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Food and Chemical Toxicology 38 (2000) 1021–1041

Food and Chemical Toxicology

www.elsevier.com/locate/foodchemtox

Assessment of the carcinogenicity associated with oral exposures to hydrogen peroxide

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(Accepted 1 May 2000)

Summary—Concern regarding hydrogen peroxide (H_2O_2) carcinogenicity arises from its ability to act as a strong oxidizing agent. In short-term genotoxicity tests, H_2O_2 has given predominantly positive results; however, these assays have been performed using either bacterial strains engineered to be exquisitely sensitive to oxidant damage, or mammalian cells deficient in antioxidant enzymes. Significantly, the addition of antioxidant protective measures (normally present *in vivo*) to these assay systems protects against H_2O_2 genotoxicity. In most whole animal studies, H_2O_2 exposure neither initiates nor promotes tumors. In mice, however, 0.4% H_2O_2 in drinking water was reported to induce hyperplastic lesions of the duodenum and to erode areas in the glandular stomach epithelium. Owing to the chemistry of dilute H_2O_2 solutions and the anatomy/physiology of the gastrointestinal tract, it is unlikely that orally ingested H_2O_2 reaches the duodenum. Instead, greatly decreased water consumption and the resultant abrasion of the luminal lining on ingestion of pelleted dry rodent chow is the most likely cause of the observed gastric and duodenal lesions following H_2O_2 administration in drinking water. Significantly, when hamsters received high doses of H_2O_2 by gastric intubation (and water intake was not affected), the gastric and duodenal epithelia appeared normal. In-depth analysis of the available data supports the conclusion that oral ingestion of H_2O_2 should not be considered a carcinogenic threat. © *2000 Elsevier Science Ltd. All rights reserved*

Keywords: cancer; duodenum; small intestine; mouse; risk assessment.

Abbreviations: CAC = Cancer Assessment Commission; DMBA = dimethylbenz[*a*]anthracene; DMH = 1,2-dimethylhydrazine dihydrochloride; DNA = deoxyribonucleic acid; ECETOC = European Centre for Ecotoxicology and Toxicology of Chemicals; FDA = United States Food and Drug Administration; GSH = reduced glutathione; GSSG = oxidized glutathione; H_2O_2 = hydrogen peroxide; IARC = International Agency for Research on Cancer; MAM = methylazoxymethanol; $\cdot O_2^-$ = superoxide anion; SCE = sister chromatid exchange; SOD = superoxide dismutase.

Introduction

First isolated in 1818 (IARC, 1999), hydrogen peroxide (H_2O_2) is most commonly used as a chemical intermediate in the production of industrial chemicals. It is also used in textile manufacturing, in the bleaching of pulp and paper, and in the potable water treatment process (ECETOC, 1993; Hess, 1995; IARC, 1999). Exposure of the general population to H_2O_2 occurs mainly as a result of its use in a variety of non-prescription compounds (Harvey, 1990; Hess, 1995; IARC, 1999). Dilute solutions of H_2O_2 are commonly used as disinfectants. In addition, H_2O_2 is widely used in dental products such as mouthwash, toothpaste and tooth-whitening systems. This latter use of H_2O_2 has become the subject of much debate in recent years due to the availability to the consumer of tooth bleaching systems for use without the supervision of a dental professional. Such systems commonly use H_2O_2 in concentrations of up to 10% or carbamide peroxide (which breaks down to H_2O_2 and urea in aqueous solutions) in concentrations of up to 22% (10% carbamide peroxide is approximately equal to 3.5% H_2O_2) (Li, 1996). Although such systems have been shown to be highly efficacious, questions regarding the safety of these products have been raised. H_2O_2 can produce

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PII: S0278-6915(00)00098-3

highly reactive oxygen radicals in the body (Fridovich, 1983; Linn, 1998); consequently, concerns regarding this chemical's mutagenic and carcinogenic potential exist. In this paper, the issue of the carcinogenic potential of H_2O_2 following oral ingestion is assessed. Background information regarding the body's endogenous production of H_2O_2 and the processes by which the body protects against oxidant damage is discussed. In addition, the genotoxic potential of H_2O_2 is briefly reviewed. Lastly, the animal chronic toxicity studies using H_2O_2 are analyzed in detail. The results of these studies, together with known biological principles, indicate that orally ingested H_2O_2, in dilute quantities (10% or less), is not of carcinogenic concern.

Background

H_2O_2 is a naturally occurring product of mammalian metabolism, produced by the body in gram quantities per day and decomposed by redundant intracellular and extracellular protective mechanisms to maintain concentrations in the range of 10^{-8} to 10^{-3} M (ECETOC, 1993). The average concentrations of H_2O_2 measured in the body include 0.3×10^{-3} M in human blood, 2.4×10^{-5} M in the human eye, and 1.2×10^{-5} M in the extracellular pool of stimulated human neutrophils (ECETOC, 1993; Nahum *et al.*, 1989; Spector and Garnel, 1981; Test and Weiss, 1984). Its steady-state concentration within cells, which has been estimated at 10^{-8} M (Chance *et al.*, 1979), is a function of its production by mitochondria and its decomposition by the enzymes catalase, peroxidase, and glutathione peroxidase.

H_2O_2 production

H_2O_2 is produced throughout the body as a by-product of cellular respiration, as a result of oxidative stress, and as an agent for microbial killing by neutrophils and macrophages (Chance *et al.*, 1979; ECETOC, 1993). Most molecular oxygen consumed by an organism is decomposed to water without the production of reactive intermediates; a small proportion, however, is metabolized via enzymatic and spontaneous redox reactions, resulting in the production of reactive oxygen species as shown in Equation 1:

$$O_2 \rightarrow \cdot O_2^- \rightarrow H_2O_2 \rightarrow \cdot OH \qquad [1]$$

These reactions often involve interaction with transition metals such as iron or copper (Linn, 1998; Sies, 1985; Vuillaume, 1987). Some of the H_2O_2-generating enzymes found intracellularly include superoxide dismutase (SOD), various oxidases, cytochrome P450-dependent monooxygenases, and flavin dehydrogenases (ECETOC, 1993). These enzymes use molecular oxygen for substrate oxidation, resulting in the production of superoxide anion ($\cdot O_2^-$) and/or H_2O_2:

$$O_2 + e^- \rightarrow \cdot O_2^- \qquad [2]$$

$$O_2 + 2e^- + 2H^+ \rightarrow H_2O_2 \qquad [3]$$

Superoxide anion can be further acted on, either spontaneously or through the catalytic actions of SOD, to form H_2O_2 (Fantel, 1996; Fridovich, 1983):

$$2 \cdot O_2\text{-} + 2H^+ \rightarrow H_2O_2 + O_2 \qquad [4]$$

Through these various pathways, the rate of H_2O_2 production in the liver has been estimated to be 3.8 g/kg/day (Boveris *et al.*, 1972). This amounts to a total production of about 6.8 g H_2O_2 each day in a 70-kg man whose liver weighs 1800 g (Snyder *et al.*, 1975).

H_2O_2 decomposition

H_2O_2 can be a potential source of damage to cells if it is decomposed through reduction to the highly reactive hydroxyl radical. As shown in the well-characterized Fenton reaction (Equation 5), this reduction requires the presence of unchelated ferrous iron:

$$Fe^{2+} + H^+ + H_2O_2 \rightarrow Fe^{3+} + \cdot OH + H_2O \qquad [5]$$

Hydroxyl radicals can potentially react with cellular nucleophiles such as DNA, causing DNA strand breaks and other genotoxic effects, as well as cytotoxicity and rapid cell death in a variety of embryonic and adult systems (DeSesso, 1979; Fantel, 1996; Wells *et al.*, 1997). Because of their extreme reactivity, however, hydroxyl radicals are short-lived, reacting within 10^{-6} sec in biological fluids (DeSesso, 1979; Ward, 1975) and travelling no more than 2–4 nm from their site of production to the site where they react (Marnett, 1987; Myers, 1973). Their high reactivity precludes their crossing biological membranes or travelling great distances within a cell. Additionally, antioxidant compounds (including ascorbic acid and glutathione in the soluble compartment of cells and α-tocopherol in lipid membranes) serve as readily available sources of electrons to detoxify hydroxyl radicals through the reduction to water (Bohinski, 1987; Chaney, 1997).

A number of cellular enzymes maintain H_2O_2 at a low steady-state concentration by catalyzing its decomposition to water and oxygen. Catalases, which deal efficiently with large concentrations of H_2O_2, are present in most mammalian cells at a wide range of intracellular concentrations (Vuillaume, 1987). These enzymes catalyze the reduction of H_2O_2 to water and oxygen:

$$2H_2O_2 \rightarrow 2H_2O + O_2 \qquad [6]$$

Peroxidases, present within cellular peroxisomes, also reduce H_2O_2, but these enzymes require the action of an electron donor cosubstrate (Vuillaume, 1987), as shown in Equation 7:

$$H_2O_2 + 2RH \rightarrow 2H_2O + R\text{-}R \quad [7]$$

Lastly, glutathione peroxidase can react with H_2O_2 as well as with other organic peroxides to produce water and oxidized glutathione (GSSG) (Meister, 1982; Vuillaume, 1987):

$$2GSH + H_2O_2 \rightarrow 2H_2O + GSSG \quad [8]$$

This enzyme is most efficient at metabolizing H_2O_2 present at low intracellular concentrations.

