OPINION OF THE SCIENTIFIC COMMITTEE ON COSMETIC PRODUCTS AND NON-FOOD PRODUCTS INTENDED FOR CONSUMERS

CONCERNING

HYDROGEN (CARBAMIDE, ZINC) PEROXIDE IN TOOTH BLEACHING / WHITENING PRODUCTS

adopted by the SCCNFP during the 21st plenary meeting
of 17 September 2002

Introduction and overview

SCCNFP adopted at the plenary session 17 February 1999 an opinion concerning *Hydrogen (carbamide) peroxide in tooth whitening products* (doc. n° SCCNFP/0058/98) to answer the following questions :

- Is an increase in the limit concentration of hydrogen peroxide (an equivalent to 3.6%) in tooth-whitening products permissible?
- Does the SCCNFP propose any restrictions or conditions for the use of these cosmetic products?

The opinion stated that "*The content of hydrogen peroxide in tooth whitening products should not exceed 3.6% (10% carbamide peroxide). Tooth whitening products containing more than 0.1% hydrogen peroxide (0.3% carbamide peroxide) should exclusively be administered under supervision of a dentist ("take home"). The products should contain a printed warning against overuse or reuse of tooth whitening products several times and that they should not be used during pregnancy or by habitual tobacco and alcohol users.".*

After the opinion was submitted, SCCNFP was asked to clarify the following questions :

- Based upon the scientific data presented to date, which level can hydrogen peroxide be safely used in cosmetic products without necessary warning statements against the use during pregnancy or by tobacco and alcohol users?
- At which concentration can hydrogen peroxide be safely used in cosmetic products that are freely available to consumers (i.e. distribution not restricted to healthcare professionals)?
- Does SCCNFP recommend any modifications or amendments to its previous opinion on the safety of hydrogen peroxide in oral cosmetic products based on the dossier submitted?
- Does the SCCNFP maintain the necessity for previous advised warning statement or restrictions on the use of hydrogen peroxide (and equivalent) in oral care cosmetic products in light of the latest submission?

The SCCNFP re-evaluated the questions raised and decided to remove the term: "during pregnancy or" from the opinion (doc. n° SCCNFP/0200/99). Based on toxicokinetic consideration, it is unlikely that hydrogen peroxide reaches the foetus.

With the above exceptions, the SCCNFP confirmed its opinion and stated based on the scientific data presented to date that hydrogen (carbamide) peroxide can be safely used in cosmetic products without necessary warning statements against the use by tobacco and alcohol users at a maximum level of 0.1%. SCCNFP maintained the necessity for the advised warning statements and restrictions on the use of hydrogen peroxide (and equivalent) in teeth bleaching products.

The SCCNFP was later asked :

- Is an increase in the limit concentration of hydrogen peroxide (and equivalent) to 3.6% in oral hygiene products (tooth whiteners, certain mouth-rinses or toothpaste) permissible?
- Does the SCCNFP propose any restrictions or conditions for use of these cosmetic products?

Since SCCNFP had recently prepared an opinion (doc. n° SCCNFP/0058/98) based on another request to increase the limit concentration of hydrogen peroxide (and equivalent) to 3.6% in tooth-whitening products, the new opinion did only cover the use of hydrogen peroxide and hydrogen peroxide releasing substances in oral products such as mouth-rinses and toothpaste.

SCCNFP adopted in the plenary meeting 23 June 1999 an opinion (doc. n° SCCNFP/00158/99) stating: *An increase of hydrogen peroxide (and equivalent) in toothpaste and mouth-rinses to 3.6% is not permissible. The margin of safety for chronic/sub-chronic toxicity and for irritation and corrosivity are not sufficiently large for an oral hygiene product. No study seems to be available concerning long term use of hydrogen peroxide containing toothpaste or mouth-rinses. A 16-month-old boy has died after in ingestion of a 3% solution of hydrogen peroxide. The content of hydrogen peroxide (and equivalent) in oral hygiene products should not exceed 0.1%.*

The previous submissions concerning the use of hydrogen peroxide (and equivalent) for tooth-whitening products did mainly employ a technique where hydrogen peroxide or a hydrogen peroxide releasing substance was used in a custom made or prefabricated tray that covered the teeth. The present submission is primarily based on the use of textured strips containing 6% hydrogen peroxide and designed to fit the front teeth.

1. Terms of Reference

1.1. Context of the question

The adaptation to technical progress of the Annexes to Council Directive 76/768/EEC of 27 July 1976 on the approximation of the laws of the Member States relating to cosmetic products.

