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SCIENCE DIRECT®
Food and Chemical Toxicology xxx (2005) xxx–xxx
www.elsevier.com/locate/foodchemtox

Review

Tooth whitening products and the risk of oral cancer

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Received 13 April 2005; accepted 21 July 2005

Abstract

Tooth whitening products (TWP) containing hydrogen peroxide (HPO) or carbamide peroxide (CPO) were evaluated in relation to potential oral cancer risk from their use. HPO is genotoxic in vitro, but such activity is not expressed in vivo. The genotoxic risk of HPO exposure of the oral mucosa encountered from TWP use is likely therefore to be vanishingly small. Available animal data on the carcinogenicity of HPO are of limited relevance to risk assessment of oral hazard of HPO exposure from TWP, and where relevant, do not indicate that there is an increased oral cancer risk for people using TWP. Clinical data on HPO-containing TWP only show evidence of mild, transient gingival irritation and tooth sensitivity, with no evidence for the development of pre-neoplastic or neoplastic oral lesions. Exposures to HPO received by the oral cavity, including areas commonly associated with oral cancer, are exceedingly low and do not plausibly pose a risk for the promotion of initiated cells or for induction of co-carcinogenic effects in conjunction with cigarette smoke or alcohol. The use of TWP was concluded not to pose an increased risk for oral cancer in alcohol abusers and/or heavy cigarette smokers. Furthermore, TWP were concluded to be safe for use by all members of the population, including potential accidental use by children.

Keywords: Tooth whitening; Hydrogen peroxide; Oral cancer

Contents

Abbreviations: bw, body weight; CHO, Chinese hamster ovary; CPO, carbamide peroxide; DMBA, 7,12-dimethylbenza[*a*]anthracene; DNA, deoxyribonucleic acid; GI, gastrointestinal; GLP, Good Laboratory Practice; HPO, hydrogen peroxide; MNNG, *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine; i.p., intraperitoneal; i.v., intravenous; MAM, methylazoxymethanol acetate; MTD, maximum tolerated dose; SCCP, European Union's Scientific Committee on Consumer Products; SCE, sister-chromatid exchanges; TWP, tooth whitening products; UDS, unscheduled DNA synthesis.

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doi:10.1016/j.fct.2005.07.012

1. Introduction

Tooth whitening products (TWP) (e.g., strips, gels, varnishes) that contain hydrogen peroxide (HPO), or carbamide peroxide (CPO), a product that degrades to form urea and HPO, have been in common use throughout North America, particularly over the past 15 years. Even though tooth whitening products have been in use for over 100 years, heightened interest in tooth whitening arose following the introduction in 1989 of a particularly popular form of dentist-supervised bleaching, called nightguard vital bleaching (Haywood and Heymann, 1989). Moreover, in North America, TWP have been available directly to the consumer since early 2001. During this time no significant health effects from use of TWP have been noted. In Europe, by contrast, TWP containing HPO or CPO, are available to consumers only from a dental practitioner. The legal status of TWP, with respect to availability directly to the consumer as cosmetic products was recently assessed by the European Union's Scientific Committee on Consumer Products (SCCP, 2005). The Committee was of the opinion that TWP containing from >0.1% to 6.0% were safe for use upon consultation and approval of the consumer's dentist. The SCCP raised concerns with respect to the potential for HPO, including HPO generated from CPO, to be associated with an increased risk of oral cancer, especially in smokers and alcohol abusers (SCCP, 2004, 2005). Smokers and alcohol abusers have a significantly elevated risk for the development of oral cancer, with a reported synergistic effect of these 2 factors (Blot et al., 1988; Maier et al., 1992; Baron et al., 1993).

Given the SCCP (2004, 2005) opinion, we undertook a review of the available safety data on various TWP, and HPO in particular, to assess the genotoxic and/or carcinogenic risks posed by HPO exposures from the use, both intended and exaggerated, of TWP. As part of this evaluation, in vitro and in vivo genotoxicity studies, experimental animal studies, clinical tolerance studies involving TWP and human pharmacokinetic studies were reviewed and assessed. In addition to these data, the results of a large number of unpublished, and several published, short- and longer-term clinical trials were critically analyzed. The following presents a review of the above safety data and conclusions with respect to the potential for HPO to influence the development of oral cancer in humans.

2. Genotoxicity

2.1. In vitro data

HPO generates reactive hydroxyl radicals that can oxidize lipid (Kanner et al., 1987; O'Brien, 1988) and produce oxidative deoxyribonucleic acid (DNA) damage (Williams and Jeffrey, 2000; Cadet et al., 2003). In particular, the hydroxyl radical formed from HPO reacts with deoxyguanosine to form 7,8-dihydro-8-oxo-2′-deoxyguanosine (8-oxo-dG) DNA adducts (Rosen et al., 1996). The 8-oxo-dG adducts are potentially promutagenic adducts and mispair during DNA replication to yield point mutations (Wood et al., 1992; Kamiya, 2003). However, for mutagenicity to occur, the DNA adducts must escape the effective DNA repair process (Asagoshi et al., 2000; Slupphaug et al., 2003), which is continuously dealing with the substantial levels of endogenous DNA oxidation that arise from cellular metabolic activity (Williams and Jeffrey, 2000; Cooke et al., 2003). In mammalian cells, the degradation of HPO is carried out by catalase and hydroxyl radicals formed from HPO are scavenged by peroxidase and the cellular stores of nucleophiles such as glutathione and protein (Griffith and Mulcahy, 1999). As noted, any 8-oxo-dG adducts that may be formed as a result of exceeding the free radical scavenging capacity of the cells, including cells of the oral mucosa, are known to be excised by DNA repair enzymes. In particular, in humans 8 oxo-dG adducts are readily repaired by such enzymes to the point that these adducts are not easily converted into a mutagenic lesion (Asagoshi et al., 2000; Lunec et al., 2002).