Thus, as a normally occurring component of cellular metabolism, H_2O_2 is regulated by cellular features that render it harmless to the cell. In addition, the total amount of H_2O_2 that is produced normally by the human body on a daily basis—and efficiently handled by various protective mechanisms—is in the range of grams. If one considers the steady-state level of H_2O_2 in the liver and the capacity of the liver to produce massive amounts of this chemical, then it becomes evident that the liver must enzymatically decompose nearly 7 g of H_2O_2 per day. Therefore, it is only when exogenous H_2O_2 levels are sufficient to overwhelm the cellular protective mechanisms that H_2O_2 could possibly present a health hazard.

Effects of H_2O_2 in short-term tests

The potential genotoxicity of H_2O_2 has been investigated extensively in studies utilizing *in vitro* short-term tests, as well as in a limited number of *in vivo* studies. These have been reviewed in detail in other publications (ECETOC, 1993; IARC, 1999). Short-term *in vitro* tests are useful in detecting genotoxic effects that may occur in intact mammalian systems; however, results of *in vitro* testing have a high potential for "false positive" findings. This is because of the unique vulnerabilities of *in vitro* systems, which lack the benefit of many of the protective mechanisms present in intact mammalian systems. On the other hand, *in vivo* tests, by their very nature, have the advantage of taking into account the absorption, distribution, and excretion of a test agent, which are of relevance to the human exposure scenario, but which are absent from short-term *in vitro* assays.

In vitro test systems

The majority of *in vitro* mutagenicity assays for H_2O_2 have used prokaryotic cells, which are much smaller than eukaryotic cells. Therefore, test agents have a much shorter distance to travel in order to interact with the DNA of a prokaryotic cell than they do to interact with that of a eukaryotic cell. In addition, prokaryotic cells lack distinct, membrane-bound nuclei; instead, their genetic material lies in the cytoplasm as a continuous loop of "naked" DNA. As such, prokaryotic DNA is extremely vulnerable to insult by xenobiotics. In contrast, the genetic material of eukaryotic cells is located within the double membrane of the nuclear envelope and is protected by histone proteins that bind to the DNA. The membranes enclosing the nucleus in eukaryotic cells protect the structural integrity of the DNA, sheltering it from the numerous chemical changes that take place in the cytoplasm. Furthermore, the volume of cytoplasm surrounding the nucleus contains its own antioxidant protective enzymes as well as numerous membrane-bound organelles, all of which are absent in prokaryotes. Because of these differences, the DNA of prokaryotic cells is much more susceptible to xenobiotic insult than that of eukaryotic cells, a mechanistic insight that is of critical importance when assessing the relevance of information derived from *in vitro* genotoxicity tests.

In addition to the above, the bacteria used in most *in vitro* mutagenicity assays have been specifically engineered to be exquisitely sensitive indicators of potential genotoxicity (Maron and Ames, 1983). For example, in many bacterial tester strains, the *rfa* gene is mutated, resulting in partial loss of the lipopolysaccharide barrier that normally acts as a deterrent to the penetration of xenobiotics. Secondly, a pAQ1 plasmid has been used to insert several copies of oxidant-sensitive genes in some bacterial strains, making them more susceptible to oxidant DNA damage. Thirdly, a deletion in the *uvrB* gene has been introduced in some bacterial strains, resulting in inadequate DNA excision repair. In addition, an R factor plasmid has been inserted in some strains of bacteria in order to enhance mutagenesis through an error-prone recombinational repair process. These various genetic alterations make the bacterial tester strains used in short-term genotoxicity assays much more sensitive to mutagenic insults than normal bacteria or normal mammalian cells. As such, they are prone to detect mutagenic activity for compounds that normally will not exhibit genotoxicity in intact mammalian systems. This fact is best exemplified by the case of glutathione, a ubiquitous tripeptide that normally acts to protect against oxidant damage in the body. Despite its protective actions *in vivo*, glutathione has been shown to be mutagenic in the *Salmonella* test strain TA100 at physiological concentrations (Ross *et al.*, 1986).

In vitro tests using mammalian cells also have many characteristics that should be carefully considered in evaluating their relevance for assessing the genotoxicity of a test agent, most specifically, their *in vitro* nature. First of all, like bacterial assays, they do not take into account the absorption, distribution and excretion of xenobiotics. Secondly, the majority of these assays use permanent and transformed (i.e. immortalized) cell lines, many with abnormal chromosome complements. Thirdly, with the exception of freshly isolated rat hepatocytes, the types of cells most often used have low xenobiotic-metabolizing capabilities. Lastly, many of these studies use extremely high concentrations of the test agent, which

can be cytotoxic to cells in culture. Cytotoxicity may result in the release of endonucleases from lysed cells (i.e. a secondary event/mechanism); this, in turn, may cause a positive genotoxic response to be observed in surviving cells. Such factors must be taken into account before the results of *in vitro* assays can be extrapolated to likely human exposure scenarios. Furthermore, information derived from these *in vitro* mammalian studies should not be used alone without the benefit of corroborative (especially, pharmacokinetic) information derived under *in vivo* conditions.

As previously stated, H_2O_2 has been tested extensively for genotoxicity using bacterial and mammalian *in vitro* assays. The results of these studies have been reviewed in detail by the European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC, 1993); consequently, they will be summarized only briefly in this paper. Evaluation of the results of these assays should include careful consideration of the way that certain inherent features of the *in vitro* systems, as discussed above, are likely to influence the test results.

For the most part, H_2O_2 has tested positive for mutagenicity in bacterial assays in the absence of metabolic activation (ECETOC, 1993). The highest mutagenic responses have been measured in bacterial strains engineered to be especially sensitive to oxidative mutagens. Although Abu-Shakra and Zeiger (1990) reported that variations in constitutive catalase content between different bacterial strains did not determine the level of mutagenicity detected in response to H_2O_2 treatment, many other studies have shown that the addition of antioxidant enzymes to bacterial assay systems reduces the mutagenic responses observed. Specifically, the addition of S9 liver fractions, exogenous catalase, or exogenous SOD to bacterial mutagenicity assays, or the induction of endogenous catalase levels prior to H_2O_2 treatment, always attenuated or completely eliminated the mutagenic responses measured in the absence of such manipulations (De Flora *et al.*, 1984; ECETOC, 1993; Kensese and Smith, 1989; Winquist *et al.*, 1984; Zhou *et al.*, 1991). These results indicate that the presence of normal mammalian protective mechanisms eliminates the mutagenic response of bacteria to H_2O_2 treatment.

Several *in vitro* H_2O_2 genotoxicity investigations have also reported varying degrees of positive results in cultured mammalian cells (ECETOC, 1993). Genotoxic endpoints reported to be positive with H_2O_2 treatment include DNA strand breaks, unscheduled DNA synthesis, sister chromatid exchanges (SCEs) and chromosomal aberrations. While mammalian cells *in vitro* express antioxidant enzymes such as SOD and catalase, these enzymes are expressed at much higher levels *in vivo* (McGregor *et al.*, 1991; Sun *et al.*, 1989, 1993a,b). For example, catalase levels in cells from normal mouse liver *in vivo* were measured at 1260 ± 26 mU/mg protein, compared to 58 ± 3 mU/mg protein in cultured cells derived from the same tissue (Sun *et al.*, 1989). This suggests that *in vitro* mammalian cells may not be able to protect against oxidant damage as well as *in vivo* mammalian cells. Few *in vitro* mammalian cell genotoxicity tests for H_2O_2 have been performed in the presence of mammalian protective enzymes. However, those studies that have included S9 liver fractions or exogenous catalase in the test systems have exhibited a complete elimination of the genotoxicity measured in the absence of such protective measures (MacRae and Stich, 1979; Mehnert *et al.*, 1984a,b; Speit *et al.*, 1982). These results again suggest that adequate levels of mammalian protective enzymes abolish the genotoxic potential of H_2O_2.

In vivo mammalian studies

Few *in vivo* investigations using H_2O_2 have been reported. Three short-term *in vivo* mammalian studies demonstrated an absence of H_2O_2-induced genotoxic activity. In one study, Chinese hamsters were given a daily oral dose of 70 mg/kg H_2O_2, 5 days/wk, for 15 wk or 6 months (Li *et al.*, 1993). No genotoxic effects were observed in these animals based on an examination of SCE frequencies in bone marrow cells obtained from control and H_2O_2-treated animals. Similarly, Adam-Rodwell *et al.* (1994) demonstrated that an oral dentifrice containing 10% urea peroxide (equivalent to 3.5% H_2O_2) administered by oral gavage at doses of 100 to 1000 mg/kg/day to Sprague–Dawley rats for 5 consecutive days did not increase the incidence of bone marrow cell SCEs over control values. Regnier *et al.* (1996) gave C57BL/6N mice H_2O_2 in drinking water for 14 days at a concentration of 6000 ppm (mean daily intakes were 536 and 774 mg/kg for male and female mice, respectively). In addition, Swiss OF1 mice were injected intraperitoneally with 250, 500 and 1000 mg H_2O_2/kg. The genotoxic endpoint tabulated in this study was the formation of micronuclei in bone marrow erythroblasts. Again, H_2O_2 was clearly shown to be negative for genotoxicity by both routes of exposure.

The results of two additional studies were also negative, although the details of these studies are not available. In a micronucleus test, H_2O_2 treatment did not induce genotoxicity in mice (Keck *et al.*, 1980). In another study, H_2O_2 treatment failed to cause chromosomal aberrations in the bone marrow cells of rats (Kawachi *et al.*, 1980).