Request to change Annex III, part 1, n° 12 to Council Directive 76/768/EEC to increase the limit concentration of Hydrogen peroxide (and equivalent) in oral care products for tooth bleaching / whitening.

1.2. Request to the SCCNFP

The SCCNFP is requested to answer the following questions :

* Does the safety profile documented in the attached submission support that hydrogen peroxide and other compounds or mixtures that release hydrogen peroxide are safe for the use in tooth bleaching/whitening products at concentrations up to 6.0% (present or released) with a limitation of a maximum of 50 mg per day?

* Does the SCCNFP propose any restrictions or conditions for the use of these increased levels of hydrogen peroxide (and equivalent) in oral care cosmetic products?

1.3. Definitions of terms where appropriate.

Not applicable

1.4. Statement on the toxicological evaluation

The SCCNFP is the scientific advisory body to the European Commission in matters of consumer protection with respect to cosmetics and non-food products intended for consumers.

The Commission's general policy regarding research on animals supports the development of alternative methods to replace or to reduce animal testing when possible. In this context, the SCCNFP has a specific working group on alternatives to animal testing which, in co-operation with other Commission services such as ECVAM (European Centre for Validation of Alternative Methods), evaluates these methods.

SCCNFP opinions include evaluations of experiments using laboratory animals; such tests are conducted in accordance with all legal provisions and preferably under chemical law regulations. Only in cases where no alternative method is available will such tests be evaluated and the resulting data accepted, in order to meet the fundamental requirements of the protection of consumer health.

2. Toxicological Evaluation and Characterisation

Executive Summary

The content of hydrogen peroxide in tooth whitening products should not exceed 6% (present or released). Tooth whitening products containing more than 0.1% hydrogen peroxide (or equivalent for hydrogen peroxide releasing substances) should exclusively be administered under supervision of a dentist ("take home"). The technique in the present submission involves the use of textured strips containing 6% hydrogen peroxide. The strips should be worn twice a day for 30 minutes over a period of 14 days. After using all of the upper strips, the process is repeated with the lower teeth. Both the dentin and the enamel change colour as a result of the easy passage of the peroxide through the tooth. Commonly observed clinical side effects include mild tooth hypersensitivity to temperature changes and irritation of oral mucosa. Overall evidence indicates that the proper use of peroxide containing tooth bleaching agents is safe if used under the supervision of a dentist. The use of tooth whitening products is not recommended prior to or immediately after dental restoration. Conditions such as pre-existing tissue injury or concurrent use of tobacco and/or alcohol may exacerbate the toxic effects of hydrogen peroxide. Few investigators have addressed the possible pathophysiological effects on oral and pulpal tissues from long-term treatment. No studies seem to be available concerning people who have overused or reused tooth-bleaching agents several times. Moreover, long-term studies seem to be lacking, except for a small study.

General information is unless not otherwise indicated taken from IARC (1985), ECETOC (1996) and EU (2001).

2.1. Identification of the substance

2.1.1. Primary names

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).

Zinc peroxide, zinc dioxide,

2.1.2. Chemical names and molecular weights

Hydrogen peroxide	:	**H_2O_2**	Mol. weight 34.0
Carbamide peroxide	:	**$CO(NH_2)_2 \cdot H_2O_2$**	Mol. weight 94.1
Zinc peroxide	:	**$Zn(O_2)$**	Mol. weight 97.4

2.1.3. Identifications

Hydrogen peroxide	:	CAS No.: 7722-84-1 EINECS No.: 231-765-0
Carbamide peroxide	:	CAS No.: 124-43-6 EINECS No.: 204-701-4
Zinc peroxide	:	CAS No.: 1314-22-3 EINECS No.: 215-226-7

2.1.4. Descriptions

Hydrogen peroxide : Colourless liquid

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 : White crystals or crystal powder

Melting point	:	75-85°C
Boiling point	:	not available

Density : 1.4 g/cm^3
Vapour pressure : not available

Zinc peroxide **:** White to yellowish powder. Gradually decomposed by water; liberates hydrogen peroxides in dilute acids.
Melting point : -
Boiling point : -
Density : 1.57 g/cm^3
Vapour pressure : -

2.1.5. Solubility

Hydrogen peroxide is miscible with water.

Carbamide peroxide is soluble in water.

Zinc peroxide is insoluble in water, soluble in dilute acids

2.1.6. 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 minumum 97% of the hydrogen peroxide – Urea adducts are available.

