was only for 6 days to a more diluted solution and the animals were examined after a recovery period of 8 days.

Haematological effects have been observed in both studies. In the study of Degussa (1989) after application of 1000 mg/kg bw/d at the end of the study red blood cell count, haemoglobin, haematocrit and number of lymphocytes was statistically significantly decreased, the number of platelets was statistically significantly increased. The spleen size and spleenic parenchyma were reduced. In contrast in the Dufour-study, (with 8 days duration instead of 28 days in the Degussa study) no changes in blood cell parameters were observed during the study but haemoglobin and haematocrit were increased up to 15 days after the end of the application. This was explained by depression of haematopoiesis during the study and overregulation at the termination of the application. In conclusion the NOEL for these endpoints is below the dose of 1000 mg/kg bw/d tested.

*Comment*

Effects after oral application of sodium perborate can be attributed to the degradation products. From the 28-day study from Degussa (1989) a NOAEL cannot be derived, because the only dose investigated was 1000 mg/kg bw/d which showed effects on the stomach, spleen and the haematopoietic system. No effects were recorded in the study of Dufour (1971). This study was only for 6 days with 3 days of recovery and only a limited number of parameters have been investigated. Therefore also from this study a NOAEL cannot be derived. Systemic effects, which have to be considered, are the effects on the haematopoietic system. Thus the LOAEL is 1000 mg sodium perborate tetrahydrate/kg bw/d (70 mg boron/kg bw/d) and no NOAEL can be derived.

Ref.: ECB (2003)

Dermal

Two dermal studies, both on sodium perborate tetrahydrate, with limited reporting of the results are available. New Zealand white rabbits received either a dermal dose of 200 mg/kg bw/d in 10% aqueous solution on the abraded skin for 3 weeks (Proctor & Gamble, 1965; 1966a) or 50 mg/kg bw/d as 2.5% solution on the intact skin for 13 weeks (Procter & Gamble, 1966b). In both studies there were no statistically significant differences compared to controls in growth, organ/body weight ratios (liver, kidney), blood parameters (all relevant parameters investigated, figures included in the report), gross pathology, or histopathology (organs required in current guidelines investigated). Only during the application of the 10 % solution to the abraded skin, some animals showed mild irritative effects.

*Comment*

The reporting of the results of the available studies (Procter & Gamble, 1965, 1966a;b) is limited. However all relevant organs have been examined by histopathology and also haematological parameters have been investigated which have shown changes in the oral studies. Therefore the studies are considered as sufficient for the risk assessment. In comparison with the results from the oral studies and as it can be assumed that sodium

perborate is not taken up by the skin very efficiently the NOAEL of this study of 200 mg/kg bw/d, which was the highest dose tested, may be too low.

Ref.: ECB (2003)

### 3.3.6. Mutagenicity / Genotoxicity

#### 3.3.6.1. Mutagenicity / Genotoxicity *in vitro*

The mutagenic potential of sodium perborate was investigated in three different assays which included the induction of DNA damage, of point mutations, and of chromosomal aberrations. The results indicated that sodium perborate was capable of producing mutagenic changes in a number of *in vitro* test systems. In an assay which was tailored to probe for oxidative damage induced by a chemical agent, the potential of sodium perborate for inflicting damage to DNA was demonstrated. Sodium perborate was able to oxidize thymidine to an appreciable extent at an incubation temperature of 80 °C, but even at 40 °C the oxidation was measurable. Point mutations were induced in the *Salmonella typhimurium* strains (TA-100) and (TA-102). There was no response with (TA-98). The mutagenic activity was abolished completely by incubation in the presence of rat liver S9. Chinese hamster ovary cells (strain CHO-K1) underwent extensive chromosomal damage when treated with sodium-perborate. Special note was taken of the rather unusual prevalence of chromosome rearrangements (Seiler, 1989).

Sodium perborate was positive in the *Escherichia coli* polA (W3119 vs P3478) rec-assay, DNA effects (bacterial DNA repair) (Leifer et al., 1981).

Sodium perborate tetrahydrate induced mutations in *Salmonella typhimurium* TA102, *Escherichia coli* WP2 (PKM101), and *Escherichia coli* WP2 UVRA (PKM101), in the absence of metabolic activation (Watanabe et al., 1998).