As expected, the in vitro genetic toxicity data clearly show genotoxic effects of HPO. In the bacterial mutagenicity assays, positive results have been reported in *Salmonella typhimurium* strains TA102 and TA104, strains that are known to be sensitive to oxidative DNA damage (Levin et al., 1982; De Flora et al., 1984; Carlsson et al., 1988; Glatt, 1989; Kensese and Smith, 1989; Abu-Shakra and Zeiger, 1990; Wilcox et al., 1990; Li et al., 1992; Nakayama et al., 1993).

The results of in vitro mammalian gene mutation assays, including the Chinese hamster ovary (CHO) V-79 *hprt* and the mouse lymphoma L5178Y *hprt* locus assays, are mixed. One (Ziegler-Skylakakis and Andrae, 1987) of 6 studies (Bradley et al., 1979; Tsuda, 1981; Bradley and Erickson, 1981; Nishi et al., 1984; Speit,

1986; Ziegler-Skylakakis and Andrae, 1987) in CHO cells reported a mutagenic effect at an HPO concentration of 17 μg/ml. The available mouse lymphoma assays reported weak mutagenic activity of HPO at concentrations of 0.17 and 0.34 μg/ml (Kruszewski et al., 1994).

HPO has been reported to induce sister-chromatid exchanges (SCE) in several mammalian cell types, including Chinese hamster V-79 cells (Bradley et al., 1979; MacRae and Stich, 1979; Sasaki et al., 1980; Speit et al., 1982; Estervig and Wang, 1984; Mehnert et al., 1984a,b; Speit, 1986; Tucker et al., 1989; Diaz-Llera et al., 2000).

In vitro exposures to HPO have also produced single stranded DNA breaks (Bradley et al., 1979; Cantoni et al., 1986; Prise et al., 1989; Kleiman et al., 1990; Djuric et al., 1993), chromosomal aberrations (e.g., Hanham et al., 1983; Estervig and Wang, 1984; Ishidate et al., 1984; Oya et al., 1986; Fenech et al., 1999), induction of unscheduled DNA synthesis (UDS) (Regnier et al., 1996), and the induction of micronuclei (Sasaki et al., 1980; Stich and Dunn, 1986).

The in vitro mutagenicity and clastogenicity data must be interpreted in light of the fact that these test systems do not contain the in vivo levels of the enzymes responsible for the detoxification of HPO. For example, the inclusion of catalase enzymes in the test preparations prevented the production of clastogenic effects (Hanham et al., 1983; Estervig and Wang, 1984; Stich et al., 1978; Tsuda, 1981). Finally, the inclusion of a metabolic activation system in the in vitro assays had the effect of reducing or negating the effects of HPO (summarized in IARC, 1999). This is due to either the direct detoxification of HPO or the reaction of hydroxyl radicals with the thiols or proteins in the metabolic activation system.

2.2. In vivo data

In vivo data on the genotoxicity of HPO are limited to a SCE assay in Chinese hamsters (Li et al., 1993), an UDS assay in rats (Regnier et al., 1997), and a bone marrow micronucleus assay in mice (Regnier et al., 1996).

In the SCE assay, Li et al. (1993) exposed groups of 20 Chinese hamsters (sex not specified) to daily intubations of HPO at a dose of 70 mg/kg body weight (bw), 5 days per week, for a period of either 15 or 26 weeks. Control groups of 20 received water. The frequency of SCE in bone marrow cells was used to evaluate genotoxicity. There was no difference ($p > 0.05$) in the SCE frequency between the groups exposed to HPO or to water for either 15 or 26 weeks. In a parallel experiment, Rembrandt dental whitening gel, containing CPO, was intubated to groups of 20 Chinese hamsters, at doses of either 500 or 2000 mg/kg bw for 5 days/week, for 15 or 26 weeks. As with HPO, the CPO-containing gel had no effect on SCE frequency in bone marrow cells in comparison to water-exposed controls.

Regnier et al. (1997) compared the activity of HPO in an ex vivo and an in vivo UDS assay in rat liver tissue. Although only reported in an abstract, all experiments were reportedly conducted according to OECD and Good Laboratory Practice (GLP) standards. In the in vivo assay, groups of five male Wistar rats were administered HPO by intravenous (i.v.) infusion at doses of 25 or 50 mg/kg bw, the maximum tolerated dose. Animals were killed and hepatocytes isolated and cultured for either 2–4 or 12–14 h after dosing. Based on a mean net grain count of <-2.1, and the finding that $<0.7\%$ of hepatocytes from the treated animals were in a state of DNA repair, Regnier et al. (1997) concluded that HPO was negative in the in vivo UDS assay. In comparison, in the ex vivo assay, incubation of HPO with rat hepatocytes at concentrations of 0.8 to 50 μg/ml for either 2–4 or 12–14 h, elicited UDS, as expected.

Regnier et al. (1996) also reported, in abstract form, that HPO was inactive in a mouse bone marrow micronucleus assay. In this study, 35% HPO (of a purity and grade approved by the FDA for use in food processing) in water was administered by intraperitoneal (i.p.) injection as a single 0, 250, 500, and 1000 mg HPO/kg bw dose to groups of 5 Swiss OF1 mice. Cyclophosphamide was used as a positive control. In an accompanying oral study, a group of 10 male and female C57BL/6N mice were administered HPO at a concentration of 6000 ppm in the drinking water (equivalent to doses of 536–774 mg/kg bw/day) as part of a 14-day subacute toxicity study. As in the i.p. study, cyclophosphamide was used a positive control. In the i.p. study, 24 and 48 h after dosing, and in the oral study, on day 14 of dosing, after sacrifice, bone marrow was harvested and 2000 polychromatic erythrocytes were counted. Scoring of 1000 total erythrocytes was conducted to establish the polychromatic: normochromatic erythrocyte ratio. By the i.p. route, all 3 dose levels at the 24-h harvest, and the 500 and 1000 mg/kg bw dose levels at the 48-h harvest, caused a decrease in the polychromatic erythrocyte: normochromatic erythrocyte ratio, indicative of toxicity of HPO to the bone marrow. No effect on the polychromatic erythrocyte: normochromatic erythrocyte ratio was reported after 14 days oral exposure to 6000 ppm HPO in the drinking water. The frequency of micronucleated erythrocytes was not increased relative to water controls in either the i.p. or the drinking water studies.