Conclusion

As suggested in the International Congress on Harmonization guidance (FDA, 1996), the assessment of a compound's genotoxic potential should incorporate all findings and knowledge relative to the intrinsic values and limitations of both *in vitro* and *in vivo* tests. The biological relevance of the positive *in vitro* test data discussed above is deemed to be lacking because they do not take into account (among other things) the ready detoxification of H_2O_2 *in vivo* and the specific features of the prokaryotic cells and

mammalian cell lines used in the *in vitro* short-term tests. The results of the *in vivo* studies, however, are relevant for assessing the potential genotoxicity of H_2O_2 in humans because they were conducted in whole animals endowed with a full complement of defence mechanisms and take into account the ability of animals to metabolize and eliminate H_2O_2. Thus, an in-depth analysis of the available data from both *in vitro* and *in vivo* short-term tests supports the conclusion that the endogenous defence mechanisms present in mammalian cells are sufficient to protect mammalian DNA from any genotoxic potential associated with exogenously administered H_2O_2.

Effects of H_2O_2 in long-term bioassays

To properly assess the safety of exogenous H_2O_2, one must carefully evaluate the information derived from long-term bioassays from both mechanistic and risk assessment perspectives. Although this paper focuses primarily on the carcinogenic potential of oral H_2O_2 exposures, studies investigating the effects associated with other routes of administration are also considered because they provide information regarding the inherent carcinogenic activity of the compound in animals. It is important to address separately the carcinogenic potential of H_2O_2 as a tumor promoter, a tumor initiator, and a complete carcinogen. These various classes of agents are generally defined as follows (Pitot and Dragon, 1996):

1. tumor initiators are genotoxic agents that produce irreversible changes in normal cells, thereby creating the potential for them to become tumors;
2. tumor promoters are non-genotoxic agents that can stimulate the growth of initiated cells into tumors; and
3. complete carcinogens are agents that possess both promoting and initiating properties and that are capable of inducing cancer by themselves.

Because these various aspects of carcinogenicity are mechanistically disparate, the methodologies used to assess them are significantly different. In general, cancer risks associated with tumor initiators (and complete carcinogens) are assessed using a linearized (non-threshold) multistage model, while a threshold approach is more appropriate for assessing the cancer risks associated with tumor promoters.

H_2O_2 as a tumor promoter

Twelve studies examined the potential tumor promoting activity of H_2O_2 in rodents (Table 1). In the study by Nagata *et al.* (1973), mice were injected subcutaneously with 3,4-benzopyrene and 0.6% H_2O_2 solution. Concurrent H_2O_2 application did not significantly increase the incidence of tumors at the site of application. In fact, compared to repeated subcutaneous application of distilled water, repeated H_2O_2 injection appeared to reduce the incidence of 3,4-benzopyrene initiated tumors in mice.

Ito *et al.* (1986) examined the ability of H_2O_2 in drinking water to promote tumors initiated by subcutaneous administration of 1,2-dimethylhydrazine (DMH) in mice. They reported that H_2O_2 administered in drinking water following DMH treatment increased the incidence of duodenal tumors in mice over that observed following DMH treatment alone (Ito *et al.*, 1986). These alterations were associated with hyperplastic changes in the duodenal villi. The authors concluded that H_2O_2 treatment promoted tumor development in three different strains of mice. However, as described later, other explanations are likely to underlie the increased incidence of duodenal tumors observed in this study.

Four studies investigated the ability of topically applied H_2O_2 to promote tumor growth in mice after exposure to the tumor initiator 9,10-dimethylbenz-[*a*]anthracene (DMBA) (Bock *et al.*, 1975; Klein-Szanto and Slaga, 1982; Kurokawa *et al.*, 1984; Shamberger, 1972). In general, these studies demonstrated a lack of any observable tumor-promoting activity associated with H_2O_2 exposure. Although the authors of one study claimed that H_2O_2 was "a very weak skin tumor promoter" (Klein-Szanto and Slaga, 1982), this promoting activity was not dose dependent, which calls into question the strength of their conclusion.

Hiroto and Yokoyama (1981) treated rats concurrently with intraperitoneal injections of methylazoxymethanol (MAM) and drinking water containing 1.5% H_2O_2. Co-treatment resulted in the production of duodenal and jejunal carcinomas. Because the incidence of these cancers following MAM administration alone was not investigated, the possible contribution of H_2O_2 treatment to the production of tumors, if any, cannot be determined. Significantly, treatment of rats with 1.5% H_2O_2 in drinking water alone for 21 wk did not result in any carcinomas of the duodenum or jejunum (Hiroto and Yokoyama, 1981). The impact of this study, however, is greatly limited by the small numbers of animals per group (three rats each in both the control and H_2O_2 groups; eight rats each in the MAM plus H_2O_2 groups).

Takahashi *et al.* (1986) examined the ability of H_2O_2 in drinking water to promote tumors initiated with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine administered orally to rats. Although H_2O_2 treatment increased the incidence of benign squamous cell papillomas in the stomach of rats, the authors concluded that it did not increase the yield of malignant tumors.

Two published studies (Marshall *et al.*, 1996; Weitzman *et al.*, 1986) and two abstracts (Marshall *et al.*, 1992, 1993) investigated the tumor-promoting potential of H_2O_2 in hamsters using the buccal pouch model. Although it is not clearly stated, it appears that the 1996 paper by Marshall *et al.* presents the

Table 1. Studies investigating the cancer-promoting activity of hydrogen peroxide in rodents[a]

Reference	Species (strain, sex)	Treatment						No. of animals
		Compound	Exposure	Route	Age at first exp.	Frequency	Duration	
Nagata *et al.*, 1973	mice (ddN, F)	0.1 ml tricaprylin, concurrently w/	–	sc	30 days	single exp.	NA	30
		0.05 ml of 0.6% H_2O_2	0.3 mg	sc	30 days	single exp.	NA	
		B(a)P in 0.1 ml tricaprylin	0.3 mg	sc	30 days	single exp.	NA	30
		B(a)P in tricaprylin, concurrently w/	0.3 mg	sc	30 days	single exp.	NA	30
		0.05 ml of 0.6% H_2O_2	0.3 mg	sc	30 days	single exp.	NA	
		B(a)P in tricaprylin, concurrently w/	0.3 mg	sc	NR	single exp.	NA	30
		0.05 ml distilled water	–	sc	NR	single exp.	NA	
		B(a)P in tricaprylin, concurrently w/	0.3 mg	sc	NR	single exp.	NA	30
		0.05 ml of 0.6% H_2O_2	0.3 mg	sc	NR	single exp.	NA	
		B(a)P in tricaprylin, concurrently w/	0.3 mg	sc	NR	single exp.	NA	32
		0.05 ml of 0.6% H_2O_2	0.3 mg	sc	NR	every 2 days	11 days	
		B(a)P in tricaprylin, concurrently w/	0.2 mg	sc	NR	single exp.	NA	32
		0.05 ml distilled water	–	sc	NR	every 2 days	11 days	
		B(a)P in tricaprylin, concurrently w/	0.2 mg	sc	NR	single exp.	NA	31
		0.05 ml of 0.6% H_2O_2	0.3 mg	sc	NR	every 2 days	11 days	
Ito *et al.*, 1986	mice (C3H/HeN, F)	no treatment	–	–	–	–	–	11
		DMH	0.4 mg	sc	6 wk	3x/2wk	2 wk	22
		0.4% H_2O_2 in drinking water	4 g/l	oral	6 wk	daily	6 mo.	21
		0.4% H_2O_2 in drinking water, w/	4 g/l	oral	6 wk	daily	6 mo.	19
		DMH	0.4 mg	sc	6 wk	3x/2wk	2 wk	
	mice (B6C3F1, F)	no treatment	–	–	–	–	–	12
		DMH	0.4 mg	sc	6 wk	3x/2wk	2 wk	21
		0.4% H_2O_2 in drinking water	4 g/l	oral	6 wk	daily	6 mo.	22
		0.4% H_2O_2 in drinking water, w/	4 g/l	oral	6 wk	daily	6 mo.	16
		DMH	0.4 mg	sc	6 wk	3x/2wk	2 wk	
	mice (C3Hbs, F)	no treatment	–	–	–	–	–	28
		DMH	0.4 mg	sc	6 wk	3x/2wk	2 wk	20
		0.4% H_2O_2 in drinking water	4 g/l	oral	6 wk	daily	6 mo.	24
		0.4% H_2O_2 in drinking water, w/	4 g/l	oral	6 wk	daily	6 mo.	18
		DMH	0.4 mg	sc	6 wk	3x/2wk	2 wk	
Kurokawa *et al.*, 1984	mice (Sencar, F)	acetone	–	dermal	4 wk	2x/wk	51 wk	15
		5% H_2O_2 in acetone	50 g/l	dermal	4 wk	2x/wk	51 wk	20
		DMBA in 0.2 ml acetone, then	20 nmol	dermal	4 wk	single exp.	NA	15
		acetone	–	dermal	1 wk after	2x/wk	51 wk	
		DMBA in 0.2 ml acetone, then	20 nmol	dermal	4 wk	single exp.	NA	20
		5% H_2O_2 in acetone	50 g/l	dermal	1 wk after	2x/wk	51 wk	
Klein-Szanto and Slaga, 1982	mice (Sencar, F)	DMBA in 0.2 ml acetone, then	10 nmoles	dermal	7–9 wk	single exp.	NA	60
		0.2 ml acetone	–	dermal	1 wk after	2x/wk	25 wk	
		DMBA in 0.2 ml acetone, then	10 nmoles	dermal	7–9 wk	single exp.	NA	58
		30% H_2O_2 in 0.2 ml acetone	60 mg	dermal	1 wk after	2x/wk	25 wk	
		DMBA in 0.2 ml acetone, then	10 nmoles	dermal	7–9 wk	single exp.	NA	59
		1:1 of 30% H_2O_2 in 0.2 ml acetone	30 mg	dermal	1 wk after	1x/wk	25 wk	
		DMBA in 0.2 ml acetone, then	10 nmoles	dermal	7–9 wk	single exp.	NA	59
		1:1 of 30% H_2O_2 in 0.2 ml acetone	30 mg	dermal	1 wk after	2x/wk	25 wk	
		DMBA in 0.2 ml acetone, then	10 nmoles	dermal	7–9 wk	single exp.	NA	59
		1:2 of 30% H_2O_2 in 0.2 ml acetone	15 mg	dermal	1 wk after	2x/wk	25 wk	
		DMBA in 0.2 ml acetone, then	10 nmoles	dermal	7–9 wk	single exp.	NA	60
		1:5 of 30% H_2O_2 in 0.2 ml acetone	10 mg	dermal	1 wk after	2x/wk	25 wk	