Whitening gel containing hydrocarbon-oxo-borate complex were compared with commercial hydrogen peroxide and carbamide peroxide products. The effects of human epithelial cell line for induction of DNA damage and subsequent induction of apoptosis and necrosis have been studied. The study was performed with MCF-7 (human breast cancer cells). The result show that the two hydrogen peroxide and the one carbamide peroxide based products induce significant DNA breakdown in MCF-7 cells while the hydrocarbon-oxo-borate complex showed much less DNA breakdown even at the highest concentration. While the hydrogen peroxide and carbamide peroxide bleaching agents induced massive necrosis at both 1 mg/ml and 10 mg/ml and no induction of apoptosis, the borate gel induce physiological cell death (apoptosis), both at 1 mg/ml and 10 mg/ml, while virtually no necrosis was found (Li and Ramaekers, 2004).

*Comment*
The *in vitro* studies on sodium perborate show a genotoxic potential. This may be due to the generation of $H_2O_2$, as similar to investigations with $H_2O_2$ the responses observed were reduced by the presence of catalase. Therefore, analogous to $H_2O_2$, the genotoxic potential may not be relevant *in vivo*. Furthermore, in contrast to $H_2O_2$, due to its ionisation, sodium perborate itself should be taken up by cells less easily than $H_2O_2$.

Ref.: ECB (2003)

#### 3.3.6.2 Mutagenicity/Genotoxicity *in vivo*

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### 3.3.7. Carcinogenicity

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### 3.3.8. Reproductive toxicity

In a developmental toxicity study according to OECD Guideline 414, groups of 25 mated Crl:Cd (SD) BR rats were dosed by gavage with 0, 100, 300 and 1000 mg/kg bw/d of sodium perborate tetrahydrate in 1% aqueous methylcellulose (Bussi, 1995; Bussi, 1996)

Since no clinical signs of toxicity were reported, the only criteria for assessment of maternal toxicity are effects on body weight gain and food intake. Significant reductions in body weight gain were observed at the two top doses. A significant reduction in food intake was observed in the top dose group (1000 mg/kg bw/d). The reduced body weight gain of the dams is partly (in later stages of pregnancy) due to reduced weights of the litters, due to reduced foetal weights and increased number of resorptions. No significant differences for the different doses and no clear dose response are found for the weight gain of the dams from day 20 except for gravid uterine weight. As maternal toxicity was apparent also in earlier times of the pregnancy the NOAEL for maternal toxicity is 100 mg/kg bw/d.

At 100 mg/kg bw/d six externally malformed foetuses with ablepharia, acrania, exencephaly, macroglossia, cleft palate, cleft lip and facial cleft were found. The increase compared to controls was statistically significant. The authors of the study considered this finding incidental, since these kinds of malformations were only present in 2 litters and not at the higher dosages. Historical control data for the years 1993-1999 (Instituto di Ricerche Biomediche, 2000) showed, that such malformations occurred, however extremely seldom, with an incidence of 0- 0.12 %.

A dose related effect was found on the ossification and the bone system. At 100 mg/kg bw/d statistical significant effects were found for unossified 5$^{th}$ sternebra and supraoccipital incomplete ossification, both being close to the historical control data. At 300 mg/kg bw/d and above, various incomplete ossifications and wavy ribs occurred. At 1000 mg/kg bw/d malformations (fused ribs) were observed.

In addition visceral changes were found. The kidney was the main target at lower dosages. At 100 mg/kg bw/d and above, the number of variants was statistically significant increased, at 1000 mg/kg bw/d also the number of anomalies and malformations were increased. The malformation included hydroneprhrosis and hypoplasia. Other viscereal malformations were microphtalmia or anophthalmia, vascular ring, displaced or double aortic arch, displaced botallus duct. The malformations were different from those observed at 100 mg/kg bw/d. Furthermore, at 300 and 1000 mg/kg bw/d dose-related increases of post implantation losses and early resorptions and dose-related lower mean foetal and placental weights were observed. The authors of the study considered 100 mg/kg bw/d as NOAEL for foetal effects.

*Comment*
In a study on developmental effects of sodium perborate tetrahydrate according to OECD Guideline 414, 100 mg/kg bw/d of sodium perborate tetrahydrate was regarded by the authors of the study as the NOAEL for both maternal and developmental toxicity. Although reduced maternal weight gain as measure of maternal toxicity may partly be due to an increased number of resorptions and reduced foetal weights, other toxicological studies support the view that doses above 100 mg/kg bw/d via gavage are toxic to the dams. Critical is the evaluation of the external malformations at 100 mg/kg bw/d. They were statistically significant but considered incidental due to lack of dose response by the authors

of the study. This is supported by the fact that with other boron compounds which have very similar developmental effects as sodium perborate at higher dose levels, this type of effect was not found. A final decision on the NOAEL or LOAEL for the teratogenicity of sodium perborate will be made after the targeted evaluation of the boron compounds.