Zinc peroxide **:** The peroxide of commerce contains 50 – 60% ZnO_2 , the remainder is ZnO

2.2. Function and use

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 contains approximately 35% hydrogen peroxide and forms hydrogen peroxide and urea in liquid solution. Zinc peroxide is insoluble in water, but decomposes gradually by water and liberate hydrogen peroxide in dilute acid. 750,000 tonnes hydrogen peroxide (calculated as 100% H_2O_2) was produced in Europe in 1995.

The main usage of hydrogen peroxide is in production of chemicals and bleaching of cellulose pulp and textiles. Small quantities are used for such purposes as disinfection of eye contact lenses, disinfections of wounds and mouth washing. Both hydrogen peroxide and carbamide peroxide are used for hair bleaching, oral antiseptics, dentifrices, oxidation of permanent waves, hair relaxer, ear drops, crank sores and tooth bleaching. Zinc peroxide is used as accelerator in rubber compounding, curing agent for synthetic elastomers and in cosmetic powders as antiseptical.

Peroxide compounds including hydrogen peroxide and carbamide peroxide have been used in various dental procedures for many years. Reports of using peroxides to bleach or whiten teeth can be traced back to more than a century ago. Current peroxide containing whiteners used in USA can be classified into 3 categories: 1) Those containing high concentration of hydrogen peroxide (30-35%) or carbamide peroxide (35%) for professional use only; 2) materials that are dispensed by dentists and used by patients at home (up to 10% hydrogen peroxide or 16% carbamide peroxide); and 3) over-the-counter products with hydrogen peroxide content up to 6% and available to consumers for home use (Li, 1996).

The first articles on bleaching teeth using night guard whitening bleaching were published in 1989 (Christensen, 1989a, b; Haywood and Heymann, 1989). The whitening effect is due to degradation of high molecular weight, complex organic molecules that reflect a specific wavelength of light and are 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 nicotine 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. It is reported in one study that after 18 months 74% and after 3 years 62% of patients whose teeth were bleached, still exhibited stable colour, and “touch-up” generally requires only one to two days of retreatment for each week taken for initial treatment (see Marshall et al., 1995, Haywood, 1997). In another study (Leonard, 1998) 17% (4 persons) responded that there were no obvious change in the colour 13-25 months after the treatment, while 57% (13 persons) stated that there was a slight darkening, but it was not noticeable by other people. 75-89 months after the treatment, 10% (3 persons) responded there were no obvious change in colour and 25% (7 persons) that there was a slight darkening, but it was not noticeable by other persons. 48% (14 persons) responded at that time that there had been some darkening, but they had retreated the teeth back to acceptable colour.

The technique that resulted in the present submission involves strips containing 6% hydrogen peroxide. The textured strip is made of polyethylene and the backing is made of polyester. The strips are designed to fit the front teeth. The strips should be worn twice a day for 30 minutes over a period of 14 days. After using all of the upper strips, the process is repeated with the lower teeth. After a limited marketing the strips have extensively marketed in USA from May 2001. Strips with higher hydrogen peroxide content are available only from dentists.

TOXICOLOGICAL CHARACTERISATION

One factor associated with the toxicity of hydrogen peroxide in addition to the oxidative damage, is the release of oxygen (1 ml of 3% hydrogen peroxide can release 10 ml oxygen)

2.3.1. Acute toxicity

Hydrogen peroxide :

* Oral LD_{50} - values for rats vary between **600-1617 mg/kg bw** (Y.Li, unpubl.; Ito et al., 1976).
* Dermal LD_{50} -values for rats vary between **700->7500 mg/kg bw** (FDA, 1983).
* Dermal LD_{50} –values in rabbits about **630 mg/kg bw** (FDA, 1983).

  - A 16-month-old boy 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 postmortem 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 were 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 estimated dose of hydrogen peroxide ingested was 7 g, about **600 mg/kg/bw** for a boy of 11.6 kg.

* 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).

2.3.2. Mucous membrane irritation

Hydrogen peroxide :

* 1 or 1.2% hydrogen peroxide applied to the gingiva or tongues of anaesthetised dogs by continuous drip caused oedema, followed by destruction and sloughing of the cornified epithelial layer of the gingiva (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 whitener containing 10% carbamide peroxide produced ulceration of gastric mucosa in the 1-hour rats; the lesions appeared to be healing after 24 hours (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). Histopathologic findings of the gastroduodenal tissue were comparable among the groups (Li et al. [Abstract], 1993).

2.3.3. Skin irritation

Hydrogen peroxide :

* Hydrogen peroxide solutions of 35% or less would not be classified as skin irritants in rabbits by the EU criteria (ECETOC, 1996).

* 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).