Findings				Remarks
No. mice w/tumors at end of study (474 days)	*Total no. tumors at end of study (474 days)*			
0	0			Data from this study also presented in Table 2.
19	63			Tumors at site of application counted at end of study (474–480 days after initiation of experiments).
22	73			
19	65			
18	62			
17	55			
21	81			
13	48			
No. mice w/duodenal tumors	*Mean no. tumors per mouse*			
0 (0%)	0			Data from this study also presented in Table 2.
0 (0%)	0			Mice examined after 6 mo. on experiment.
2 (9.5%)	0.1± 0.07			In C3H and B6C3F1 mice, duodenal weights also significantly increased in cotreatment *vs* H_2O_2 treatment alone.
10 (52.6%)*	0.7± 0.3*			
0 (0%)	0			Total 1,2-dimethylhydrazine dose of 1.2 mg.
0 (0%)	0			
7 (31.8%)	0.4± 0.1			
15 (93.8%)*	2.0± 0.3*			
0 (0%)	0			
1 (5%)	0.1± 0.1			
22 (91.7%)	2.6± 0.4			
18 (100%)	4.0± 0.5			
No. mice w/tumors at end of study	*Max. no. skin tumors per mouse*	*No. mice w/squamous cell carcinoma*	*No. mice w/epidermal hyperplasia*	
0	0	0	0	Data from this study also presented in Table 2.
1	0.1	0	1 (5%)	Dorsal hair removed from animals 48 hrs before first treatment and 1x/wk thereafter.
0	0	0	0	
3	0.6	1 (5%)	9 (45%)*	Mice examined for skin tumors weekly.
				No. mice with epidermal hyperplasia significantly higher with H_2O_2 treatment.
% of mice w/ papillomas at end of study	*No. papillomas per mouse at end of study*			
0	0			Data from this study also presented in Table 2.
6	0.06			
5	0.1			
8	0.08			
10	0.1			
10	0.15			

(continued)

Table 1 (*continued*)

Reference	Species (strain, sex)	Treatment						No. of animals
		Compound	Exposure	Route	Age at first exp.	Frequency	Duration	
Bock *et al.*, 1975	mice (ICR Swiss, F)	no treatment	–	–	–	–	–	30
		DMBA in 0.25 ml acetone, then	125 mg	dermal	NR	single exp.	NA	30
		3% H_2O_2 in 0.2 ml water	6 mg	dermal	3 wk after	5x/wk	56 wk	
Shamberger *et al.*, 1972	mice (ICR Swiss, F)	DMBA in 0.25 ml acetone alone	125 mg	dermal	55–60 days	single exp.	NA	36
		DMBA in 0.25 ml acetone, then	125 mg	dermal	55–60 days	single exp.	NA	30
		3% H_2O_2 in acetone	7.5 mg	dermal	20 days after	daily	40 wk	
Hiroto and Yokoyama, 1981	rats (Fischer F344, M)	no treatment	–	–	–	–	–	3
		1.5% H_2O_2 in drinking water	15 g/l	oral	8 wk	daily	21 wk	3
		1.5% H_2O_2 in drinking water, then	15 g/l	oral	8 wk	daily	8 wk	8
		MAM acetate	25 mg/kg	ip	4 wk after	biweekly	4 wk	
		1.5% H_2O_2 in drinking water, then	15 g/l	oral	8 wk	daily	8 wk	8
		MAM acetate (no H_2O_2), then	25 mg/kg	ip	4 wk after	biweekly	4 wk	
		1.5% H_2O_2 in drinking water	15 g/l	oral	4 wk after	daily	13 wk	
Takahashi *et al.*, 1986	rats (Wistar, M)	no treatment	–	–	7 wk	–	40 wk	10
		100 mg/l MNNG in drinking water, then no treatment	100 mg/l	oral	7 wk	daily	8 wk	30
			–	–	8 wk after	–	32 wk	
		100 mg/l MNNG in drinking water, then	100 mg/l	oral	7 wk	daily	8 wk	21
		1% H_2O_2 in drinking water	10 g/l	oral	8 wk after	daily	32 wk	
		no treatment, then	–	–	7 wk	–	8 wk	10
		1% H_2O_2 in drinking water	10 g/l	oral	8 wk after	daily	32 wk	
Weitzman *et al.*, 1986	hamsters (Syrian, M)	no treatment	–	–	–	–	–	NR
		mineral oil alone	–	buccal	3 mo.	2x/wk	19 wk	NR
		25% DMBA in mineral oil	250 g/l	buccal	3 mo.	2x/wk	19 wk	9
		25% DMBA in mineral oil, concurrently w/	250 g/l	buccal	3 mo.	2x/wk	19 wk	6
		3% H_2O_2	30 g/l	buccal	3 mo.	2x/wk	19 wk	
		25% DMBA in mineral oil, concurrently w/	250 g/l	buccal	3 mo.	2x/wk	19 wk	10
		30% H_2O_2	300 g/l	buccal	3 mo.	2x/wk	19 wk	
		30% H_2O_2 alone	300 g/l	buccal	3 mo.	2x/wk	19 wk	9
		no treatment	–	–	–	–	–	NR
		mineral oil alone	–	buccal	3 mo.	2x/wk	22 wk	NR
		25% DMBA in mineral oil	250 g/l	buccal	3 mo.	2x/wk	22 wk	7
		25% DMBA in mineral oil, concurrently w/	250 g/l	buccal	3 mo.	2x/wk	22 wk	11
		3% H_2O_2	30 g/l	buccal	3 mo.	2x/wk	22 wk	
		25% DMBA in mineral oil, concurrently w/	250 g/l	buccal	3 mo.	2x/wk	22 wk	5
		30% H_2O_2	300 g/l	buccal	3 mo.	2x/wk	22 wk	
		30% H_2O_2 alone	300 g/l	buccal	3 mo.	2x/wk	22 wk	9

Findings							Remarks
No. mice w/tumors at end of study		*No. mice w/skin cancer*					
0		0					Dorsal hair removed from animals in order to prevent interference with absorption.
0		0					Mice examined weekly. Lesion classified as a skin tumor if at least 1 mm in diameter and persisted at least 3 successive weeks. Tumors classified as cancers if they had invaded tissues below panniculus carnosus.
Total no. of tumors at end of study		*% of mice w/tumors at end of study*					
NR		NR					Mice freshly shaven at start of experiment and 2x/mo. thereafter. Mice examined weekly for skin tumors. Some numbers reported here estimated from graphs in text.
0		0					
No. rats w/duodenal carcinomas at 21 wk		*No. rats w/jejunal carcinomas at 21 wk*		*No. rats w/colon carcinomas at 21 wk*			
0		0		0			Data from this study also presented in Table 2. Tap water given for 2-day intervals following MAM injections. Animals sacrificed at age 29 wk Methods as reported are unclear and conflicting.
0		0		0			
2 (2 carcinomas)		2 (2 carcinomas)		1 (1 carcinoma)			
8 (21 carcinomas)		5 (7 carcinomas)		0			
		Fundus		Pylorus			
No. rats w/carcinoma	*No. rats w/forestomach papilloma*	*No. rats w/adenocarcinoma*	*No. rats w/adenomatous hyperplasia*	*No. rats w/adenocarcinoma*	*No. rats w/adenomatous hyperplasia*	*No. rats w/duodenal adenocarcinoma*	
0	0	0	0	0	0	0	Data from this study also presented in Table 2. Rats administered 10% sodium chloride in diet during initiation with MNNG. Rats on H_2O_2 treatment gained less body weight over time than did other groups of rats.
4 (13.3%)	0	0	0	1 (3.3%)	7 (23.3%)	3 (10%)	
2 (9.5%)	21 (100%)	0	8 (38.1%)	2 (9.5%)	6 (28.6%)	0	
0	5 (50%)	0	0	0	0	0	
No. hamsters w/hyperchromatic cells		*No. hamsters w/chronic inflammation*		*No. hamsters w/dysplasia*		*No. hamsters w/carcinoma*	
0		0		0		0	Data from this study also presented in Table 2. The number of animals used is too small to draw firm conclusions. Methods are not well described.
0		0		0		0	
3		4		6		1	
1		0		3		0	
5		5		4		1	
1		2		1		0	
0		0		0		0	
0		0		0		0	
3		3		5		3	
6		6		7		6	
5		2		5		5	
4		8		4		0	

(continued)

Table 1 (*continued*)