Ref.: ECB (2003)

It is proposed by ECB that sodium perborate should be classified as toxic to reproduction category 2; R61 *May cause harm to the unborn child* and category 3; R62 *Possible risk of impaired fertility* (http://ecb.jrc.it/classification-labelling/search-classlab/ (Search Working Database)).

### 3.3.9. Toxicokinetics

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### 3.3.10. Photo-induced toxicity

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### 3.3.11. Human data

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### 3.3.12. Special investigations

Lim et al. (2004) compared the bleaching efficacy of 35% carbamide peroxide, 35% hydrogen peroxide and sodium perborate (2 g per ml of distilled water to form a thick paste) for intracoronal bleaching of root filled discoloured teeth. Extracted premolars were artificially stained using whole blood then root canal treatment was performed. After obturation, a 2 mm intermediate base was placed 1 mm below the buccal amelocemental junction. Intracoronal bleaching was performed in 11 teeth per group, using either 35% carbamide peroxide gel (group CP), 35% hydrogen peroxide gel (group HP) or sodium perborate mixed with distilled water (group SP). The bleaching agents were replaced after 7 days. It was concluded that 35% carbamide peroxide and 35% hydrogen peroxide were equally effective for intracoronal bleaching, and significantly better than sodium perborate after 7 days. After 14 days, there were no significant differences between the groups.

Timpswat et al. (2005) determined the effect of intracoronal bleaching agents on adhesion of bonding agents to pulp chamber dentine. Forty extracted human maxillary anterior teeth were randomly divided into four groups of 10 teeth each. Bleaching agents were sealed in pulp chambers for 7 days, as in clinical use. Group 1 (control): distilled water, group 2: 35% hydrogen peroxide, group 3: sodium perborate (2 g per ml water) mixed with water, and group 4: sodium perborate (2 g per ml liquid) mixed with 35% hydrogen peroxide. Teeth were stored in saline at 37 $^{o}$C for 7 days. After the bleaching agent was removed, teeth were leached in water for a further 7 days prior to bonding. The crown was cut vertically from mesial to distal and the labial pulp chamber dentine was prepared for bonding with Clearfil SE-Bond and filled with resin composite (Clearfil AP-X). The bonded specimens were kept moist at 37 $^{o}$C for 24 h. Microtensile bond strengths were determined using a universal testing machine. Additional teeth were prepared using the same bleaching procedures to investigate the scanning electron microscopic appearance of the dentine surface. Mean values (±SD) of microtensile bond strength for the experimental groups were: group 1: 5.29 ± 2.21 MPa, group 2: 5.99 ± 1.51 MPa, group 3: 9.17 ± 1.65 MPa and group 4: 3.99 ± 1.31 MPa. Dentine treated with sodium perborate in water (group 3) had significantly higher mean bond strength when compared with the other three groups ($P < 0.05$). Mean bond strength was lowest when dentine was treated with sodium perborate

plus hydrogen peroxide (group 4). It was concluded that in terms of subsequent bond strength during restoration, sodium perborate mixed with distilled water appears to be the best intracoronal bleaching agent.

Chng et al. (2004) compared the effect of traditional and alternative bleaching agents on microhardness of human dentine when used intracoronally. Thirty-six premolars were divided into six groups and bleaching agents were sealed into the pulp chambers as follows: group 1 – distilled water (control), group 2–30% hydrogen peroxide solution, group 3 – sodium perborate mixed with distilled water to a consistency of wet sand, group 4 – sodium perborate mixed with 30% hydrogen peroxide solution, group 5–35% carbamide peroxide gel, group 6–35% hydrogen peroxide gel. Access cavities were sealed and the teeth were stored in distilled water at 37 °C. After 7 days, each tooth was sectioned at the cemento-enamel junction level and micro-hardness testing was carried out on dentine. The results showed that treatment with 35% hydrogen peroxide gel, 30% hydrogen peroxide solution and 35% carbamide peroxide gel reduced the micro-hardness of outer dentine to a small extent while treatment with sodium perborate mixed with water and sodium perborate mixed with 30% hydrogen peroxide solution did not significantly alter the micro-hardness of dentine.

### 3.3.13. Safety evaluation (including calculation of the MoS)

/

### 3.3.14. Discussion

*Chemistry*
Sodium perborate monohydrate and tetrahydrate contain theoretically about 34% and 22% hydrogen peroxide, respectively. It is soluble in water giving a solution of hydrogen peroxide and sodium borate. It contains < 2% borax, < 200 ppm total metal content, and $\leq$ 1.2% $MgSO_4$ (stabilizer) as impurity. The stability of sodium perborate in products is not known.