2.3.4. Eye irritation

Hydrogen peroxide :

* Eye irritation studies with rabbits indicate that a 5% hydrogen peroxide solution is non-irritant to mild-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 hyperemia, tearing, and eyelid spasm (Knoph, 1984).

* In 10 human volunteers, the threshold of detection for irritation 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).

2.3.5. 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 single 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. The author reported that 156 other hairdresser patch tested with hairdresser series tested negatively to 3% hydrogen peroxide. This report indicates that some individuals may be

extremely sensitive to the ingredients of the hairdyes. In general, the data do not provide adequate evidence that hydrogen peroxide is a skin sensitiser in man (Aguire et al., 1994).

2.3.6. Repeated dose oral toxicity

Hydrogen peroxide:

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).

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. Mild-minimal duodenal mucosal hyperplasia was noted in animals receiving 1000 and 3000 ppm hydrogen peroxide and in one male receiving 300 ppm hydrogen peroxide for 13 weeks. All effects noted during treatment period, including the duodenal hyperplasia, were reversible during the 6 weeks recovery period. The NOAEL was 100 ppm or 26 and 37 mg/kg/day hydrogen peroxide for males and females respectively (Weiner et al., 1998).

When rats were administered hydrogen peroxide by oral gastric tube 6 days weekly for 90 days, the dose of 506 mg/kg suppressed bodyweight gain, decreased food consumption, and caused changes in haematology, blood chemistry, and organ weights. Principle 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).

In another rat study (Kawasaki et al., 1969) the NOAEL of hydrogen peroxide was 30 mg/kg/day, when the animals were treated by oral gastric tubing daily for 100 days. The same study showed no adverse effect in rats receiving a diet containing 6 mg hydrogen peroxide in 20 g of food (about 12 mg/kg/day).

Humans are exposed to hydrogen peroxide (0.75%) in dentifrice products, as commercially available tooth pastes. Monitoring of adverse reactions by the U.S Cosmetic, Toiletry and Fragrance Association (CTFA, 1994) indicated one adverse reaction for every 100,000 units sold. Reported reactions were primarily burning mouth and oral irritation. Symptoms generally subsided with cessation of the product used. The incidence and types of reactions were typical also of other dentifrices on the market without hydrogen peroxides.

Several studies have reported the use of 0.75 or 1.5% hydrogen peroxides as a mouthwash or mouth rinse for periods of up to three months. Tombes et al. (1993) reported discoloration of the mucosal surfaces and the tongue following 5 weeks of rinsing 4 times daily with 0.75% or 1.5% hydrogen peroxide-containing solutions. Shibly et al. (1997) reported no adverse effects from a

1.5% hydrogen peroxide mouthwash, used for 60 days. Winer et al. (1991) reported that 4 times a day use of a 1.5% hydrogen peroxide mouthwash for 90 days resulted in no intraoral soft tissue adverse effects.

Daily use of a 0.75% hydrogen peroxide-containing dentifrice for a 6-month treatment period or a 1.5% hydrogen peroxide-containing mouthrinse for 18 months or two years resulted in no reported adverse effects on oral health. Adults who brushed with a 0.87% hydrogen peroxide/5% baking soda dentifrice twice per day and once per day with a control dentifrice for 6 months showed no differences in gingival health relative to controls (Fischman et al., 1992). Daily rinsing with a mouthwash of 1.5% hydrogen peroxide and 0.05% sodium fluoride for 18 months resulted in an improvement in gingival health, relative to baseline (Boyd, 1989). Use of a 1.5% hydrogen peroxide-containing mouthwash twice daily for a two-year period resulted in significant improvements in gingival health (Gangler et al., 1985).

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 mouth wash 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 filform papillae of the tongue (Miller et al., 1938).

Tooth whiteners containing 10% carbamide peroxide :

The majority of the published peroxide based teeth whitening studies are done with a type of product only available via the dental office. In 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 a daytime exposure to 8-10 hours for an 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. (1994a) 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.

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. None of the subjects reported oral soft tissue irritation (Kozlovsky et al., 1996).

Beyond one month

A 38% incidence of adverse events (tooth sensitivity) 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.

2.3.7. Genotoxic effects

Hydrogen peroxide :

The *in vitro* and *in vivo* genotoxic potential of H_2O_2 is summarised in Table 3.1.