Reference	Species (strain, sex)	Treatment: Compound	Exposure	Route	Age at first exp.	Frequency	Duration	No. of animals
Marshall *et al.*, 1992	hamsters (NR, M & F)	mineral oil alone	–	buccal	NR	5x/wk	20 wk	50
		0.5% DMBA in 0.1 ml mineral oil	0.5 mg	buccal	NR	5x/wk	20 wk	50
		dentifrice containing 0.75% H_2O_2	NR	buccal	NR	5x/wk	20 wk	50
		0.5% DMBA in 0.1 ml mineral oil, concurrently w/ commercial dentifrice	0.5 mg –	buccal buccal	NR NR	5x/wk 5x/wk	20 wk 20 wk	50
		0.5% DMBA in 0.1 ml mineral oil, concurrently w/ dentifrice containing 0.75% H_2O_2	0.5 mg NR	buccal buccal	NR NR	5x/wk 5x/wk	20 wk 20 wk	50
Marshall *et al.*, 1993	hamsters (NR, M & F)	0.25% DMBA in 0.1 ml mineral oil	0.25 mg	buccal	4 wk	3x/wk	16 wk	50
		0.50% DMBA in 0.1 ml mineral oil	0.5 mg	buccal	4 wk	3x/wk	16 wk	50
		0.25% DMBA in 0.1 ml mineral oil, w/ dentifrice w/ 1.5% H_2O_2	0.25 mg 15 g/L	buccal buccal	4 wk 4 wk	3x/wk 5x/wk	16 wk 16 wk	50
		0.50% DMBA in 0.1 ml mineral oil, w/ dentifrice w/ 1.5% H_2O_2	0.5 mg 15 g/l	buccal buccal	4 wk 4 wk	3x/wk 5x/wk	16 wk 16 wk	50
		0.25% DMBA in 0.1 ml mineral oil, w/ 3% H_2O_2/NaHCO3 solution	0.25 mg 30 g/l	buccal buccal	4 wk 4 wk	3x/wk 5x/wk	16 wk 16 wk	50
Marshall *et al.*, 1996	hamsters (Syrian, M & F)	0.1 ml mineral oil alone	–	buccal	8–10 wk	5x/wk	20 wk	50
		0.5% DMBA in 0.1 ml mineral oil	0.5 mg	buccal	8–10 wk	5x/wk	20 wk	50
		dentifrice w/0.75% H_2O_2 & 5% $NaHCO_3$	1.5 mg	buccal	8–10 wk	5x/wk	20 wk	50
		0.5% DMBA in 0.1 ml mineral oil, w/ dentifrice w/ 0.75% H_2O_2 & 5% $NaHCO_3$	0.5 mg 1.5 mg	buccal buccal	8–10 wk 8–10 wk	5x/wk 5x/wk	20 wk 20 wk	50
		0.25% DMBA in 0.1 ml mineral oil	0.25 mg	buccal	8–10 wk	3x/wk	16 wk	50
		0.5% DMBA in 0.1 ml mineral oil	0.5 mg	buccal	8–10 wk	3x/wk	16 wk	50
		0.25% DMBA in 0.1 ml mineral oil, w/ dentifrice w/ 1.5% H_2O_2 & 7.5% $NaHCO_3$	0.25 mg 3 mg	buccal buccal	8–10 wk 8–10 wk	3x/wk 5x/wk	16 wk 16 wk	50
		0.25% DMBA in 0.1 ml mineral oil, w/ 3% H_2O_2/NaHCO3 solution	0.25 mg 6 mg	buccal buccal	8–10 wk 8–10 wk	3x/wk 5x/wk	16 wk 16 wk	50
		0.5% DMBA in 0.1 ml mineral oil, w/ dentifrice w/ 1.5% H_2O_2 & 7.5% $NaHCO_3$	0.5 mg 3 mg	buccal buccal	8–10 wk 8–10 wk	3x/wk 5x/wk	16 wk 16 wk	50

[a]B(a)P = Benzo[*a*]pyrene, a tumor initiator; DMBA = dimethylbenz[*a*]anthracene, a tumor initiator; DMH = 1,2-dimethylhydrazine dihydrochloride, a tumor initiator; F = female; H_2O_2 = hydrogen peroxide; ip = intraperitoneal injection; M = male; MAM = methylazoxymethanol, a tumor initiator; MNNG = *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine, a tumor initiator; NA = not applicable; NR = not reported; sc = subcutaneous injection; *statistically significant, as determined by original authors.

same experimental data as the two preceding abstracts (Marshall *et al.*, 1992, 1993); therefore, only the results of the 1996 paper will be discussed here. In the study by Weitzman *et al.* (1986), male Syrian hamsters (n = 5–11) were exposed for 22 wk to 30% H_2O_2 plus DMBA or to DMBA only. The authors reported a marginally significant ($P = 0.054$) increase in the incidence of carcinomas in the H_2O_2 plus DMBA-treated animals. However, this effect was not dose dependent. Additionally, treatment with 30% H_2O_2 alone did not result in carcinomas. In the study by Marshall *et al.* (1996), a 3% H_2O_2 solution (supplemented with sodium bicarbonate to mimic the active ingredients found in a common dentifrice product) was applied to the buccal pouches of hamsters for 16 wk following DMBA treatment. This treatment failed to promote tumor formation. Additionally, application of a dentifrice containing 0.75 or 1.5% H_2O_2 or application of a solution containing 3% H_2O_2 increased the tumor latency period and increased the survival rate of hamsters treated concurrently with 0.25–0.5% DMBA applied within the buccal pouch. The authors noted that 97% of the hamsters treated with DMBA alone had tumors or had died between wk 10 and 13. These results, like those of Nagata *et al.* (1973), suggest a protective effect of H_2O_2 against cancer induction.

When considered as a whole, these studies suggest that H_2O_2, administered under chronic conditions, does not act as a tumor promoter in rodents.

H_2O_2 as a tumor initiator or a complete carcinogen

Klein-Szanto and Slaga (1982) investigated the ability of H_2O_2 to act as a tumor initiator (Table 2). H_2O_2 (15% in acetone) was applied topically to mice (single exposure), followed by dermal application of the tumor promoter 12-*O*-tetradecanoyl-phorbol-13-acetate (2 μg) twice weekly for 25 wk. H_2O_2 pretreatment did not significantly increase the percentage of mice with papillomas relative to that induced with acetone-only pretreatment.

Fourteen studies examined the potential of H_2O_2 to act as a complete carcinogen in rodents (Table 2). Many of these studies used H_2O_2 administration alone as only one of the treatments in a series of regimens designed to examine the ability of H_2O_2 to act as a tumor promotor. Therefore, some of these data can be also found in Table 1. Only data derived from H_2O_2

Findings	Remarks
No tumors in the animals treated with mineral oil alone or with 0.75% hydrogen peroxide-containing dentifrice. 97% of DMBA-treated animals had tumors or died between wk 10 and 13. No difference in tumor latency time between animals treated with DMBA alone and those treated with DMBA and dentifrice or dentifrice with 0.75% hydrogen peroxide. Lower tumor incidence and increased survival of animals treated with DMBA and dentifrice with 0.75% hydrogen peroxide compared to animals treated with DMBA alone.	Data from this study also presented in Table 2. Results reported in abstract presented at IADR meeting in 1992 and published in *J. Dent. Res.*
DMBA administration increased tumor incidence in a dose-dependent manner. Hydrogen peroxide administration had no effect on DMBA-induced tumor incidence or on latency period to tumor formation.	Hamster buccal cheek pouch assay. Survival >94% with 0.25% DMBA, 44–52% with 0.5% DMBA. Results reported in abstract presented at IADR meeting in 1993 and published in *J. Dent. Res.*
No. hamsters w/ carcinomas 0 27 0 25	Data from this study also presented in Table 2. Hamster buccal cheek pouch assay. Few animals in treatment groups receiving DMBA survived to termination of study. Coadminstration of DMBA and 0.75% H_2O_2 dentifrice increased tumor latency period vs. DMBA alone.
No. hamsters w/ carcinomas 48 48 48 38 50	Co-administration of 0.5% DMBA and 0.75% H_2O_2 dentifrice increased tumor latency period *vs* 0.5% DMBA alone. Coadminstration of 0.25% DMBA and 3% H_2O_2 solution increased tumor latency period *vs* 0.25% DMBA alone.

treatment alone are presented Table 2 (with the exception of data from the tumor initiator study, discussed above). Of these 14 studies, 10 demonstrated that H_2O_2 is not a complete carcinogen. These 10 studies include two subcutaneous injection studies in mice (Nagata *et al*., 1973; Nakahara and Fukuoka, 1959), two dermal application studies in mice (Klein-Szanto and Slaga, 1982; Kurokawa *et al*., 1984), two drinking water studies in rats (Hiroto and Yokoyama, 1981; Ishikawa and Takayama, 1984), one oral intubation study in hamsters (Li *et al*., 1993), and three hamster buccal pouch studies (Marshall *et al*., 1992, 1996; Weitzman *et al*., 1985).

Only the four studies by Ito *et al*. (1981, 1982, 1984, 1986) concluded that H_2O_2 is carcinogenic. With regard to these studies, several important features should be carefully considered in evaluating the relevance of these experiments for assessing the potential carcinogenic activity of H_2O_2. First, carcinomas were only observed in the proximal duodenum; no carcinomas occurred in the oral cavity, esophagus, forestomach or glandular stomach as a result of exposure to H_2O_2. Secondly, the incidence and average number of duodenal lesions drastically decreased or fell to zero when the H_2O_2 treatment was stopped for 10–30 days after 150–210 days of exposure (Ito *et al*., 1982), indicating reversibility. Thirdly, the type of duodenal lesions and their sequential development are typical of those seen following exposure of rapidly regenerating tissues to corrosive/cytotoxic agents acting on tissues. Finally, the C57BL mouse strain used in many of the experiments is deficient in catalase (Ito *et al*., 1984), a key protective enzyme in the degradation of H_2O_2.