*Acute toxicity*
The oral LD50 in mice and rats is > 1000 mg/kg. The dermal LD50 in rabbits is > 2000 mg/kg.

*Irritation and corrosivity*
Solutions of 10% sodium perborate are mildly skin irritating. Sodium perborate caused strong eye irritation in animal studies.

*Skin sensitisation*
Sodium perborate was not skin sensitising in a Buehler test.

*Dermal / percutaneous absorption*
There are no valid quantitative data on the absorption of sodium perborate following dermal exposure.

*Repeated dose toxicity*
Effects after oral application of sodium perborate to rats can be attributed to the degradation products. Systemic effects, which have to be considered on the basis of a 28 day study, are the effects on the haematopoietic system. The LOAEL is 1000 mg sodium perborate tetrahydrate/kg bw/d (70 mg boron/kg bw/d) (only dose tested). The NOAEL from a 3 week skin painting study on rabbits is 200 mg/kg bw/d, which was the highest dose tested.

*Mutagenicity / Genotoxicity*
The *in vitro* studies on sodium perborate show a genotoxic potential, which may be due to the generation of $H_2O_2$. No *in vivo* studies are available.

*Carcinogenicity*
No data found.

*Reproductive toxicity*
In a study on developmental effects of sodium perborate tetrahydrate according to OECD Guideline 414, 100 mg/kg bw/d of sodium perborate tetrahydrate was regarded by the authors of the study as the NOAEL for both maternal and developmental toxicity.

It is proposed by ECB that sodium perborate should be classified as toxic to reproduction category 2; R61 *May cause harm to the unborn child* and category 3; R62 *Possible risk of impaired fertility.*

*Toxicokinetics*
No data found.

*Human data*
Sodium perborate has been used in vital tooth bleaching systems (e.g. Supersmile Whitening Toothpase, Vitint Tooth Gel, Vitint Mouth Contitioner, Vitint Irresistible, Vitint Safe & White Whitening Gel, Vitint System A [for use by dentist only]) The concentration of sodium perborate in the different products are not given.

Only a few studies are available. The results suggest that the effects are similar to those found with the use of hydrogen peroxide and carbamide peroxide on tooth bleaching products.

*Conclusion*
It is proposed by ECB for inclusion in ATP31 that sodium perborate should be classified as toxic to reproduction category 2; R61 *May cause harm to the unborn child* and category 3; R62 *Possible risk of impaired fertility* (http://ecb.jrc.it/classification-labelling/search-classlab/ (Search Working Database)). This will imply that sodium perborate can not be used in tooth whitening products.

## SODIUM PERCARBONATE

### 3.1. Chemical and Physical Specifications

| 3.1.1. | Chemical identity |
|---|---|

| 3.1.1.1. | Primary name and/or INCI name |
|---|---|

Sodium carbonate peroxide (INCI)

| 3.1.1.2. | Chemical names |
|---|---|

Tetrasodium hydrogen peroxide dicarbonate
Sodium percarbonate
Sodium carbonate hydrogen peroxide
Carbonoperoxoic acid, sodium salt
Peroxy sodium carbonate

| 3.1.1.3. | Trade names and abbreviations |
|---|---|

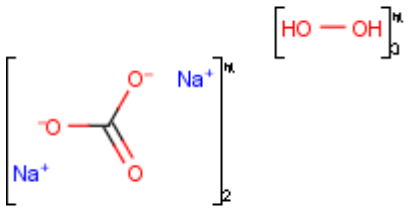
Caperox, Oxyper, Perdox,

| 3.1.1.4. | CAS / EINECS/ELINCS number |
|---|---|

CAS : 15630-89-4 (carbonic acid, disodium salt, compd. with hydrogen peroxide (2:3))
14455-47-1 (sodium percarbonate)
EINECS: 239-707-6