The in vivo genotoxicity studies indicate that activity demonstrated in vitro is not expressed in vivo. This is likely related to the rapid detoxification of HPO and scavenging of radicals prior to any opportunity to interact with DNA.

Overall, the genotoxicity data indicate that while HPO is predictably genotoxic under conditions that

allow oxidative attack on DNA (i.e., high concentrations and lack of detoxification systems), such activity is not expressed in vivo. Taking into consideration the foregoing, the genotoxic risk of exposures of the oral mucosa (having considerable catalase activity in saliva as well as the oral mucosa) to HPO encountered from TWP under recommended conditions of use is likely to be vanishingly small.

3. Carcinogenicity

Studies to assess the carcinogenicity of HPO in rodents include an unpublished drinking water study of the carcinogenicity of HPO in F344 rats (Takayama, 1981), oral administration to several strains of mice (Ito et al., 1981a,b, 1982, 1984), several dermal skin painting assays (Klein-Szanto and Slaga, 1982; Kurokawa et al., 1984), and an oral initiation–promotion study in rats (Takahashi et al., 1986). Since these studies utilized various designs and protocols, they are summarized below according to study type.

3.1. Standard bioassays

In an unpublished study (Takayama, 1981), F344 rats (50 per sex per group) were administered HPO in the drinking water at dose levels of 0, 0.3 (195–306 mg/kg/day), or 0.6% (433–677 mg/kg/day) for 18 months, followed by a 6-month recovery period. This study was thorough and collected data pertaining to mortality, serum biochemistry, as well as of the histopathology of all key organs (skin, mammary glands, pituitary, thyroid, lung, pancreas, liver, adrenal, kidney, small intestine, testis, muscle, peritoneum, eye spleen, stomach, uterus, vagina, lymph nodes, as well as the oral cavity and esophagus). Survival of treated rats was similar to the controls (41/50), except for males treated at 0.3% (36/50). Following 45 weeks of treatment, body weights were reduced by approximately 10% in the high-dose groups, and by 6% in the low-dose group. During the course of the study some nasal bleeding was observed, the significance of which was not clear since no additional data were reported regarding the histopathology of the nasal epithelium. There were no reported statistically significant differences in tumor incidence between the treated and control animals for animals that died prior to termination or for animals killed at the end of the recovery period. No tumors, or any other adverse effects were reported to occur in the oral cavity or esophagus, the 2 sites having initial contact with HPO in drinking water. The authors concluded that HPO was not carcinogenic to F344 rats. Findings of reduced body weight gain of about 6 and 10% in the 0.3 and 0.6% HPO groups, respectively, indicate that an adequate, near maximum tolerated dose (MTD), was delivered in the study.

In a series of studies in mice, which included catalase-deficient strains, Ito et al. (1981a,b, 1982, 1984) reported weak pre-carcinogenic and carcinogenic effects of HPO following administration in the drinking water for periods of up to 2 years. Administration of HPO to groups of 48–50 male and female C57BL/6J mice (Ito et al., 1981a,b, 1982) at 0%, 0.1%, or 0.4% (w/v) in the drinking water (approximately 0, 250, or 1000 mg/kg/day) for 2 years yielded a slight increase in the incidence of duodenal adenocarcinomas, but only when the results for both sexes were combined (5/99, males and females) ($p = 0.05$). The study authors concluded that the oral administration of HPO to mice induced gastric erosion, duodenal hyperplasia, and, at the high dose, duodenal carcinoma. These results must be interpreted with caution as C57BL mice have low levels of catalase, and may, therefore, be especially susceptible to HPO (Ito et al., 1984). This fact was highlighted by the finding in further studies (Ito et al., 1984) that preneoplastic/neoplastic lesion development in the duodenum following HPO treatment was inversely correlated with the catalase activity of each strain of mouse tested; mice with the highest catalase activity developed a low incidence of duodenal hyperplastic/preneoplastic lesions. No pathological findings in the oral cavity or in the esophagus were reported despite the oral administration of high HPO concentrations. The incidence of duodenal tumors in four different strains of female mice administered 0.4% HPO in the drinking water is shown below in Table 1.

The relevance of the Ito et al. studies to humans is limited because healthy humans are likely to have sufficient peroxidase/catalase activity in saliva (Tipton et al., 1995) and in the oral mucosa to deal with the extremely low amounts of HPO released from TWP. Tipton et al. (1995) have reported that human saliva effectively inhibits the cytotoxic effects of 0.05% HPO on human gingival fibroblasts in vitro.

Table 1
Incidence of duodenal tumours in 4 strains of female mice administered HPO in the drinking water at a concentration of 0.4% for about 6 months

Mouse strain	Number	Catalase activity[a]	Number with tumours (%)	Total number of tumours
C3H/HeN	18	5.3 ± 1.4	2 (11.1)	2
$B6C3F_1$	22	1.7 ± 0.2	7 (31.8)	8
C57BL/6N	21	0.7 ± 0.3	21 (100)	82
C3H/C	24	0.4 ± 0.1	22 (91.7)	63

Source: Ito et al. (1984) and SCCP (2004).

[a] Duodenal catalase activity expressed as 10^{-4} k/mg proteinin 6–8 week old mice.

3.2. Sequential exposure studies

In an initiation–promotion experiment in rats, in which one of the study groups consisted of non-initiated control rats dosed with 1.0% HPO in the drinking water for 32 weeks, forestomach papillomas developed in 5/10 of the treated rats as compared to 0/10 in the non-initiated controls not administered HPO (Takahashi et al., 1986). No tumors of the oral cavity or esophagus were reported.

In this study, enhanced tumor development was reported in rats that were pre-treated with *N*-methyl-*N*-nitro-*N*-nitrosoguanidine (MNNG) and sodium chloride, and then exposed to 1.0% HPO in the drinking water for 32 weeks. The HPO-treated rats had a 100% incidence of forestomach papillomas compared to 0% in the initiated comparison group. The incidence of adenocarcinoma of glandular stomach and duodenum was not increased by HPO in comparison to initiation-only controls, although the incidence of adenomatous hyperplasia in the glandular stomach was increased in the initiated and HPO-treated group (8/21 or 38%) compared to the initiated controls (0%).