Table 3.1. *Summary of genotoxicity of hydrogen peroxide (ECETOC 1996).*

End-point	Test system	Results
IN VITRO		
Gene mutation	*Salmonella typhimurium*	+ve without activation decrease of the genotoxic potential in presence of exogenous S9 or catalase
	Escherichia coli	+ve without activation
	Saccharomyces cerevisae	+/-ve without activation
	Bacillus subtilis	+ve without activation
	Mammalian cells	+ve without activation
Primary DNA damage		
DNA-repair	*Salmonella typhimurium*	+ve without activation
	Escherichia coli	+ve without activation - ve with activation
Sister chromatid exchanges	Mammalian cells	+ve without activation
	Mammalian cells	+ve with activation

End-point	**Test system**	**Results**
	Mammalian cells	+ve without activation decrease of the SCE induction in presence of exogenous S9 or catalase
Chromosomal aberration	Mammalian cells	+ve without activation
IN VIVO		
Gene mutation	*Drosophila melanogaster*	-ve
	Salmonella typhimurium (host mediated assay in mice)	+ve
Chromosomal aberration		
Micronucleus or metaphase analysis	Mice and rats	-ve

In addition to the studies reported by ECETOC (1996) 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-tumorigenic 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).

Conclusion on mutagenicity (EU, 2001)

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. Regarding *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 damages 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.

Tooth whiteners containing 10% carbamide peroxide :

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 to 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). Several *in vivo* studies on peroxide containing tooth whiteners detected no genotoxicity. No increase in frequency of micronuclei were 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 were incubated with up to a dose of 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 administrated 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).

2.3.8. Carcinogenicity

2.3.8.1. Animal studies

Hydrogen peroxide :

Mice

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 3.2) (Ito et al., 1981).

Table 3.2. *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 were 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 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 3.3 (Ito et al., 1984). (The IARC Group noted that the pathology of the tumours was not well documented.)

Table 3.3. *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

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

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 (once and twice weekly), 1:2 or 1:5 in 0.2 ml acetone twice weekly for 25 weeks. Controls received acetone alone. The proportions of mice with papillomas at 25 weeks were 0/60 (0%) (controls), 3/58 (5%), 5/59 (8%), 6/59 (10%) and 6/60 (10%), respectively. Mice in experiment (B) received a single topical application of hydrogen peroxide diluted 1:1 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) received twice-weekly topical applications of hydrogen peroxide diluted 1:1 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).

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

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. Adenocarcinomas were observed in the pyloric stomach and duodenum of the MNNG-treated rats, and "preneoplastic hyperplasia" was observed in the pylorus (Table 3.4). 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.

Table 3.4. *Effect on gastro-duodenal carcinogenesis induced by MNNG (Takahashi et al, 1986)*

Treatment group (n)	**Forestomach papilloma**	**Fundus hyperplasia** [a]	**Glandular stomach** **Pylorus adeno-carcinoma**	**Pre-neoplastic hyperplasia**	**Duodenum adeno-carcinoma**
MNNG controls (30)	0 (0%)[b]	0 (0%)	1 (3.3%)	7 (23.3%)	3 (10%)
MNNG-H_2O_2 (21)	21 (100%)	8 (38.1%)	2 (9.5%)	6 (28.6%)	0 (0%)
H_2O_2 (10)	5 (50%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)

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

Hirota and Yokoyama (1981) examined the tumour promotion effect of 1.5% hydrogen peroxide in drinking water in the duodenum and jejunum of Fischer 344 rats. After 4 weeks of administration, methylazoxymethanol acetate (MAM) was administered i.p.; hydrogen peroxide administration was continued except for 2 days following injection. At the end of 8 weeks, one group of rats continued on hydrogen peroxide whereas a second group was given tap water to drink for an additional 5 weeks. A third group of 3 rats received only hydrogen peroxide throughout the study. A fourth group of 3 rats received only tap water. There was no control MAM group. Animals were killed 21 weeks after the study started. Proximal duodenal (Ito et al., 1984) and upper jejunal tumours were observed in groups 1 and 2, with a higher incidence in Group 1 (100% incidence) compared to Group 2 (25%). Tumours were classified as adenocarcinomas, mucosal or invasive. No tumours were observed in tap water control animals or animals treated only with hydrogen peroxide, although duodenal and upper jejunal hyperplasia were noted in the latter group. 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

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 longterm, 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 was observed only with 30% hydrogen peroxide. The experiment demonstrated a promoting effect of hydrogen peroxide (Weitzman et al., 1986).

Carbamide peroxide

Mice

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).

2.3.8.2. Human studies

No data are available.

2.3.8.3. Evaluation

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. Some tumour promotion studies indicate that hydrogen peroxide may act as a weak promoter.