(both CAS numbers exist; only 15630-89-4 is listed in INCI dictionary and has an EINECS no)

| 3.1.1.5. | Structural formula |
|---|---|




| 3.1.1.6. | Empirical formula |
|---|---|

Formula: $C_2H_6O_{12}Na_4$

| 3.1.2. | Physical form |
|---|---|

Solid white powder

| 3.1.3. | Molecular weight |
|---|---|

Molecular weight: 313.96

| 3.1.4. | Purity, composition and substance codes |
|---|---|

Purity: /

| 3.1.5. | Impurities / accompanying contaminants |
|---|---|

Impurities: /

| 3.1.6. | Solubility |
|---|---|

In water: 130g/l

| 3.1.7. | Partition coefficient (Log $P_{ow}$) |
|---|---|

Log $P_{ow}$: /

| 3.1.8. | Additional physical and chemical specifications |
|---|---|

| | | |
|---|---|---|
| Appearance | : | Solid white powder |
| Melting point | : | Decompose above 50 °C |
| Boiling point | : | / |
| Density | : | 700 – 1100 kg/m$^3$ |
| Rel. vap. dens. | : | / |
| Vapour Press. | : | / |

Conversion factor: Sodium percarbonate dose x 0.28 = equivalent dose (hydrogen peroxide)

| 3.1.9. | Stability |
|---|---|

Sodium percarbonate dissociates into sodium carbonate and hydrogen peroxide rather easily in fresh water. A degradation of 30% within 24 hours was reported.

## 3.2. Function and uses

The structure of sodium percarbonate is a carbonate perhydrate: 2 $Na_2CO_3$ • 3 $H_2O_2$. Dissolved in water, it releases hydrogen peroxide and carbonate.

$$[Na_2CO_3]_2 \cdot 3H_2O_2 \rightarrow 2Na_2CO_3 + 3H_2O_2$$

Sodium percarbonate contains theoretically about 28% hydrogen peroxide.

Sodium percarbonate has been used in vital tooth bleaching systems (e.g. Crest Night Effects [19% sodium percarbonate, equivalent to 5.3% hydrogen peroxide]).

## 3.3. Toxicological Evaluation

| 3.3.1. | Acute toxicity |
|---|---|

***Oral***

LD50 mouse: 2050 - 2200 mg/kg (IUCLID dataset)
LD50 rat: 2000 mg/kg (IUCLID dataset)
LD50 rat: 1034 mg/kg (IUCLID dataset)

***Dermal***

LDLo > 2000 mg/kg in rabbit (IUCLID dataset)

| 3.3.2. | Irritation and corrosivity |
|---|---|

**Rabbit**

Skin irritation
Solid or 1% in water solution application repeated for 12 days.
Powder: Mild irritation with slight oedema, desquamation by day 4 but no progression afterwards.
1% Solution: Slight erythema and desquamation during the last 2 days of the test (IUCLID dataset)

Eye irritation
Powder: Moderate initial pain, severe irritation of cornea, iris, conjunctiva for 7 days, pannus reaction from day 3. Score 7 (max =8)
1% Solution: Slight irritation in 1 rabbit during 2 hours. Score 1 (IUCLID dataset)

| 3.3.3. | Skin sensitisation |
|---|---|

Skin painting test, guinea pig
Buehler test (1965). 75% solution used for induction phase, 25% solution for challenge test. Very slight dermal reaction during induction phase. No reaction during challenge. Conclusion: not sensitizing (IUCLID dataset).

| 3.3.4. | Dermal / percutaneous absorption |
|---|---|

/

| 3.3.5. | Repeated dose toxicity |
|---|---|

/

| 3.3.6. | Mutagenicity / Genotoxicity |
|---|---|

/

### 3.3.7. Carcinogenicity

/

### 3.3.8. Reproductive toxicity

/

### 3.3.9. Toxicokinetics

/

### 3.3.10. Photo-induced toxicity

/

### 3.3.11. Human data

Date et al. (2003) studied the whitening efficacy of a bleaching system containing 19% sodium percarbonate (Crest Night Effects). Nine adult volunteers participated in a study to determine overnight retention of the product. A non-reactive blue pigment was added to the product. 84.7% of the subject teeth surfaces were covered in the average with the blue pigment-containing film immediately after application. Following overnight use, very good retention was found as 77.4% of the initially covered surfaces still had pigment-containing film on them. The product was also evaluated in a 2-week efficacy study with 16 adult volunteers. The product was left on the teeth overnight and then removed in the morning via tooth brushing. A significant colour improvement was reported. The primary adverse events are oral irritation (13%) and tooth sensitivity (6%).

### 3.3.12. Special investigations

Mahony et al. (2003) studied the hydrogen peroxide degradation kinetics of a 19% sodium percarbonate (5.3% hydrogen peroxide released) direct application bleaching gel in the tooth surface and in saliva during use. 14 persons participated in the study. The median peroxide concentrations on the teeth at 10 minutes, 30 minutes, 1 hour, 2 hours, and 4 hours of daytime wear were 4.7, 3.5, 1.5, 0.3, and 0.1%, respectively. The median peroxide concentration in the saliva at 5, 10, and 20 minutes wear were 0.001, 0.0001, and 0.0001%, respectively. By 30 minutes, the median salivary concentration of peroxide was below the limit of detection (0.00007%).