The significance of forestomach tumors is questionable given the fact that humans have no corresponding organ. In the rat, the forestomach acts as a storage organ rather than a digestive one. As a result, locally high and longer duration exposures to forestomach epithelium/mucosa would be expected. For this reason, tumors of the forestomach, especially if related to chronic tissue irritation, are generally considered to be of little relevance to human carcinogenic risk (Wester and Kroes, 1988; Grasso et al., 1991; Wurtzen, 1993; IARC, 2003). Moreover, the effects in the forestomach may represent a sequential syncarcinogenic effect of DNA-reactive agents given in sequence rather than a true promoting effect (Williams and Iatropoulos, 2001).

Despite the use of a strong alkylating agent and high drinking water concentrations of HPO over a 32-week span, no tumors or other adverse effects were reported to occur in tissues proximal to the forestomach (i.e., the oral cavity and the esophagus). Strong alkylating agents and other carcinogens have been shown to produce tumors of the oral cavity and esophagus, thus indicating that these sites in rodent respond to genotoxic insult (Gold et al., 2001).

The available skin painting initiation–promotion studies in which mice were pre-treated with 7,12-dimethylbenz[*a*]anthracene (DMBA) followed by treatment with HPO, failed to elicit any clear evidence of a tumor promoting effect (Shamberger, 1972; Bock et al., 1975; Klein-Szanto and Slaga, 1982; Kurokawa et al., 1984). Although the dermal studies were negative, it should be acknowledged that mouse skin, although a standard assay (Enzmann et al., 1998), is not a perfect surrogate for oral mucosa. Both are squamous epithelia, but mouse skin has a greater degree of keratinisation as compared to the oral mucosa. Thus, oral mucosa could be more sensitive due to a greater degree of HPO penetration. The gingiva, however, is more highly keratinized than the floor of the mouth, and thus is more similar to mouse skin. The studies cited above generally used acetone as the dosing vehicle for HPO. This vehicle, based on data available for benzoyl peroxide (Binder et al., 1997) likely increased the absorption of HPO into the skin.

3.3. Combined exposure studies

In another rat experiment, Hirota and Yokoyama (1981) studied the interactive effects of methylazoxymethanol acetate (MAM) treatment in conjunction with HPO. Male Fischer 344 rats were exposed to 1.5% HPO in the drinking water for 8 or 21 weeks, during which time MAM was administered by i.p. administration (25 mg/kg) in weeks 4, 6, and 8 of the study. No gastrointestinal (GI) tract tumors were observed in rats treated with HPO alone for 21 weeks or in untreated controls. Treatment of rats with HPO for 8 weeks, during which time MAM was administered, and for a further 25 weeks resulted in a 100% incidence of duodenal carcinomas. In rats exposed only to HPO during the first 8 weeks plus the MAM treatments, a 25% incidence of duodenal carcinomas was reported. The lack of a MAM only initiation control group limits the interpretation of the study; however, the data are suggestive of a syncarcinogenic effect (Williams and Iatropoulos, 2001) of HPO given following co-administration of HPO and MAM. In a small group ($n = 3$) of rats given only HPO, no tumors were recorded. As in the other oral studies, including protocols for carcinogenicity (Ito et al., 1981a,b, 1982, 1984; Takayama, 1981) and initiation–promotion (Takahashi et al., 1986), no evidence of any tumors in the upper alimentary tract was reported in rats treated with HPO and MAM, or HPO alone.

In a study in hamsters, the buccal pouches were painted with 0.25 DMBA 2 times per week for 19–22 weeks together with 30% HPO applied 2 times per week the day following DMBA (Weitzman et al., 1986). A marginally significant ($p = 0.054$) increase was reported in the trend for cheek carcinoma incidence in hamsters treated with DMBA and HPO for 22 weeks ($5/5 = 100\%$) versus DMBA alone ($3/7 = 43\%$). These results are uncertain given their marginal significance due to the low numbers of animals used in the experiment. Co-treatment of the buccal pouches with DMBA and 3% HPO did not increase the incidence of carcinoma ($6/11 = 55\%$) in comparison to the DMBA only controls. Also, no tumors were seen in hamsters ($n = 9$) treated with 30% hydrogen peroxide alone twice per week. Treatment at the high-concentration of 30% HPO in uninitiated controls resulted in clear evidence

of tissue irritation and toxicity as shown by the observation of chronic inflammation and cellular dysplasia. The fact that tumors developed within the time frame of the study (19–22 weeks) with DMBA application indicates that the negative finding with HPO alone reflects non-carcinogenicity.

In a later study, using groups of 25 hamsters of each sex, Marshall et al. (1996) exposed the cheek pouches to a solution containing 0.75% HPO along with 5% baking soda 5 times per week for 20 weeks. Another group received the same solution along with 0.5% DMBA. A third group received a commercial dentifrice containing 3% HPO along with 0.5% DMBA. A control group received 0.1 ml mineral oil. At the end of treatment, 0/50 hamsters receiving only the 0.75% HPO/5% baking solution presented with cheek pouch masses. In contrast, 40/50 of those exposed to DMBA alone had cheek pouch masses. The incidence of cheek pouch masses in the hamsters treated with DMBA and the 0.75% HPO/5% baking soda solution (37/50) or the DMBA and 0.5% HPO commercial dentifrice preparation (41/50) were not significantly different from the group treated with DMBA alone.

In a second phase of the previous study, Marshall et al. (1996) applied HPO (1.5%) 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% HPO/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 preparations/solutions were administered 5 times per week. In this second phase, there was no HPO/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/HPO co-exposed groups, this phase of the study was not capable of detecting any potential enhancing effect of HPO, if it existed.