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

2.3.9. Toxicity to reproduction

2.3.9.1. Animal studies

Hydrogen peroxide :

Mice

Male albino mice were given 0.33%, 1.0% or 3.0% hydrogen peroxide in drinking water. There were no controls. The mice at the highest dose would not drink the solution and were taken off the study. Mice were mated after 7 and 21 days on hydrogen peroxide. All females became pregnant within a few days and delivered litters of normal size. The concentration, morphology and mobility of the spermatozoa of the male mice receiving hydrogen peroxide in the drinking water over 3 and 6 weeks, remained normal. *In vivo*, hydrogen peroxide had not significant spermaticidal action in mice at concentration up to 1% in solution (Wales et al., 1959).

Rats

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/kgbw. 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 bodyweigth 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.

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).

2.3.9.2. Human studies

No data are available.

2.3.9.3. Evaluation

Data on the teratogenic potential and reproductive toxicity are limited and do not allow a complete evaluation.

2.3.10. Toxicokinetics after dermal exposure

2.3.10.1. Metabolism

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. 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.

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

$H_2O_2 + H_2O_2 \rightarrow 2H_2O + O_2$

Catalases are present at a wide range of concentrations in nearly all mammalian cells. Catalases are located in the subcellular compartments, mainly in peroxisomes. Soluble catalases were 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.

Peroxidases decompose hydrogen peroxide through the reaction:

$H_2O_2 + 2RH \rightarrow 2H_2O + R- R$

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, 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.

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 reduces hydrogen peroxide to water with formation of oxidised glutathione which is regenerated by glutathione reductase by consuming NADPH.

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 presence of transition metals, particularly ferrous ions (Fe^2), hydrogen peroxide can be reduced to hydroxyl radicals:

$$H_2O_2 + Fe^{2+} \rightarrow OH^{\cdot} + OH^- + Fe^{3+}$$

The hydroxyl radical is highly reactive and will attack most molecules in living cells.

Product based peroxide degradation kinetic data

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). 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 the average 56% remained after 4 hours and 23% after 10 hours (Matis et al., 1999).

2.3.10.2. 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.

Later it has been reported two Hungerian acatalasaemic subjects (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 peroxidase. It is estimated that about 400 million people throughout the world are deficient in G6PD.

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, two studies (Aguire et al., in press) indicate the degradation of hydrogen peroxide in the oral cavity is not affected by xerostomia. 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 a Procter & Gamble 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.

Procter & Gamble 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.

2.3.11. Elements influencing safety

2.3.11.1. Assessment of human exposure

A study (study no. 2000045 and 2000143) was 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), a 10% carbamide peroxide (CP) tray that delivers ≈ 22-48 mg hydrogen peroxide (600-900 mg of a 3.6% hydrogen peroxide gel; 10, 30, 60 or 120 minute treatments) or a 20% CP tray that delivers ≅40-60 mg hydrogen peroxide (600-900 mg of a 6.7% hydrogen peroxide gel; 10, 30, 60 or 120 minute treatments). Treatment was on maxillary teeth only. It is concluded that hydrogen peroxide delivered at 6% in tooth whitening products (films, gels or varnished) 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 3.5. *Available hydrogen peroxide in the salvia 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 median is used to calculate exposure this will correspond to (4/60) 0.067 mg/kg/d while if a maximum exposure of 10 mg is (Max observation = 11.4; mean + 2SD = 10.2) used, this will correspond to (10/60) 0.17 mg/kg/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, 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. On the other hand, Matis et al. (2002) has concluded that 25% of carbamide peroxide in the tray is swallowed. This will correspond to about 0.07 mg/kg/day.

The above calculation 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.

It is difficult to assess the exposure. In the case of the gel strips it ha been reported that the user occasionally may swallow the strip. This will result in an exposure of about 12 mg hydrogen peroxide.

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.

2.3.11.2. Clinical side effects of treatments with tooth whiteners

There is little information available on the adverse effects of at-home bleaching agents. The number of patient enrolled in the studies is to small to detect adverse events of low frequency and in many studies, control group is not included.

In a survey by Clinical Research Associates, 91% of 8,143 dentists stated that they had used vital tooth bleaching (Christensen, 1997), 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.

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).

A study involving 13 adults with teeth stained by tetracycline ingestion and treated with tooth bleaching agent nightly for six calendar 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 patients experienced gingival sensitivity or tooth hypersensitivity since completion of the treatment.

Nachnani ([Report] 1997) reported that there were no statistically significant difference between the placebo group and the group using Nite White 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).

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.

Procter & Gamble have 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.

In studies on teenagers (10-18 years) by Procter & Gamble, it was reported that 5.3%-6.5% hydrogen peroxide gel strips, and 10% carbamide peroxide delivered in a tray based system produced similar types of mild adverse events (tooth sensitivity and oral soft tissue irritation), similar occurrence of adverse events and were equally well tolerated.