Kaneko et al. (2000) evaluated the bleaching effect of sodium percarbonate on artificially stained pulpless teeth. Twenty extracted human mandibular premolers were stained by immersing them in human blood mixed with iron sulfide, and divided into three test groups and one control group. The following bleaching agents were used: sodium perborate (about 2 g/ml) mixed with 30% hydrogen peroxide (WB), sodium percarbonate mixed (about 2 g/ml) with distilled water (PW) or 30% hydrogen peroxide (PH). On days 5,10, and 15, each pulp chamber was refilled with fresh bleaching paste. The L* (average lightness) of the midbuccal area of specimens was measured before and after staining, and on days 5, 10, 15, and 20 by colorimeter. The bleaching effect was evaluated by the lightness recovery rate (LRR) calculated from L*. WB showed the highest LRR among all groups, and PV and PH showed significantly higher LRR when compared with the control group. PW and PH were not significantly different. It was concluded that sodium percarbonate had an obvious bleaching effect without hydrogen peroxide and that it could be a safe intracoronal bleaching agent.

Karpinia et al. (2003) evaluated the whitening effect of Crest Night Effects. 50 adults were randomized to Crest Night Effects or a placebo gel. Subjects were evaluated biweekly during the 6-week treatment period to evaluate initial and cumulative colour change, and again, 4 weeks after completion of the treatment to evaluate colour relapse. Relative to the baseline, the sodium percarbonate film group had significantly whiter teeth after 2 weeks overnight use. There was a cumulative benefit with additional treatment. Approximately 90% of the 6-week cumulative color improvement was still evident after 4 week post-treatment. Tooth sensitivity (20% of the peroxide group) represented the only adverse event with increased occurrence over placebo. These events were fully resolved during/after treatment.

Gerlach and Barker (2003) compared the whitening efficacy of Crest Night Effects, a 19% sodium percarbonate system packaged in unit dose sachets that when applied, dries to form an adherent film, and Colgate Simply White Night, a paint-on liquid in an applicator bottle at a concentration of 8.7% hydrogen peroxide. 57 adult volunteers were randomly selected in two groups. Both groups applied their assigned gel on the facial surfaces of the six maxillary anterior teeth for 14 nights. Significantly greater whitening was found for the 19% sodium percarbonate film compared to the 8.7% hydrogen peroxide gel.

Gökay et al. (2005) investigated peroxide penetration from newer bleaching products into the pulp chamber. Fifty extracted human maxillary central incisor teeth were separated into five groups (n = 10). All the teeth were sectioned 3 mm apical to the cemento-enamel junction; the intracoronal pulp tissue was removed, and the pulp chamber filled with acetate buffer. Buccal crown surfaces of teeth in the experimental groups were subjected to either a whitening strip (containing 5.3% hydrogen peroxide-G1) or one of three paint-on liquid whiteners (containing 19% sodium percarbonate peroxide-G2, 18% carbamide peroxide-G3 and 8.7% hydrogen peroxide-G4). The teeth in the control group (G5) were exposed only to distilled water. The acetate buffer solution in each tooth was then transferred to a glass test tube after 30 min and leuco-crystal violet and enzyme horseradish peroxidase were added, producing a blue solution. The optical density of the resultant blue colour in the tubes was measured by a UV-visible spectrophotometer at a wavelength of 596 nm. The values were converted into microgram equivalents of hydrogen peroxide using a spectrophotometric calibration curve. Statistically significant differences were found between all of the groups ($P < 0.05$). Pulpal peroxide was not observed in the control group (G5). G1, the whitening strip containing 5.3 % hydrogen peroxide showed the highest pulpal peroxide penetration. The differences in peroxide penetration were also observed within the paint-on whitener groups. Amounts of hydrogen peroxide found in the pulp chamber of G4 (8.7% hydrogen peroxide) was significantly higher when compared with G2 (19% sodium percarbonate, equivalent to 5.3% hydrogen peroxide) and G3 (18% carbamide peroxide, equivalent to 6.5% hydrogen peroxide) specimens. G3 showed the higher pulpal peroxide than G2. It was concluded that the peroxides from the whitening strip and paint-on whiteners penetrated into the pulp chamber to varying degrees.