Overall, the combined exposures studies, including the hamster cheek pouch studies, can be considered to provide limited evidence of a weak interactive effect of relatively high concentrations of HPO (1.5–30% in aqueous solution) with co-treatment with potent DNA-reactive initiating agents such as MAM and DMBA. It is noteworthy that no carcinogenic effect of HPO alone in the oral cavity was noted in the hamster cheek pouch assays or in the oral dosing studies in mice and rats.

4. Clinical studies

There are over 100 clinical studies, most as yet unpublished, comprising approximately 4000 subjects in total, that have been conducted on HPO-containing (5.33–16%) TWP. In addition, Leonard et al. (2003) reported a 7.5-year follow-up study on a small group of TWP users. In this follow-up study of 15 subjects who received 6 months of continuous HPO treatment for tetracycline stains, no evidence of adverse effects in the oral cavity were noted in 9 of the 15 who agreed to a clinical examination. While the study is small in terms of number of subjects, thus limiting the value of statistical analyses, none of the 15 participants reported any side effects that they believed to have been treatment-related. Studies have evaluated the effects of TWP under recommended use conditions (1–2 weeks) and under conditions of extended (up to 6 months) and exaggerated use (four times application per day). Summaries of the longer-term (i.e., 90–180 days of HPO exposure through use of TWP) and of studies that incorporated long-term follow-up of subjects following TWP product use are provided in Tables 2 and 3, respectively.

The incidence of adverse effects, while quite variable, is in all cases mild and transient and limited to gingival irritation and tooth sensitization. These effects resolve within a few days of ending product use.

Mild gingival irritation has not been reported to be a risk factor for the development of oral cancer. Moreover, the gingiva is a very rare site for the development of oral cancers. The most common sites, the floor of the mouth and the lateral edge of the tongue (Cawson, 1975; Mashberg and Meyers, 1976) have not been reported to be adversely affected in any of the clinical studies on TWP. Also, at these sites, HPO concentrations in saliva [maximum concentration of 0.03% 1-min post application (Slezak et al., 2002)] are very low in comparison to HPO concentrations achieved on the gingiva [maximum median concentrations of 0.65% within 5 min of application of 10% HPO TWP strips (unpublished clinical trial)], the site adjacent to the application of TWP. Even the highly variable incidence of gingival irritation reported in the clinical studies may not entirely be the result of HPO since many TWP contain dehydrant vehicles such as glycerol. In addition, subjects in clinical trials often traumatize gingival tissues through over zealous brushing prior to dental visits.

Beyond the published and unpublished clinical data accumulated to support the safety of TWP, over the last 4–5 years, millions of tooth whitening kits have been sold directly to consumers, and, bleaching procedures have been extensively conducted under the supervision of dental professionals for the last 15 years, yet no published reports of preneoplastic or neoplastic lesions associated with their use have appeared in the scientific literature to date. Since the incidence of gingival cancer is likely less than 1 per 100,000 population (Sugerman and Savage, 2002), if TWP were to cause neoplasms of the gingiva, it would be expected that changes in the background incidence rate would be relatively easy to detect.

Table 2
Clinical studies on the use of HPO-containing TWP for 31–180 days

Reference or unpublished study number	Number of subjects enrolled	Formulation tested	Exposure	Safety outcomes
Study number 1998073	108	5.3% HPO gel strips, pH 5.5, low viscosity	3-Phases	Tooth sensitivity was most frequent adverse event
Bleaching regimen comparison			Treatment phase: 4 weeks, 30 min per day 8 weeks, 30 min per day	Tooth sensitivity incidence was not related to treatment duration
			Maintenance phase: 30 min, once per week 30 min, 4 consecutive days in the month	4-Week treatment: 0.33 adverse events/subject; 14% of subjects had tooth sensitivity 8-Week treatment: 0.35 adverse events/subject; 15% of subjects had tooth sensitivity
			Re-treatment phase: 4 weeks, 30 min per day 8 weeks, 30 min per day	
Study number 2000101	40	6.0% HPO gel strip	Maxillary application only	6% HPO strip: 20% of subjects had oral soft tissue adverse events, 40% had tooth sensitivity
University of North Carolina		Placebo	30 min per application, twice daily, every day for 6 weeks	Placebo strip: 10% of subjects had oral soft tissue adverse events, 10% had tooth sensitivity
				No subjects experienced any adverse events other than oral soft tissue irritation and tooth sensitivity
Karpina et al. (2003)	50	5.3% HPO paint-on gel	Subjects used product once daily, with the first day's use under supervision	All adverse events were mild in severity and resolved during treatment or upon completion of treatment
		Placebo paint-on gel	Product used overnight only, on days 1–42	7 (14%) subjects reported possible/probable treatment related oral symptom adverse events in the 5.3% HPO paint-on gel group, of which 5 (20%) were tooth sensitivity and 2 (8%) were oral soft tissue adverse events
				One of the subjects discontinued treatment after week 4 due to tooth sensitivity
				Placebo: 4 (16%) subjects reported oral soft tissue adverse events
Study number 2002096	152	5.3% HPO paint-on gel	Used overnight for 6 weeks	All adverse events were mild in severity, except 1 oral soft tissue event which was moderate in severity
				Resolution of all adverse events occurred during treatment or upon cessation of treatment
				All oral soft tissue adverse events that were deemed possibly or probably treatment related were reported by subjects, with 1 exception, a mild aphthous stomatitis, which the examiner observed

(*continued on next page*)

Table 2 (*continued*)

