

In the oral cavity, toxic effects of hydrogen peroxide vary from pulpal alterations (Ref. 146) to gingival lesions (Refs. 147 and 148) and oral irritation in rats (Ref. 149) under certain conditions. Adding a 1- to 1.5-percent solution to drinking water resulted in apparent enamel demineralization in rats over an 8-week period (Ref. 149). This effect on enamel was possibly due to the hydrogen-ion (pH) concentration of the solution used rather than true carious lesions. In addition, no enamel solubility was found from an in-vitro experiment using a 1.5-percent aqueous solution on human enamel (Ref. 141).

The Subcommittee's discussion of mutagenicity is not intended to be a complete review of the literature concerning the mutagenic nature of hydrogen peroxide, but is intended to point out the apparent mutagenic safety concerns associated with hydrogen peroxide. Any mutagenic role of hydrogen peroxide will be further discussed with sodium bicarbonate and hydrogen peroxide in combination.

Numerous reports indicate a mutagenic role for hydrogen peroxide (Refs. 150, 151, and 152). Reviews on the genotoxicity of hydrogen peroxide can be found in reports by the European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) (Refs. 153 and 154) and in an overview of hydrogen peroxide genotoxicity presented at the Subcommittee meeting on December 4, 1995 (Ref. 155).

Hydrogen peroxide can produce hydroxyl radicals which are reactive but short-lived (Refs. 155 and 156). In vitro superoxide and hydroxyl radicals caused chromatic exchanges in mammalian cells and preneoplastic changes (Refs. 153 and 154). Although hydroxyl radicals and singlet oxygen can damage DNA in vitro, the genotoxic potential of hydrogen peroxide depends on the proximity of unprotected DNA. In vitro genotoxicity tests enhance the opportunity for DNA damage and are conducted in cells with defective DNA repair systems. Genotoxic effects are not seen with hydrogen peroxide in the presence of protective enzyme systems that are normally present intracellularly, in the presence of iron chelating agents, and in the presence of hydroxyl radical scavengers.

The mechanism of mutagenesis through superoxide radical production was also suggested by MacRae et al. (Ref. 157). In contrast to most of the references available, Taylor et al. (Ref. 158) suggested that hydrogen peroxide itself and not hydroxyl radicals was responsible for DNA strand breaks in epithelial and fibroblast cultures. Most carefully controlled in vitro studies

have shown that the participation of transition metal ions, such as iron or copper, is required for DNA damage to occur (Ref. 159).

In some bacterial mutagenesis studies, hydrogen peroxide was found to be a weak mutagenic agent (Refs. 160 through 167). Many strains are not sensitive to hydrogen peroxide and hydroxyl radicals and mutations are only seen in certain bacterial strains that are sensitive to oxidative damage (Ref. 168). The addition of an external enzymatic metabolic source resulted in abolition of the weak genotoxic effects seen in sensitive bacterial strains. These enzyme sources are normally present throughout the body, and the presence of detoxifying enzymes may explain the lack of genotoxicity seen in whole animals that have been administered hydrogen peroxide. In the oral cavity, salivary peroxidase serves as the initial line of defense against hydrogen peroxide (Ref. 169).

Additional studies were conducted to evaluate systemic effects of long-term administration of hydrogen peroxide, and the endpoint measured was sister chromatic exchange (SCE), a very sensitive assay for genotoxic damage. Hydrogen peroxide was administered to hamsters for 6 months at 70 mg/kg (Ref. 170) and to mice for 3 months (Ref. 171). In both studies, there was no increase in SCE formation following long term ingestion of hydrogen peroxide. A single administration of a carbamide peroxide-containing dentifrice to rats at 1,000 mg/kg daily for 5 days did not increase the incidence of SCE (Ref. 172). Woolverton also examined two commercial carbamide peroxide-containing dental products for micronucleus formation. After two exposures, these products did not increase the incidence of micronucleated erythrocytes (Ref. 173).

Similar results were seen in a micronucleus assay for chromosomal damage in mice that were given hydrogen peroxide intraperitoneally or in drinking water at 0.6 percent for 2 weeks (Refs. 174 and 175). The SCE and micronucleus studies consistently demonstrated a lack of genotoxicity following hydrogen peroxide ingestion or intraperitoneal injection.

Hydrogen peroxide was reported to promote carcinomas in rodents following intraperitoneal injections (Ref. 176) and through its addition to drinking water (Refs. 177, 178, and 179). Duodenal hyperplasia has been found in the rat model following the addition of 1.5 to 3 percent hydrogen peroxide to drinking water (Ref. 176). Ito *et al.* (Ref. 140) observed similar toxicity with higher doses of hydrogen peroxide. In

mice with reduced catalase activity, hyperplastic and neoplastic duodenal nodules were found (Ref. 179). Ito's report of the carcinogenicity of hydrogen peroxide has been evaluated by FDA toxicologists who concluded that the results of the study did not provide sufficient evidence to designate hydrogen peroxide as a carcinogen (53 FR 53176, December 30, 1988). Similar conclusions were drawn by a panel of toxicologists who reviewed the potential carcinogenicity of hydrogen peroxide for the International Agency for Research on Cancer (IARC) (Refs. 180 and 181).

A long-term study was conducted in F344 rats in which hydrogen peroxide was administered in drinking water for 18 months at concentrations of up to 0.6 percent, the maximal tolerated dose in F344 rats (Ref. 182). All surviving animals were sacrificed at 24 months of age. Hydrogen peroxide ingestion in the 0.6-percent hydrogen peroxide group was 677 mg/kg/day for females and 433 mg/kg/day for males, with a total ingestion of 72.7 g hydrogen peroxide in females and 81.4 g hydrogen peroxide in males during the course of the study. There was no evidence of carcinogenicity at any organ site in this study following hydrogen peroxide ingestion.

In Syrian hamsters, applications of 3 percent and 30 percent hydrogen peroxide produced pathogenic changes associated with preneoplastic lesions. Preneoplastic lesions are reversible following cessation of exposure (Ref. 178). When combined with DMBA, a known carcinogen, hydrogen peroxide, at a concentration of 30 percent, appeared to augment the carcinogenic effects associated with DMBA (Ref. 183). No carcinogenicity was seen in this study resulting from hydrogen peroxide alone at concentrations of 3 or 30 percent.

Marshall et al. (Ref. 184) conducted two carcinogenesis studies of 16 weeks and 20 weeks in hamsters to compare the effects of similar dentifrices with and without the combination of hydrogen peroxide and sodium bicarbonate in the presence of DMBA. The authors reported that the results demonstrated that an oral product containing hydrogen peroxide and sodium bicarbonate was not carcinogenic and that the combination did not enhance the tumorigenicity of DMBA. In summary, these robust animal studies (Refs. 183 and 184) indicate that hydrogen peroxide does not increase the incidence of oral cavity tumors in combination with a known carcinogen.