Interpretation of the results of the Ito studies

An assessment of the relationship between the oral administration of H_2O_2 and the duodenal lesions reported in the Ito studies (1981, 1982, 1984, 1986) requires an understanding of the comparative gastrointestinal anatomy and physiology of mice and humans. One must consider the chemical nature of dilute aqueous solutions of H_2O_2, as well as the different ways in which the gastrointestinal contents interact with the digestive systems of mice and humans. From such an analysis, two conclusions can be drawn. First, H_2O_2, whether ingested by mice in drinking water or by human beings using oral care products, is likely to decompose before reaching the

Table 2. Studies investigating hydrogen peroxide as an initiator or complete carcinogenic in rodents[a]

Reference	Species (strain, sex)	Treatment: Compound	Exposure	Route	Age at First exp.	Frequency	Duration	No. of Animals
Initiation study:								
Klein-Szanto and Slaga, 1982	mice (Sencar, F)	1:1 of 30% H_2O_2 in 0.2 ml acetone	30 mg	dermal	7–9 wk	daily	50 wk	57
		0.2 ml acetone, then	–	dermal	7–9 wk	single exp.	NA	57
		TPA	2 μg	dermal	1 wk after	2x/wk	25 wk	
		1:1 of 30% H_2O_2 in acetone, then TPA	30 mg 2 μg	dermal dermal	7–9 wk 1 wk after	single exp. 2x/wk	NA 25 wk	58
Complete carcinogen studies:								
Ito *et al.*, 1981	mice (C57BL/6J, M & F)	no treatment		–	–	–	–	98
		0.1% H_2O_2 in drinking water	1 g/l	oral	8 wk	daily	100 wk	101
		0.4% H_2O_2 in drinking water	4 g/l	oral	8 wk	daily	100 wk	99
Ito *et al.*, 1982	mice (C57BL/6J, M & F)	0.4% H_2O_2 in drinking water	4 g/l	oral	NR	daily	30 days	7
		0.4% H_2O_2 in drinking water	4 g/l	oral	NR	daily	60 days	5
		0.4% H_2O_2 in drinking water	4 g/l	oral	NR	daily	90 days	6
		0.4% H_2O_2 in drinking water	4 g/l	oral	NR	daily	120 days	6
		0.4% H_2O_2 in drinking water	4 g/l	oral	NR	daily	150 days	17
		0.4% H_2O_2 in drinking water	4 g/l	oral	NR	daily	180 days	9
		0.4% H_2O_2 in drinking water	4 g/l	oral	NR	daily	210 days	5
		0.4% H_2O_2 in drinking water	4 g/l	oral	NR	daily	300 days	10
		0.4% H_2O_2 in drinking water	4 g/l	oral	NR	daily	360 days	7
		0.4% H_2O_2 in drinking water	4 g/l	oral	NR	daily	420 days	14
		0.4% H_2O_2 in drinking water	4 g/l	oral	NR	daily	490 days	12
		0.4% H_2O_2 in drinking water	4 g/l	oral	NR	daily	560 days	7
		0.4% H_2O_2 in drinking water	4 g/l	oral	NR	daily	630 days	4
		0.4% H_2O_2 in drinking water	4 g/l	oral	NR	daily	700 days	29
		0.4% H_2O_2 in drinking water	4 g/l	oral	NR	daily	150 days	9
		0.4% H_2O_2 in drinking water, then distilled water	4 g/l –	oral –	NR –	daily –	140 days 10 days	5
		0.4% H_2O_2 in drinking water	4 g/l	oral	NR	daily	160 days	5
		0.4% H_2O_2 in drinking water, then distilled water	4 g/l –	oral –	NR –	daily –	140 days 20 days	5
		0.4% H_2O_2 in drinking water	4 g/l	oral	NR	daily	170 days	5
		0.4% H_2O_2 in drinking water, then distilled water	4 g/l –	oral –	NR –	daily –	140 days 30 days	5
	mice (DBA, M & F)	0.4% H_2O_2 in drinking water	4 g/l	oral	NR	daily	90 days	2
		0.4% H_2O_2 in drinking water	4 g/l	oral	NR	daily	150 days	10
		0.4% H_2O_2 in drinking water	4 g/l	oral	NR	daily	210 days	10
	mice (BALB, M & F)	0.4% H_2O_2 in drinking water	4 g/l	oral	NR	daily	90 days	3
		0.4% H_2O_2 in drinking water	4 g/l	oral	NR	daily	120 days	10
		0.4% H_2O_2 in drinking water	4 g/l	oral	NR	daily	150 days	10
		0.4% H_2O_2 in drinking water	4 g/l	oral	NR	daily	210 days	16
	mice (C57BL/6J, M & F)	0.4% H_2O_2 in drinking water	4 g/l	oral	NR	daily	90 days	6
		0.4% H_2O_2 in drinking water	4 g/l	oral	NR	daily	120 days	6
		0.4% H_2O_2 in drinking water	4 g/l	oral	NR	daily	150 days	17
		0.4% H_2O_2 in drinking water	4 g/l	oral	NR	daily	210 days	5
Ito *et al.*, 1984	mice (M & F)	0.4% H_2O_2 in drinking water	4 g/l	oral	6 wk	daily	6 mo.	18 C3H
		0.4% H_2O_2 in drinking water	4 g/l	oral	6 wk	daily	6 mo.	22 B6C3F1
		0.4% H_2O_2 in drinking water	4 g/l	oral	6 wk	daily	7 mo.	21 C57
		0.4% H_2O_2 in drinking water	4 g/l	oral	6 wk	daily	6 mo.	24 C3H/Cbs

Findings								Remarks
% mice w/papillomas at end of study		*No. papillomas per mouse at end of study*						
6		0.06						Data from this study also presented in Table 1.
6		0.06						
10		0.1						
Glandular stomach				Duodenum				
% mice w/ erosions	*% mice w/ hyper plasia*	*% mice w/ adenoma*	*% mice w/ carcinoma*	*% mice w/ erosions*	*% mice w/ hyper plasia*	*% mice w/ adenoma*	*% mice w/ carcinoma*	
4	7	0	0	2	9	1	0	
20*	13	1	0	1	40*	6	1	
42*	10	0	0	4	62*	2	5*	
Stomach			Duodenum					
% mice w/ lesions	*% mice w/ erosions*	*% mice w/ nodules*	*% mice w/ lesions*	*% mice w/ plaques*	*% mice w/ nodules*			
29	29	0	14	14	0			Authors state in Methods section that the intake of H_2O_2 solution and of food was the same for all treatment groups (data not shown). In experiment in which C57Bl/6N mice were administered either 0%, 0.1%, or 0.4% H_2O_2 in drinking water, dose-dependent and time-dependent induction of gastric and duodenal lesions observed.
40	40	0	80	80	0			
33	0	33	100	100	67			
67	17	50	83	33	83			
71	41	41	82	65	47			
67	56	22	89	78	22			
60	60	0	100	80	100			
90	20	70	100	70	90			
86	71	0	100	86	14			
93	79	43	100	43	93			
100	83	33	100	58	92			
100	86	43	100	57	86			
100	75	25	100	75	75			
83	76	34	100	66	100			
78	33	56	89	67	56			Cessation of H_2O_2 treatment decreased percent of mice with stomach erosions and percent of mice with duodenal lesions (plaques and nodules).
60	0	60	100	60	60			
40	0	40	60	20	60			
40	0	40	40	20	20			
60	60	40	100	100	60			
20	0	20	0	0	0			
0	0	0	100	50	100			No. of mice having duodenal lesions did not differ significantly between the three different strains of mice.
30	30	10	60	10	60			
10	0	10	80	30	60			
0	0	0	67	33	33			The average no. of lesions per mouse was higher in the C57BL mice (2.6–4.0) than in the DBA mice (1.5–1.6) or in the BALB mice (1.0–2.0).
0	0	0	40	40	0			
10	10	0	60	40	20			
6	0	6	69	44	25			
33	0	33	100	100	67			
67	17	50	83	33	83			
71	41	41	82	65	47			
60	60	0	100	80	100			
No. mice w/duodenal tumors		*Total no. tumors*		*Mean no. tumors per mouse*				
2 (11.1%)		2		0.11±0.076				Lesions preferentially developed in proximal portion of duodenum between pyloric ring and Vater's papilla. Tumor incidence was inversely related to catalase activity in the mice. No control animals used in experiment.
7 (31.8%)		8		0.36±0.124				
21 (100%)		82		3.91±0.316				
22 (91.7%)		63		2.63±0.403				

(continued)

Table 2 (*continued*)