Reference or unpublished study number	Number of subjects enrolled	Formulation tested	Exposure	Safety outcomes
				Overall, 8 (5.3%) subjects reported oral soft tissue adverse events determined by the investigator to be possibly or probably treatment related
				4 (2.6%) subjects reported tooth sensitivity determined by the investigator to be possibly or probably treatment related
Kugel et al. (2002) and Study number 2000043	40	6.3% HPO gel strips ($N = 30$)	Gel strips: 30 min, twice daily for 6 months	6.3% HPO strip: 43% of subjects had oral soft tissue adverse events, 47% had tooth sensitivity
		10% CPO (~3.3% HP) Opalescence in dental tray ($N = 10$)	10% CPO dental tray: overnight use	10% CPO dental tray: 30% of subjects had oral soft tissue adverse events, 40% had tooth sensitivity
			All of the subjects had tetracycline stained teeth	All adverse events were reported; none were observed by the examiner No subject left the study due to adverse events
Study number 20022063	35	Crest White Strips Retail Kit (6% HPO gel strips gel strips)	Maxillary application only	Crest White Strips: 6% of subjects had oral soft tissue adverse events, 44% had tooth sensitivity
Safety/efficacy on Tetracycline Dental Stain Loma Linda Univ.		9.5% HPO gel strips	30 min per application, 2 applications per day for 3 months	9.5% HPO gel strips: 6% of subjects had oral soft tissue adverse event, 59% had tooth sensitivity
				Overall, 73% adverse events were mild in severity, 23% moderate, and 4% severe. No serious adverse events
				One severe case of tooth sensitivity occurred in the 9.5% group
Haywood et al. (1997)	10	10% CPO in a custom-fitted dental tray	Overnight use for a period of 6 months	Four subjects discontinued product use during the first 2 weeks due to gingival irritation (oral soft tissue), tooth sensitivity, and/or throat irritation/taste
				All symptoms resolved within 24 h

Table 3
Clinical studies on the use of HPO-containing TWP that included long-term follow-up

Reference or unpublished study number	Number of subjects enrolled	Formulation tested	Exposure	Safety outcomes
Kugel and Kastali (2000)	70	5.3% HPO gel strips	Maxillary application only	Tooth sensitivity was the only adverse event reported
		Placebo	30 min per application, twice daily for 2 weeks	5.3% HPO strip: 6% of subjects had oral soft tissue adverse events
			Follow-up visits at approximately 3- and 6-months post-treatment	Placebo strip: No adverse events were reported
				Gingival Index and Plaque Index were the same for both groups at the 3- and 6-month visits
Study number 1999091	70	5.3% HPO gel strips	Maxillary application only	5.3% HPO strip: 3% of subjects had oral soft tissue adverse events, 0% had tooth sensitivity
Hill Top, Florida		Placebo	30 min per application, twice daily for 4 weeks	Placebo strip: No subjects experienced any oral soft tissue irritation or tooth sensitivity
			Follow-up visits at approximately 3- and 6-months post-treatment	No subjects experienced any effects other than oral soft tissue irritation and/or tooth sensitivity
				Gingival Index and Plaque Index were the same for both groups at the 3 and 6-month visits
Study number 1999113	95	5.3% HPO gel strips	Maxillary application only	5.3% HPO strip (2 weeks): 39% of subjects had oral soft tissue adverse events, 3% had tooth sensitivity, 3% had other, non-oral, types of adverse events
Hill Top, Ohio		Placebo	30 min per application, twice daily for 2 or 4 weeks	
			Follow-up visits at about 3- and 6-months post-treatment	5.3% HPO strip (4 weeks): 15% of subjects had oral soft tissue adverse events, 15% had tooth sensitivity, but no other types of adverse events
				Placebo strip: 3% of subjects had oral soft tissue adverse events, 0% had tooth sensitivity or other types of adverse events
				Gingival Index and Plaque Index were the same for both groups at the 3 and 6-month visits
Leonard et al. (1999)	21 initial, 12 completed the follow-up period	10% CPO gel in custom-fitted mouth guard	10% CPO gel in custom-fitted mouth guard, overnight for 6 months	Eighty percent of subjects reported adverse events during the 6 month treatment period
			Follow-up visits at 6, 12, and 54 months post-treatment	There were no reports of tooth whitener related adverse events at the 6-month post-treatment visit
				One subject reported having tooth sensitivity or gingival irritation at the 12 month post-treatment visit
				Three subjects reported having tooth sensitivity or gingival irritation at 54 months

(*continued on next page*)

Table 3 (*continued*)

Reference or unpublished study number	Number of subjects enrolled	Formulation tested	Exposure	Safety outcomes
Leonard et al. (2003)	21 initial, 15 assessed at follow-up	10% CPO in custom-fitted mouth guard	10% CPO gel in custom-fitted mouth guard, overnight for 6 months	One subject reported having tooth sensitivity at the 90-month post-treatment visit; however, this subject also reported pre-treatment tooth sensitivity
			Follow-up visit 90 months post-treatment	No pathological alterations were seen on the radiographs for these subjects
			Follow-up was an extension of the Leonard et al. (1999) study	SEM photomicrographs indicated no obvious differences between the facial surfaces of the treated maxillary teeth and the untreated surfaces of the mandibular teeth at 90 months post-treatment
Ritter et al. (2002)	30 of 38 participants originally in the study were assessed at post-treatment follow-up	10% CPO gel in a custom-fitted night guard	10% CPO gel in a custom-fitted night guard tray, overnight for 6 weeks	For the examined teeth, 93% had a normal gingival index score, 5% had a gingival index = 1 (mild inflammation) and 1% had a gingival index = 2 (moderate inflammation). No evidence of ECR was found during an evaluation of the X-rays and no apical lesions were observed. Gingival index and ECR findings were considered normal suggesting minimal post whitening effects at 10 years post treatment
			Subjects were evaluated for gingival index and external cervical resorption (ECR) by radiographic examination 10 years post-treatment	

An unpublished meeting abstract (Burningham et al., 2004) suggested a possible association between the development of oral cancer and use of TWP in younger adults (<45 years of age). This abstract recorded the use of TWP in 19 young adult patients with primary oral cancer. Three persons (16%) reported a history of use of TWP. The ages of the three persons who used TWP (mean of 34.3 years) tended to be lower than those who did not (mean of 52.4 years). The use of alcohol and tobacco products was similar between users and non-users of TWP. The oral cancers in three subjects who used TWP had spread to regional lymph nodes, while in the remaining 16 patients who did not use TWP, three of the cancers were reported to have spread to regional lymph nodes. The authors concluded that their case reports do not show any causative association between TWP use and oral cancer risk. Among the three cases of primary oral cancer in young adults who had used TWP, two were cases of tongue cancer in patients who reported using TWP 2–3 years prior to diagnosis; that is not a sufficient interval for induction of malignant, metastatic tumors. The abstract provides no biologically plausible basis for any potential association between the use of TWP and the two cases of tongue cancer. Moreover, in no clinical studies on TWP have adverse effects on the tongue been reported to occur.