### 3.3.13. Safety evaluation (including calculation of the MoS)

/

### 3.3.14. Discussion

*Chemistry*

Sodium percarbonate contains theoretically about 28% hydrogen peroxide. The structure of sodium percarbonate is a carbonate perhydrate: 2 $Na_2CO_3$ • 3 $H_2O_2$. Dissolved in water, it releases hydrogen peroxide and carbonate. Impurities in sodium percarbonate and its stability in products are not known.

*Acute toxicity*
The oral LD50 in mice and rats > 1000 mg/kg. The dermal LD50 in rabbits is > 2000 mg/kg.

*Irritation and corrosivity*
Solutions of 1% sodium percarbonate caused slight erythema and desquamation during the last 2 days of the test. Powder caused strong eye irritation. Solutions of 1% sodium percarbonate caused slight eye irritation in 1 rabbit during 2 hours.

*Skin sensitisation*
Sodium perborate was not skin sensitising in a Buehler test.

*Dermal / percutaneous absorption*
No data found

*Repeated dose toxicity*
No data found

*Mutagenicity / Genotoxicity*
No data found

*Carcinogenicity*
No data found

*Reproductive toxicity*
No data found

*Toxicokinetics*
No data found

*Human data*
Sodium percarbonate has been used in vital tooth bleaching systems (e.g. Crest Night Effects [19% sodium percarbonate, equivalent to 5.3% hydrogen peroxide]).

Only a few studies are available. The results suggest that the effects are similar to those found with the use of hydrogen peroxide and carbamide peroxide on tooth bleaching products.

## POTASSIUM PEROXYMONOSULFATE

Most of the chemical and toxicological information is from DuPont Oxone Monopersulfate Compound Technical Information (http://www.dupont.com/oxone/techinfo/index.html)

### 3.1. Chemical and Physical Specifications

| 3.1.1. | Chemical identity |
|---|---|

| 3.1.1.1. | Primary name and/or INCI name |
|---|---|

Potassium peroxymonosulfate
Potasium monoperoxide
Potasium caroate

| 3.1.1.2. | Chemical names |
|---|---|

Monopotassium peroxymonosulfate,
Peroxymonosulfuric acid, monopotassium salt
Potassium persulfate

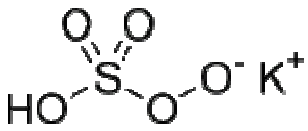
| 3.1.1.3. | Trade names and abbreviations |
|---|---|

Caroat (2 $KHSO_5$ • $KHSO_4$ • $K_2SO_4$)
Oxone (2 $KHSO_5$ • $KHSO_4$ • $K_2SO_4$)

| 3.1.1.4. | CAS / EINECS/ELINCS number |
|---|---|

| | | |
|---|---|---|
| CAS: | 10058-23-8 | Potassium peroxymonosulfuric acid |
| | 37222-66-5 | **(**Peroxymonosulfuric acid, monopotassium salt, mixture with dipotassium sulfate and potasssium hydrogen sulphate) |
| | 70693-62-8 | (2 $KHSO_5$ • $KHSO_4$ • $K_2SO_4$) |
| EINECS: | 233-187-4 | Potassium peroxymonosulfuric acid |
| | 274-778-7 | (2 $KHSO_5$ • $KHSO_4$ • $K_2SO_4$) |

| 3.1.1.5. | Structural formula |
|---|---|




| 3.1.1.6. | Empirical formula |
|---|---|

$KHSO_5$
$K_5H_3O_{18}S_4$

| 3.1.2. | Physical form |
|---|---|

White powder

| 3.1.3. | Molecular weight |
|---|---|

Mol.wt: 152.2
614.7 (2 $KHSO_5$ • $KHSO_4$ • $K_2SO_4$)

| 3.1.4. | Purity, composition and substance codes |
|---|---|

/

| 3.1.5. | Impurities / accompanying contaminants |
|---|---|

/

| 3.1.6. | Solubility |
|---|---|

256 g/l in water 20°C

| 3.1.7. | Partition coefficient (Log $P_{ow}$) |
|---|---|

Log $P_{ow}$: /

| 3.1.8. | Additional physical and chemical specifications |
|---|---|

| | | |
|---|---|---|
| Appearance | : | White powder |
| Melting point | : | |
| Boiling point | : | / |
| Density | : | 0.95-1.25 (2 $KHSO_5$ • $KHSO_4$ • $K_2SO_4$) |
| Rel. vap. dens. | : | / |
| Vapour Press. | : | / |

| 3.1.9. | Stability |
|---|---|

Oxygen loss < 1% per month. The decomposition is accelerated on contact with moisture.