Procter & Gamble have performed a number of studies from which they conclude: “Several factors (increasing frequency of daily exposure and brushing immediately prior to gel strip application) were found to influence the incidence and severity of adverse events from tooth whitening products. Hydrogen peroxide gel strips were well tolerated in clinical subject with pre-existing tooth sensitivity. Gel strips with 6.5% hydrogen peroxide were as well tolerated as tray products with 10% carbamide peroxide. Hydrogen peroxide gel strips were well tolerated in clinical subjects with artificially induced xerostomia.

Procter & Gamble 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.

2.3.11.3. Effects of concern

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. Claims of safety have been based largely on previous use of these

peroxides as a short-term mouth-rinse adjunctive to routine oral hygiene procedures. One concern of the present evaluation is the long-term effect on pulp. The dental pulp is vulnerable through exposed dentin in patients with gingival retraction, attrition, cervical abrasion, and leaking restorations, and the gingiva is exposed directly to gels which leak from the trays. Significant amounts of hydrogen peroxide diffuse through dentin after application of carbamide peroxide and hydrogen peroxide-based bleaching agents (Hanks et al., 1993). 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 dentine of rat incisors caused emphysema and capillary stasis, and slowed down the blood circulation in the underlaying 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.

In two studies, extracted human teeth were sectioned above the cementoenamel junction and oriented so that an enamel surface was immersed in a solution of hydrogen peroxide (Bowles et al., 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).

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 et al., 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-Ej, 1992; Nathoo et al., 1994). Results are dependent on the methodology used and the materials or products tested.

Most scanning electron microscopy showed little or no morphological changes in enamel surfaces treated with carbamide peroxide tooth whitening agents (Haywood et al., 1990, Haywood et al., 1991, Scherer et al., 1991, Sterret et al., 1995, Ernst et al., 1996). A six-month clinical study reported that long-term use of a whitening gel containing 10% carbamide peroxide did not adversely affect the surface morphology of the human enamel (Haywood and Robinson, 1997).

On the other hand, 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 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). A number of 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, 1996, Nathoo et al., 1994, Murchison et al., 1992, McCracken and Haywood, 1995).

Shannon et al. (1993) subjected enamel slabs to different bleaching agents containing10 % 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

In studies where samples of amalgam were treated for 14 and 28 days, either with 10% carbamide peroxide or 10% hydrogen peroxide solution and compared with phosphate buffer controls, a significant increase in mercury levels occurred. It was concluded that prolonged treatment with bleaching agents might cause microstructural changes in amalgam surfaces, possibly increasing exposure of patients to mercury (Rotstein et al, 1997). Although, 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).

Rotstein et al. (1996) have studied the effect of commonly used bleaching materials on the dental hard tissues of extracted human premolars. Significant reductions in Ca/P ratio in dentin as well as cementum were found following treatment. The authors conclude that the bleaching materials may adversely affect the dental hard tissues and should be used with caution. It has been pointed out, however (McCracken and Haywood, 1996) that the calcium loss was equivalent to the loss during a 2.5 minutes exposure to Cola beverage.

Procter & Gamble has summarized their studies: In *in vitro* studies, hydrogen peroxide penetrates human tooth pulp at levels well below those adversely affected pulpal enzymes. *In vivo* exposures to hydrogen peroxide did not adversely affect the pulpal tissue. Clinically relevant changes in tooth hardness, fracture sensitivity, dentin morphology or ultrastructure will not result from repeated surface exposures (14-70 hours) of teeth to 5.3% or 6.5% hydrogen peroxide gels or 10% to 20% carbamide peroxide gels. Exposure of freshly prepared dental restoration materials to peroxide-containing gels for periods of 14-70 hours may adversely affect the hardness of some restoration materials and the use of such whitening products is not recommended prior to or immediately after dental restoration.

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.

Most studies on adverse effects are small and have short observation times. Potential adverse effects occur. At home tooth bleaching should be monitored by dental professionals to maximise the benefit, while minimising adverse effects (Li, 1998).

2.4. Legislative Controls

The US Food and Drug Administration (FDA) has concluded that there is insufficient evidence of carcinogenicity in humans and IARC that there is *"limited"* evidence of carcinogenicity of hydrogen peroxide in experimental animals (FDA, 1988; IARC, 1985). FDA has approved both hydrogen peroxide and carbamide peroxide as an oral antiseptic agents in 1988 (FDA, 1988). 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 and effective. The most recent FDA classification listed three therapeutic categories of hydrogen peroxide and carbamide peroxide for oral uses. Applications of hydrogen peroxide for débriding/oral wound cleansing (7 day) and antiseptic (2 day) agents were considered safe. FDA seems not to have considered the use of hydrogen peroxide or carbamide peroxide in tooth whitening products.