5. Risk factors for the development of oral cancer

Oral cancer, more specifically squamous cell carcinoma of the oral mucosa, is a multifactorial disease, the primary risk factors for which are smoking and alcohol consumption/abuse. Other contributory factors that have been identified include poor nutrition, poor oral hygiene, viral infections, and exposure (occupational) to carcinogens. The most common sites at which squamous cell carcinoma is reported to occur in the oral cavity is along the floor of the mouth and on the soft palate (Cawson, 1975). The gingiva is only rarely identified as a site for oral cancer development (Lesch et al., 1989; Mashberg and Meyers, 1976).

More than 90% of persons who develop oral cancer are smokers (Blot et al., 1988; Merletti et al., 1989; Baron et al., 1993). In persons who smoke more than 1 pack of cigarettes per week, the relative risks for the development of oral cancer (7.3) are especially high in comparison to persons who smoke less than 1 pack per week (Maier et al., 1992). Life-long smokers of unfiltered cigarettes have been reported to have approximately double the risk for development of oral cancer compared to life-long smokers of filtered cigarettes (Mashberg et al., 1993; Andre et al., 1995). Cessation of smoking results in a sharp decline in the risk for oral cancer development, with no increased risk detectable

after 10–15 years (Merletti et al., 1989; Franceschi et al., 1992; Andre et al., 1995).

Excessive consumption of alcohol is a pronounced risk factor for oral cancer development of nearly the same order of magnitude as smoking status (Blot et al., 1988; Maier et al., 1992; Mashberg et al., 1993; Andre et al., 1995). As with smoking, a distinct linear dose-response relationship between the amount of alcohol consumed and the relative risk for the development of oral cancer has been reported. The type of alcoholic beverage (e.g. wine, beer, spirits) consumed appears to have little influence on the degree of risk for the development of oral cancer (Blot et al., 1988; Kabat and Wynder, 1989).

In contrast to cigarette smoke, which is known to contain a number of carcinogenic chemicals, alcohol is itself not a carcinogen, but functions as a co-carcinogen or promoter (Seitz et al., 1998). The co-carcinogenic effect of alcohol may be mediated by CYP 2E1 metabolism to acetaldehyde. Although most metabolism of ingested alcohol occurs in the liver, the oropharyngeal mucosa contains relatively high concentrations of metabolizing enzymes (Seitz et al., 1998).

The combination of smoking and heavy alcohol consumption appears to have a synergistic, or greater than additive (i.e., multiplicative), effect on the risk for development of oral cancer (Blot et al., 1988; Maier et al., 1992; Baron et al., 1993).

Given the potential, if not likely, use of TWP by smokers and/or alcohol consumers, it is of interest to evaluate the potential (exacerbation of) oral cancer risks by TWP use, and HPO exposure, in these subjects. As with smoking and alcohol consumption, increased cancer risk from combined exposures can arise when both exposures each convey a cancer risk. For example, combined smoking and asbestos exposures, which individually present cancer risks (IARC, 1977, 2002), convey greatly increased risks for lung cancer (IARC, 1977). However, since there is no established human cancer risk from TWP or HPO, there is no basis to postulate that there would be an increased risk from use of TWP by individuals with exposure to products associated with risk of oral cancer, such as in smokers and/or heavy drinkers.

The clinical studies on TWP, many of which would have included smokers and/or alcohol consumers, provide no evidence to indicate that the rate or severity of the adverse effects of TWP, namely mild, transient gingival irritation and tooth sensitivity are significantly different from non-smokers/alcohol consumers. Although, there is no long-term follow-up (e.g., greater than 7.5 years) in smokers and non-smokers, no visible pathological changes that could plausibly be related to future preneoplastic or neoplastic lesion development were seen in any of the subjects in the over 100 clinical trials.

6. Discussion and conclusions

As would be expected, HPO is genotoxic in vitro, under conditions that allow oxidative attack on DNA (i.e., high concentrations and lack of detoxification systems). Nevertheless, such activity is not expressed in vivo. HPO at high concentrations is weakly carcinogenic to the duodenum of mice, especially those that are catalase deficient (Ito et al., 1981a,b, 1982, 1984). This animal model is of limited relevance to humans because humans have high levels of catalase activity, especially in the oral cavity where exposure to HPO would occur with the use of TWP. Similarly, the relevance of forestomach tumors induced by a high drinking water concentration of 1% HPO in rats (Takahashi et al., 1986) is highly questionable given the lack of a human correlate for this organ and the fact that chronic tissue irritation over a sustained period often underlies tumor development in this organ (Wester and Kroes, 1988; Grasso et al., 1991; Wurtzen, 1993; Kraus et al., 1995; IARC, 2003).

The available sequential exposure study (Takahashi et al., 1986) and combined exposure studies (Hirota and Yokoyama, 1981; Weitzman et al., 1986) document interactive effects of HPO with DNA-reactive carcinogens. The effects in the forestomach (Takahashi et al., 1986) and duodenum (Hirota and Yokoyama, 1981) are not relevant to risk assessment since the experimental conditions (i.e., use of potent DNA-reactive carcinogens for stomach and duodenum) are highly artificial. The hamster cheek pouch experiments (Weitzman et al., 1986; Marshall et al., 1996) are conceptually more relevant, but did not reveal a clear effect of HPO.