T1/2 pH 7 about 5 hours at 23 °C
T1/2 pH 9 about 10 min at 23 °C

Decomposition reaction:
2 $KHSO_5$ • $KHSO_4$ • $K_2SO_4$ $\rightarrow$ 5 $K^+$ + 2 $HSO_5^-$ + $HSO_4^-$ + $SO_4^{2-}$
2 $HSO_5^-$ $\rightarrow$ 2 $HSO_4^-$ + $O_2$

## 3.2. Function and uses

Potassium peroxymonosulfate is widely used as an oxidising agent.
Peroxymonosulfate has been used in vital tooth bleaching systems. Tooth bleaching products containing peroxymonosulfate have not been identified.

## 3.3. Toxicological Evaluation

### 3.3.1. Acute toxicity

**Oral**

Rat LD50 = 802 mg/kg
Rat LD50 = 2050 mg/kg (IUCLID)
Rat LD50 = 1204 mg/kg (IUCLID)
Rat LD50 = 2000 mg/kg (IUCLID)

**Dermal**

Rat LD50 > 2050 mg/kg (IUCLID)
Rabbit LD50 > 11000 mg/kg (IUCLID)

### 3.3.2. Irritation and corrosivity

Potassium peroxymonosulfate is corrosive to the eyes and skin due to its acidity and oxidizing properties.

### 3.3.3. Skin sensitisation

Guinea pig uehler test (1985). Conclusion: not sensitizing (IUCLID dataset) (no details given).

Guinea pig Maximization test (1985). Conclusion: not sensitizing (IUCLID dataset) (no details given).

Guinea pig Maximization test (1992). Conclusion: not sensitizing (IUCLID dataset) (no details given).

Potassium peroxymonosulfate may cause allergic reactions (Dupont).

### 3.3.4. Dermal / percutaneous absorption

/

### 3.3.5. Repeated dose toxicity

**Rat**
Rats received 680 mg/kg feed of 2 $KHSO_5$ • $KHSO_4$ • $K_2SO_4$ for 10 days (1954). Autopsies revealed a healing gastritis in 3 animals, sacrificed on the day of final treatment. The remaining 3 animals were sacrificed 10 days later and showed no pathology. No further data available (IUCLID)

### 3.3.6. Mutagenicity / Genotoxicity

#### 3.3.6.1. Mutagenicity / Genotoxicity *in vitro*

Salmonella typhimurium TA1535, TA1537, TA1538, TA98, TA100, up to 200 µg/plate, with and without metabolic activation.

Results: negative (IUCLID)

3.3.6.2 Mutagenicity/Genotoxicity *in vivo*

/

3.3.7. Carcinogenicity

/

3.3.8. Reproductive toxicity

/

3.3.9. Toxicokinetics

/

3.3.10. Photo-induced toxicity

/

3.3.11. Human data

/

3.3.12. Special investigations

Ellingsen et al. (1982) performed experiments where a rabbit model were used for examination of the potential of chlorhexidine and other denaturing agents to induce dental stain together with ferric ions. On teeth with established discoloration a 4% solution of a peroxomonosulphate compound (Caroat®; 2 $KHSO_5$ • $KHSO_4$ • $K_2SO_4$) was applied for 5 minutes to remove the stain. The authors point out that the observations that the stain formation can be affected by the use of the red-ox principle or by oxidizing agents may be of future clinical interest. The latter principle has been tested in a clinical experiment recently, with promising results.

3.3.13. Safety evaluation (including calculation of the MoS)

/

3.3.14. Discussion

*Chemistry*
Potassium peroxymonosulfate is widely used as anoxidising agent. It releases oxygen when dissolved in water. Impurities and stability in products are not found.

*Acute toxicity*
The oral LD50 in rats > 1000 mg/kg. The dermal LD50 in rats and rabbits is > 2000 mg/kg.

*Irritation and corrosivity*
Potassium peroxymonosulfate is corrosive to the eyes and skin due to its acidity and oxidizing properties.

*Skin sensitisation*
Potassium peroxymonosulfate was not skin sensitising in a Buehler test or in two Maximization tests. A company has stated that potassium peroxymonosulfate may cause allergic reactions.

*Dermal / percutaneous absorption*
No data found.

*Repeated dose toxicity*
No data found.

*Mutagenicity / Genotoxicity*
One Salmonella study was negative.
*Carcinogenicity*
No data found.

*Reproductive toxicity*
No data found.

*Toxicokinetics*
No data found.

*Human data*
Peroxymonosulfate has been used in vital tooth bleaching systems, but commercial products containing peroxymonosulfate appear not to be on the market.

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