Reference	Species (strain, sex)	Treatment: Compound	Exposure	Route	Age at first exp.	Frequency	Duration	No. of animals
Ito, *et al.*, 1986	mice	no treatment	–	–	–	–	–	11
	(C3H/HeN, F)	0.4% H_2O_2 in drinking water	4 g/l	oral	6 wk	daily	6 mo.	21
	mice	no treatment	–	–	–	–	–	12
	(B6C3F1, F)	0.4% H_2O_2 in drinking water	4 g/l	oral	6 wk	daily	6 mo	22
	mice	no treatment	–	–	–	–	–	28
	(C3Hbs, F)	0.4% H_2O_2 in drinking water	4 g/l	oral	6 wk	daily	6 mo.	24
Nakahara and Fukuoka, 1959	mice (NR, NR)	0.1 ml of 0.5% H_2O_2	0.5 mg	sc	NR	3x/wk	NR	NR
Nagata *et al.*, 1973	mice	0.1 ml tricaprylin (vehicle), w/	–	sc	30 days	single exp.	NA	30
	(ddN, F)	0.05 ml of 0.6% H_2O_2	0.3 mg	sc	30 days	single exp.	NA	
Klein-Szanto and Slaga, 1982	mice	0.2 ml acetone alone	–	dermal	7–9 wk	single exp.	NA	12
	(Sencar, F)	0.2 ml of 30% H_2O_2 solution	60 mg	dermal	7–9 wk	single exp.	NA	16–21
		1:1 of 30% H_2O_2 in 0.2 ml acetone	30 mg	dermal	7–9 wk	single exp.	NA	16–21
		1:5 of 30% H_2O_2 in 0.2 ml acetone	10 mg	dermal	7–9 wk	single exp.	NA	16–21
Kurokawa *et al.*, 1984	mice	acetone	–	dermal	4 wk	2x/wk	51 wk	15
	(Sencar, F)	5% H_2O_2 in acetone	50 g/l	dermal	4 wk	2x/wk	51 wk	20
Hiroto and Yokoyama, 1981	rats	no treatment	–	–	–	–	–	3
	(Fischer F344, M)	1.5% H_2O_2 in drinking water	15 g/l	oral	8 wk	daily	21 wk	3
Ishikawa and Takayama, 1984	rats	no treatment	–	–	–	–	–	100
	(Fischer, M & F)	0.3% H_2O_2 in drinking water	3 g/l	oral	8 wk	daily	2 yr	100
		0.6% H_2O_2 in drinking water	6 g/l	oral	8 wk	daily	2 yr	100
Li *et al.*, 1993	hamsters	water	–	–	–	–	–	20
	(Chinese, NR)	H_2O_2	70 mg/kg	oral intubation	NR	5 days/wk	15 wk	20
		water	–	–	–	–	–	20
		H_2O_2	70 mg/kg	oral intubation	NR	5 days/wk	6 mo.	20

Findings				Remarks
No. mice w/duodenal tumors	*Mean no. tumors per mouse*			
0 (0%)	0			Data from this study also presented in Table 1. Mice examined after 6 mo. on experiment. Duodenal weights also significantly increased in cotreatment vs H_2O_2 treatment alone in C3H and B6C3F1 mice.
2 (9.5%)	0.1±0.07			
0 (0%)	0			
7 (31.8%)	0.4±0.1			
0 (0%)	0			
22 (91.7%)	2.6±0.4			
Five mice survived over 200 days; no tumors were found in any of these animals.				
No. mice w/tumors at end of study	*Tumor incidence at end of study*			Data from this study also presented in Table 1. Tumors at site of application counted at end of study (474-480 days after initiation of experiments).
0	0			
30% H_2O_2 and 1:1 dilution of 30% H_2O_2 caused marked tissue necrosis, followed by regeneration and hyperplasia.				Data from this study also presented in Table 1. Mice shaved 2 days before treatment. Animals sacrificed 1, 2, 4, 6, 8, and 10 days after treatment and the number of dark basal skin cells counted.
No. mice w/tumors at end of study	*Max. no. skin tumors per mouse*	*No. mice w/squamous cell carcinoma*	*No. mice w/epidermal hyperplasia*	Study data also presented in Table 1. Dorsal hair removed from animals 48 hr before first treatment and 1x/wk thereafter. Mice examined for skin tumors weekly. No. mice with epidermal hyperplasia significantly higher with H_2O_2 treatment.
0	0	0	0	
1	0.1	0	1 (5%)	
No. rats w/carcinomas in the duodenum	*No. rats w/carcinomas in the jejunum*	*No. rats w/carcinomas in the colon*		Data from this study also presented in Table 1. Tap water given for 2-day intervals following MAM injections. Animals sacrificed at age 29 wk. Methods as reported are unclear and conflicting.
0	0	0		
0	0	0		
Negative results reported for carcinogenicity.				Data as cited from table in secondary source (IARC, 1984).
Animals grew normally, regardless of treatment.				Results reported in abstract presented at IADR meeting in 1993 and published in *J. Dent. Res.*
No differences in SCE frequencies between treatment groups. Gastroduodenal tissue findings comparable between treatment groups.				

(continued)

Table 2 (*continued*)

Reference	Species (strain, sex)	Treatment						No. of animals
		Compound	Exposure	Route	Age at first exp.	Frequency	Duration	
Weitzman *et al.*, 1986	hamsters (Syrian, M)	no treatment	–	–	–	–	–	NR
		30% H_2O_2 alone	300 g/l	buccal	3 mo.	2x/wk	19 wk	9
		no treatment	–	–	–	–	–	NR
		30% H_2O_2 alone	300 g/l	buccal	3 mo.	2x/wk	22 wk	9
Marshall *et al.*, 1992	hamsters (NR, M & F)	mineral oil alone	–	buccal	NR	5x/wk	20 wk	50
		dentifrice containing 0.75% H_2O_2	NR	buccal	NR	5x/wk	20 wk	50
Marshall *et al.*, 1996	hamsters (Syrian, M & F)	0.1 ml mineral oil alone	–	buccal	8-10 wk	5x/wk	20 wk	50
		dentifrice w/0.75% H_2O_2 & 5% $NaHCO_3$	1.5 mg	buccal	8-10 wk	5x/wk	20 wk	50

[a]F = female; H_2O_2 = hydrogen peroxide; M = male; NA = not applicable; NR = not reported; sc = subcutaneous injection; TPA = 12-*O*-tetradecanoyl-phorbol-13-acetate a tumor promoter; *statistically significant, as determined by original authors.

duodenum. Second, the duodenal and gastric lesions observed in treated mice in the Ito studies are most likely caused by physical abrasion of the stomach and duodenal linings by their contents.

Fate of H_2O_2 in the alimentary canal. H_2O_2 is a relatively unstable substance in dilute aqueous solutions and decomposes rapidly into water and molecular oxygen (Stecher *et al.*, 1968). In addition, since it is a naturally occurring product of metabolism, abundant intracellular and extracellular enzymes that catalyze the decomposition of H_2O_2 exist in mammals to protect against any adverse effects. In order to assess the potential toxicity of orally administered H_2O_2 in humans and mice, it is necessary to understand the chemical properties of H_2O_2 itself and to appreciate the salient anatomical and physiological differences between the upper gastrointestinal tracts of the two species.

H_2O_2 is a weak acid with a pK_a of 11.75 (Hess, 1995). This means that, despite the great difference in the pH of stomach secretions in humans *vs* mice, H_2O_2 will exist in the nonionized state in both species. In aqueous solutions, the rate of H_2O_2 decomposition increases with temperature from 25°C up to 100°C. Its stability in aqueous solutions is optimum at a pH of 3.5-4.5 and decreasing concentration favors decomposition (Hess, 1995). These factors suggest that following oral ingestion, H_2O_2 is rather short-lived in both species, although it is likely to be more stable in mice than in humans.

Humans, like most mammals, possess an efficient salivary peroxidase system that begins metabolizing H_2O_2 in the oral cavity (Banerjee and Datta, 1986; Tenovuo and Pruitt, 1984). H_2O_2 that is encountered in dentifrice has a residence time in the oral cavity of approximately 1.5–2 min during brushing. On swallowing, a bolus is transmitted to the stomach. The human stomach is a relatively capacious single-chambered organ (physiological capacity of 1300 ml) that is intermittently empty of food but retains watery, secreted fluid that has a resting pH of approximately 2.0 (Johnson, 1985), but which can reach a pH as low as 0.7 during times of active secretion (Granger *et al.*, 1985). Its low pH and irregular contractions, which mix the stomach contents, keep the human stomach essentially sterile (Granger *et al.*, 1985; Johnson, 1985; DeSesso and Jacobson, 2000). The acidity of the gastric environment inactivates salivary peroxidase (Granger *et al.*, 1985); however, mixing of the bolus with the dilute watery contents and an increase in temperature favor the chemical decomposition of any remaining H_2O_2. Residence time in the empty stomach is likely to be about 12 or more min (Granger *et al.*, 1985), providing ample time for a diluted solution of H_2O_2 to become chemically decomposed.

Administration of H_2O_2 to mice via drinking water, with the attendant brief residence time in the oral cavity, precludes significant breakdown by salivary peroxidase. The rodent stomach differs from the human stomach in both its structure and the nature of its contents (DeSesso and Jacobson, 2000; Kararli, 1995). Not only is the volume of the mouse stomach smaller (approx. 2 ml) than that of the human, but also the gastric contents of all rodents retain a rather thick, pasty consistency with a moderate pH of 3.8–5.0 (Smith, 1965, from Calabrese, 1983; DeSesso and Jacobson, 2000). The stomach contents contain a rich population of microorganisms (Smith, 1965, from

Findings				Remarks
No. hamsters w/hyperchromatic cells	*No. hamsters w/chronic inflammation*	*No. hamsters w/dysplasia*	*No. hamsters w/carcinoma*	
0	0	0	0	Data from this study also presented in Table 1.
1	2	1	0	
0	0	0	0	The number of animals used is too small to draw firm conclusions. Methods are not well described.
4	8	4	0	
No tumors in the animals treated with mineral oil alone or with 0.75% hydrogen peroxide-containing dentifrice.				Data from this study also presented in Table 1.
				Results reported in abstract presented at IADR meeting in 1992 and published in *J. Dent. Res.*
No. hamsters w/ carcinomas				
0				Data from this study also presented in Table 1. Hamster buccal cheek pouch assay.
0				

Calabrese, 1983). While such conditions are not conducive to the dilution and chemical decomposition of H_2O_2, rhythmic contractions of the stomach will thoroughly mix the contents and bring the resident bacteria into contact with H_2O_2 so that bacterial peroxidases can destroy it. Transit time for liquids through the stomachs of rodents has been measured to take at least 36 min (Marcus and Lengeman, 1962), which is ample time for H_2O_2 administered in drinking water to be destroyed enzymatically.