No labelling is required in EU for hydrogen peroxide solutions of less than 5%. Solutions containing 5 – 20% hydrogen peroxide are labelled harmful due to eye and skin irritation. According to Annex III of the Cosmetic Products Directive oral hygiene products must not contain more than 0.1% hydrogen peroxide.

2.5. Safety Evaluation

Estimated daily exposure of hydrogen peroxide (see *2.3.11.1. Assessment of human exposure)* when using daily 4 teeth whitener strips containing 6% hydrogen peroxide is 0.067 mg/kg body weight. The maximum exposure (mean + 2xSD) has been estimated to be about 2.5 times higher (0.17 mg/kg/day). In the safety evaluation below, calculations based on estimated daily exposure is given in **bold** and calculation based estimated maximum exposure in *italic.* As indicated in *2.3.11.1. Assessment of human exposure* the exposure data are probably to low as they are based on a saliva flow of 0.3 ml/min. The real exposure may thus be closer to the estimated maximum exposure.

Acute and repeated dose toxicity

NOAEL of hydrogen peroxide from a rat study (repeated dose oral toxicity (*see 3.6)* was 30 mg/kg bw/day (Kawasaki et al., 1969) and from a mice study was 26 mg/kg/day (Weiner et al., 1998). In one mice experiment an oral LD_{50} of 87.2 mg/kg bw with tooth whitener containing 10-22% carbamide peroxide is reported (Woolverton et al., 1993). This corresponds to about 9

mg/kg bw carbamide peroxide or 3 mg/kg bw hydrogen peroxide. This number is much lower than found in the repeated dose study and is probably not correct.

MOS = 26/0.067 = 388.
MOS = 26/0.17 = 153.

Mucous membrane irritation

Stomach gavage of 15 mg/kg bw of carbamide peroxide produced ulceration of gastric mucosa in rats observed after 1 hour; the lesions appeared to be healing after 24 hours (Dahl and Becher, 1995).

MOS = 15/0.067 = 224.
MOS = 15/0.17 = 88.

Procter & Gamble 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.

Eye and skin irritation

No labelling is required in EU for hydrogen peroxide solutions in consumer products containing of less than 5%.

Sensitisation

Not detected to be allergenic.

Genotoxic and carcinogenic effects

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

Due to degradation of hydrogen peroxide in the oral cavity, it is unlikely that the use of tooth whitener will represent a cancer risk in persons that do not have an increased risk of oral cancer due to tobacco use, alcohol abuse or genetic predisposition. The risk of oral cancer may increase with repeated treatments with tooth whiteners.

Toxicity to reproduction

Data on the teratogenic potential and reproductive toxicity are limited and do not allow a complete evaluation.

Other effects of concern

The most commonly observed clinical side effects of treatments with tooth whiteners include 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.

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. Most scanning electron microscopy showed little or no morphological changes in enamel surfaces treated with carbamide peroxide tooth whitening agents. Some authors have however, reported alterations of enamel surfaces, including shallow depression, and increased porosity and slight erosion, associated with whitening treatments. Two clinical cases of serious adverse effects on enamel associated with whitening agents, both of which involve the use of over-the-counter products, have been reported.

It has been noted that prolonged treatment with bleaching agents might cause microstructural changes in amalgam surfaces and possibly increasing exposure of patients to mercury.

Studies designed to detect adverse effects of low frequency (e.g doubling the risk of the reference group) are lacking. No studies seem to be available concerning people that have reused tooth bleaching agents several times. Moreover, long-term studies seem to be lacking, except for a smaller study where less than 40 patients have been followed for up to 7 years after treatment.

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. 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

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3. Opinion of the SCCNFP

The content of hydrogen peroxide in tooth whitening products should not exceed 6% (present or released) with a limitation of maximum 50 mg hydrogen peroxide per day. The use of tooth whitening products is not recommended prior to or immediately after dental restoration. Conditions such as pre-existing tissue injury or concurrent use of tobacco and/or alcohol may exacerbate the toxic effects of hydrogen peroxide.

Overall evidence indicates that the proper use of tooth bleaching agents containing 0.1 to 6.0 % hydrogen peroxide (or equivalent for hydrogen peroxide releasing substances) is safe if used under the supervision of a dentist.