Beyond the limitations of the design of these studies with respect to human relevance, the results must be interpreted in light of the exposure conditions experienced by humans with TWP use. Based on available data, salivary concentrations of HPO following application of a TWP rapidly decline to near undetectable levels within 15–60 min (Slezak et al., 2002; Mahony et al., 2003). Moreover, based on a surface area exposure analysis (to account for the fact that effects in the human oral mucosa would, if they were to occur, be associated with site of contact concentrations, not systemic mg/kg body weigh/day exposure rates), exposures in the carcinogenicity/tumor promotion/interaction studies are orders of magnitude higher than would be experienced by humans using TWP. Specifically, exposure of the floor of the mouth to HPO from TWP use was calculated to be >400-fold lower than the dose used in mouse dermal tumor initiation–promotion skin painting studies (Shamberger, 1972; Bock et al., 1975; Kurokawa et al., 1984) and >100-fold lower than the dose used in a hamster check pouch tumor initiation and promotion studies in which no carcinogenic effects were observed (Marshall et al., 1996).

A comparison of the maximal drinking water HPO concentrations of 0.4%, 1.0%, and 1.5% utilized in the Ito et al. (1981a,b, 1982, 1984) mouse studies, in the Takahashi et al. (1986) rat initiation–promotion study, and in the Hirota and Yokoyama (1981) combined exposure (with MAM) study, respectively, with HPO concentrations in saliva after application of TWP also reveals large differences in exposure. The above drinking water HPO concentrations (continuous exposure) are from 13-fold to 33-fold-greater than the peak concentrations of HPO in the saliva of 0.03% (representative data) achieved 1 min after application of TWP (Slezak et al., 2002). Given the rapid disappearance of HPO in the saliva (undetectable within 15–60 min), daily exposures in the animal studies were in fact likely 1000 s fold greater than exposure to HPO from TWP since during the time that the animals consumed water, and during residency time in the stomach and duodenum, HPO concentrations would remain near the nominal concentrations used in each study (i.e., constant exposure to 0.4–1.0% HPO concentrations during periods of water consumption and storage/transit through the stomach and duodenum).

In addition to the low rates of exposure of the human oral mucosa to HPO from the use of TWP, exposures are generally short-term (minutes post-application) and intermittent in nature (e.g., exposure periods of up to 14 days 2 or 3 times per year). Accordingly, the weak carcinogenic, promoting and or enhancing effect of repeated or sustained exposures to much higher concentrations of HPO as was the case in the development of duodenal, gastric and forestomach tumors in rodents are not at all comparable to the very low, short-term and intermittent exposures to HPO from TWP use in humans.

The more than the 100 published and unpublished clinical studies on HPO-containing TWP demonstrate that the only findings of clinical significance are variable incidences of tooth sensitivity and of mild temporary gingival irritation. In each case the effects are transient and usually disappear within a few days. No long-term sequelae or pathology has been reported in any of these studies. Similarly, a 7.5-year follow-up study on a small group of TWP users (Leonard et al., 2003) found no evidence of any adverse effect, even after use of TWP for six continuous months. The clinical data provide no evidence to indicate that HPO in TWP has preneoplastic or neoplastic potential in humans.

Mild gingival irritation has not been reported to be a risk factor for the development of oral cancer and the gingiva is a very rare site for the development of oral cancers (Cawson, 1975; Mashberg and Meyers, 1976). The most common sites, the floor of the mouth and the lateral edge of the tongue have not been reported to be adversely affected in any of the clinical studies on TWP.

While smoking and alcohol use have a synergistic effect on the risk for development of oral cancer, the theoretical risk from the use of TWP, even under exaggerated use conditions, to smokers and/or heavy drinkers must be put in perspective with the fact that risks for oral cancers are significantly increased only after prolonged and sustained high-level usage of known carcinogenic agents. Exposures of gingival cells, and cells of the oral mucosa at other sites as well, to HPO are exceedingly low and very brief, thus are highly unlikely to pose an added risk for the development of cancer by cells initiated by tobacco smoke carcinogens or by the co-carcinogenic effects of alcohol.

A classical ''promoting'' effect of TWP can be discounted because such an effect in animals typically involves high and sustained exposures. For promoters to be effective, continuous long-term sustained high-level exposures are required and, interruption of which generally results in the lack of initial development of preneoplastic/neoplastic lesions or regression of any lesions formed (Burns et al., 1976; Williams and Whysner, 1996). Similarly, such exposures typically produce clear signs of tissue injury at the affected site [e.g., forestomach (Wester and Kroes, 1988; Grasso et al., 1991; Wurtzen, 1993; IARC, 2003) and skin (summarized in Kraus et al., 1995)]. TWP use is not associated with any clinical signs of sustained tissue injury. Therefore, the results of initiation–promotion or combined exposure studies, typically conducted to address mechanisms of carcinogenesis and generally not used in human cancer risk assessment (Kraus et al., 1995), should not be extrapolated to suggest a potential risk of HPO to the oral mucosa from TWP under recommended, exaggerated or extended, conditions of use. This conclusion is also supported by the fact that there are many common rodent tumor promoters, including food ingredients such as sodium chloride, ascorbates, butylated hydroxyanisole, glycerine, and sucrose, but very few, if any, are known human tumor promoters (Kraus et al., 1995).

There exists the possibility of accidental use of TWP by children. Such use however, would be rare and for the most part would be a ''one-time'' occurrence. No excess cancer risk due to tumor promoting effects would occur since long-duration and continuous exposures are required for the interaction of tumor promoters with initiating carcinogens.

In conclusion, the available genetic toxicity and animal toxicology data do not indicate that HPO poses a carcinogenic risk to the human oral mucosa. This conclusion is further bolstered by the results of the dosimetric exposure analyses from TWP users showing margins-of-safety on the order of 100–1000 s of fold between no effect levels in animal studies and transient peak HPO concentrations in saliva at the floor of the mouth. Moreover, HPO concentrations are highest in the gingiva, a site where oral cancer is rarely found and humans have

sufficient catalase activity in saliva and oral mucosa to effectively detoxicate HPO at such low exposure levels.

Acknowledgements

This review was funded by a consortium of companies belonging to the European Cosmetic Toiletry and Perfumery Association (COLIPA), Avenue Herrmann Debroux 15A B-1160 Auderghem—Brussels, Belgium.

The authors would like thank Dr. Donna McMillan of Procter & Gamble (Mason, OH USA 45040-9762) for providing the cited unpublished clinical trial data.

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