Thus, despite major species-specific differences in the structure and physiological environment of the upper gastrointestinal tracts of humans and mice, the environmental conditions of each species are such that H_2O_2 is unlikely to persist long enough to be transmitted to the duodenum.

Epithelial irritation as a cause of duodenal lesions in mice. If H_2O_2 administered in drinking water does not survive long enough to be transmitted to the duodenum of treated mice, then an alternative explanation that is both biologically plausible and consistent with other findings must account for the occurrence of duodenal lesions in the Ito studies. Such an explanation must take into consideration the comparative gastrointestinal anatomy and physiology of mice and humans. In humans, there exist large crescentic folds positioned perpendicular to the long axis of the duodenum (Warwick and Williams, 1973). These folds serve to agitate the contents and to increase the ability of the mucosa to be in contact with watery luminal contents. In mice, there are no crescentic folds (Hummel *et al*., 1968). The presence of such structures would impede the progress of the pasty chyme through the murine intestine and would be the likely site of injury caused by impaction of contents during normal intestinal motility.

Despite these anatomical differences, the duodenal linings of mice and humans are similar in that both consist of a single epithelial layer of cells that is replaced approximately every 3 days (Granger *et al*., 1985; Haschek and Rousseaux, 1991). Such a lining is rather delicate and poorly suited to withstand abrasion or other stresses induced by friction with the luminal contents. When injury to the epithelium occurs resulting in cell loss, whether from abrasion, irritation or chemical attack, the remaining cells respond by increasing their rate of proliferation in order to replace lost tissue (Haschek and Rousseaux, 1991). This phenomenon is known as regenerative (compensatory) hyperplasia. Friction in the duodenum is minimized through the maintenance of a watery ''unstirred'' layer between the chyme and the epithelium. In order to maintain this unstirred fluid layer, the water content of the chyme must be sufficient to prevent absorption of additional fluid from the luminal mucosa.

The Ito studies did not report water consumption data, although one paper did make a subjective comment that water and food intake appeared to be similar across treatment groups (Ito *et al*., 1982). In studies performed by other investigators (Hankin, 1958; Kihlström *et al*., 1986; Weiner *et al*., 1996), however, rats or mice that received 0.1–0.6% H_2O_2 as their only source of fluid exhibited dramatically reduced water intake compared to controls. For example, in an investigation of rats that received 0.45% H_2O_2 in drinking water, fluid intake was reduced by 48% relative to controls (Hankin, 1958); this decreased fluid intake was reflected in decreased

body weights of treated animals compared to controls. Presumably, the reduced fluid intake was due to unpalatability, and there is no reason to suspect that the mice in the Ito studies, which received 0.1–0.4% H_2O_2, reacted differently. Reduced liquid intake would decrease the fluidity of the stomach contents, making the normally pasty chyme coarser than usual. The overly coarse texture of the chyme would irritate and abrade both the gastric and duodenal mucosa, resulting in cell loss and subsequent regenerative hyperplasia (Cotran *et al.*, 1999). The Ito studies, in fact, reported erosions of the gastric mucosa and hyperplasia of the duodenal epithelium (Ito *et al.*, 1981, 1982, 1984, 1986). These duodenal lesions occurred exclusively between the pylorus (where the stomach empties into the duodenum) and the papilla of Vater (Ito *et al.*, 1982, 1984).

The restriction of duodenal hyperplasia to the portion of the duodenum proximal to the papilla of Vater is a key finding. The duodenal papilla is the site where the pancreatic duct enters the duodenum (Warwick and Williams, 1973). The pancreas releases copious secretions that mix with the chyme and make it more fluid as it passes from that point down through the duodenum (Guyton and Hall, 1996). The proximal portion of the duodenum (that preceding the papilla of Vater) does not benefit from the pancreatic fluid secretion. The confinement of the epithelial changes to only those portions of the alimentary tract (glandular stomach and proximal duodenum) that were exposed to the relatively dehydrated, coarse-textured chyme provides a biologically plausible explanation for these findings that does not invoke chemical toxicity.

This explanation is further supported by the fact that the forestomachs of treated mice did not exhibit any lesions (Ito *et al.*, 1982). The forestomach, which is lined by a resilient stratified squamous epithelium capable of withstanding irritation (Green, 1968), has been shown to be sensitive to a variety of chemical carcinogens (Soderman, 1982), but was unaffected by H_2O_2. In contrast, the epithelial surface of the glandular stomach, unlike that of the forestomach, is more delicate. It consists of a single layer of cells that is continuously desquamated into the lumen, with a half-life of 12–24 hr (Snyder *et al.*, 1975), making it more susceptible to cell loss by abrasion. In treated mice, the glandular stomach showed eroded areas consistent with abrasion by the less moist, rough-textured contents (Ito *et al.*, 1981, 1982, 1984, 1986). The fact that the forestomach was not a target organ in these studies adds credence to the concept that a physical mechanism, rather than a chemical one, is responsible for the hyperplastic changes observed in the duodenum of mice administered H_2O_2 in drinking water.

Further experimental support for this explanation can be found in the experiments reported by Li *et al.* (1993). These investigators administered a very high dose of H_2O_2 (70 mg/kg) by gastric intubation to hamsters 5 days per wk for 6 months. This method of exposure allowed high concentrations of H_2O_2 to reach the stomach without dilution. Furthermore, since the material was not in drinking water, palatability was not an issue and water intake was not reduced. The treated animals exhibited no increases in epithelial lesions of the glandular stomach or duodenum compared to controls. These results lend further support to the concept that reduced water intake was the cause of the lesions reported in the Ito studies.

Conclusions

H_2O_2 reacts with DNA in selected *in vitro* systems and, thus, presents a potential carcinogenic concern. The addition of exogenous antioxidant enzymes in such assay systems, however, diminishes or eliminates the DNA damage associated with H_2O_2 treatment (De Flora *et al.*, 1984; ECETOC, 1993; Kensese and Smith, 1989; Winquist *et al.*, 1984; Zhou *et al.*, 1991). Furthermore, the endogenous presence of H_2O_2 in cells establishes that (1) it is a normal constituent of cellular metabolism, and (2) mammalian cells *in vivo* are adequately protected against the potential DNA damage associated with endogenous H_2O_2. The potential of exogeneously applied H_2O_2 to overwhelm the cellular defences that protect against oxidative damage *in vivo* has been assessed in several studies in hamsters, rats and mice (Adam-Rodwell *et al.*, 1994; ECETOC, 1993; Kawachi *et al.*, 1980; Keck *et al.*, 1980; Li *et al.*, 1993; Regnier *et al.*, 1996). These studies consistently showed a lack of genotoxicity associated with oral exposure to exogenous H_2O_2, suggesting that H_2O_2 does not act as a genotoxic carcinogen *in vivo*. Numerous long-term bioassays further support this hypothesis. The majority of studies performed in mice, rats and hamsters have shown no tumor-initiating or -promoting activity under conditions relevant to human H_2O_2 exposure. In disagreement with these findings are a hamster buccal pouch study (Weitzman *et al.*, 1986) and a series of communications from a single laboratory involving oral administration of H_2O_2 in drinking water to mice (Ito *et al.*, 1981, 1982, 1984, 1986). In the study by Weitzman *et al.* (1986), co-administration of DMBA and 30% H_2O_2 was reported to cause a slight increase in the incidence of carcinomas in hamsters. However, this dose of H_2O_2 was associated with severe tissue necrosis and regenerative hyperplasia, which probably contributed to the increased incidence of carcinomas observed. Significantly, 3% H_2O_2, which is a dose more relevant to that associated with human exposures, had no effect on the incidence of carcinomas.

In the studies by Ito *et al.* (1981, 1982, 1984, 1986), mice were administered 0.4% H_2O_2 in drinking water for extended periods of time. The authors reported a lack of carcinogenic effects in the mouth, esophagus

and stomach, but a significant increase in the incidence of duodenal lesions as compared to control animals was observed. The duodenal effects observed in these studies, however, are not relevant to human health risk assessment. When one considers the chemical nature of aqueous H_2O_2 solutions, the anatomy and physiology of the mouse gastrointestinal tract, and how chyme contents interact with gastrointestinal surfaces, it becomes evident that the effects observed in the Ito studies are not due to any direct chemical interaction of H_2O_2 with the target tissues. Rather, decreased water intake and abrasion of the duodenal surfaces by the rough gastrointestinal contents probably caused the duodenal lesions observed in these experiments.

In 1988, the results of the Ito studies (1981, 1982, 1984, 1986) were thoroughly evaluated by the Cancer Assessment Committee (CAC) of the US Food and Drug Administration (FDA). The conclusion of that committee was that the studies did not provide evidence that H_2O_2 is a carcinogen (FDA, 1988). In summary, on the basis of the results of *in vivo* genotoxicity assays and long-term bioassays, it can be concluded that tumor production will not occur in humans as a result of exposure to H_2O_2, particularly from oral care products for daily use. No hazard is likely to be associated with the long-term use of H_2O_2 at concentrations found in oral care products.

Acknowledgements—The authors would like to thank Dr Catherine Jacobson for her editorial and scientific suggestions, and Ms Mary Helen Malow for her help in gathering the references used in this paper. This work was funded by the Nonprescription Drug Manufacturers Association (now the Consumer Healthcare Products Association) and by the Mitretek-Sponsored Research Program.

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