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EVALUATION OF THE HEALTH ASPECTS OF HYDROGEN
PEROXIDE AS A FOOD INGREDIENT

1979

Prepared for

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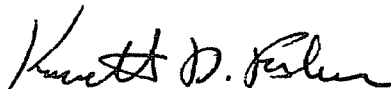
NOTICE

This report is one of a series concerning the health aspects of using the Generally Recognized as Safe (GRAS) or prior-sanctioned food substances as food ingredients, being made by the Federation of American Societies for Experimental Biology (FASEB) under contract no. 223-75-2004 with the Food and Drug Administration (FDA), U.S. Department of Health, Education, and Welfare. The Federation recognizes that the safety of GRAS substances is of national significance, and that its resources are particularly suited to marshalling the opinions of knowledgeable scientists to assist in these evaluations. The Life Sciences Research Office (LSRO), established by FASEB in 1962 to make scientific assessments in the biomedical sciences, is conducting these studies.

Qualified scientists were selected as consultants to review and evaluate the available information on each of the GRAS substances. These scientists, designated the Select Committee on GRAS Substances, were chosen for their experience and judgment with due consideration for balance and breadth in the appropriate professional disciplines. The Select Committee's evaluations are being made independently of FDA or any other group, governmental or nongovernmental. The Select Committee accepts responsibility for the content of each report. Members of the Select Committee who have contributed to this report are named in Section VII.

Tentative reports are made available to the public for review in the Office of the Hearing Clerk, Food and Drug Administration, after announcement in the Federal Register, and opportunity is provided for any interested person to appear before the Select Committee at a public hearing to make oral presentation of data, information, and views on the substances covered by the report. The data, information, and views presented at the hearing are considered by the Select Committee in reaching its final conclusions. Reports are approved by the Select Committee and the Director of LSRO, and subsequently reviewed and approved by the LSRO Advisory Committee (which consists of representatives of each constituent society of FASEB) under authority delegated by the Executive Committee of the Federation Board. Upon completion of these review procedures the reports are approved and transmitted to FDA by the Executive Director of FASEB.

While this is a report of the Federation of American Societies for Experimental Biology, it does not necessarily reflect the opinion of all of the individual members of its constituent societies.


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I. INTRODUCTION

This report concerns the health aspects of using hydrogen peroxide as a food ingredient. It has been based partly on the information contained in a scientific literature review (monograph) furnished by FDA (1), which summarizes the world's scientific literature from 1920 through 1973.* To ensure completeness and currency as of the date of this report this information has been supplemented by searches of over 30 scientific and statistical reference sources and compendia that are generally available; use of new, relevant books and reviews and the literature citations contained in them; consideration of current literature citations obtained through computer retrieval systems of the National Library of Medicine; searches for relevant data in the files of FDA; and by the combined knowledge and experience of members of the Select Committee and the LSRO staff. In addition, an announcement was made in the Federal Register of April 6, 1979 (44 FR 20797-20800) that opportunity would be provided for any interested person to appear before the Select Committee at a public hearing to make oral presentation of data, information and views on the health aspects of using hydrogen peroxide as a food ingredient. The Select Committee received no requests for such a hearing on hydrogen peroxide.

As indicated in the Food, Drug, and Cosmetic Act [21 USC 321(s)], GRAS substances are exempt from the premarketing clearance that is required for food additives. It is stated in the Act and in the Code of Federal Regulations (2) [21 CFR 170.3 and 170.30] that GRAS means general recognition of safety by experts qualified by scientific training and experience to evaluate the safety of substances on the basis of scientific data derived from published literature. These sections of the Code also indicate that expert judgment is to be based on the evaluation of results of credible toxicological testing or, for those substances used in food prior to January 1, 1958, on a reasoned judgment founded in experience with common food use, and is to take into account reasonably anticipated patterns of consumption, cumulative effects in the diet, and safety factors appropriate for the utilization of animal experimentation data. FDA (2) recognizes further that it is impossible to provide assurance that any substance is absolutely safe for human consumption.

*The document (PB-241 957/OWF) is available from the National Technical Information Service, U.S. Department of Commerce, P.O. Box 1553, Springfield, Virginia 22161.

The Select Committee on GRAS Substances of LSRO is making its evaluation of these substances in full recognition of the foregoing provisions. In reaching its conclusions on safety, the Committee, in accordance with FDA's guidelines, is relying primarily on the absence of substantive evidence of, or reasonable grounds to suspect, a significant risk to the public health. While the Committee realizes that a conclusion based on such reasoned judgment is expected even in instances where the available information is qualitatively or quantitatively limited, it recognizes that there can be instances where, in the judgment of the Committee, there are insufficient data upon which to base a conclusion. The Committee is aware that its conclusions will need to be reviewed as new or better information becomes available.

In this context, the LSRO Select Committee on GRAS Substances has reviewed the available information on hydrogen peroxide and submits its interpretation and assessment in this report, which is intended for the use of FDA in determining the future status of this substance under the Federal Food, Drug, and Cosmetic Act.

II. BACKGROUND INFORMATION

Hydrogen peroxide (H_2O_2) is a strong oxidizing agent that is used extensively in industry and medicine. It is usually available as aqueous solutions in concentrations of 3, 30, or 90 percent by weight. The 3 percent solution is used as a topical antiseptic and cleansing agent, and as a constituent in mouthwashes, dentifrices, and sanitary lotions; the 30 percent as an effective bleaching agent and for other industrial uses; and the 90 percent as a vigorous oxidizer of rocket fuels. The anhydrous form is a colorless, bitter-tasting liquid with an ozone-like odor. In the absence of stabilizing agents (e.g., phosphates, tin), hydrogen peroxide solutions are unstable and decompose upon standing, agitation, exposure to light, or heating. Hydrogen peroxide reacts vigorously with many oxidizing as well as reducing agents. Concentrated solutions are highly caustic to the skin (3).

The Food Chemicals Codex (4) specifies that the composition of solutions of hydrogen peroxide used in foods must be within the range stated on the label or contain not less than the stated amount. The limits of impurity in parts per million are as follows: acidity (as H_2SO_4), 300; arsenic (as As), 3; heavy metals (as Pb), 10; iron, 0.5; phosphate, 50; tin, 10; and residue on evaporation, 60. It should be stored in a cool place in containers with vented stoppers.

The Code of Federal Regulations (2) lists hydrogen peroxide as a multiple purpose GRAS food substance specifically listed as GRAS when used as a bleaching agent in accordance with good manufacturing practice [21 CFR 182.1366]. It is also considered GRAS as a substance migrating to food from cotton and cotton fabrics used in dry food packaging [21 CFR 182.70]. It has been accorded unpublished GRAS status for bleaching of lecithin, provided that no residual hydrogen peroxide remains after treatment (5). Additional authorized uses are summarized in Table I.

In addition to its effectiveness as a bleach, hydrogen peroxide has proved to be a useful antimicrobial agent. This latter property has been utilized in some countries as a preservative of milk (8) and whey (9). Thus, treatment of milk with hydrogen peroxide is officially recognized in Italy as a substitute for pasteurization (10). The Joint FAO/WHO Expert Committee on Food Additives has approved the use of hydrogen peroxide in milk as an emergency measure when other methods of microbiological control, such as pasteurization, are not available (11). In the United States hydrogen peroxide cannot be used as a substitute for pasteurization (10), but it is used in the manufacture of certain cheeses (Table I) and in whey processing (12).

TABLE I

Authorized Uses of Hydrogen Peroxide

Use	Limitations	Authorization	Comment
<u>GRAS</u>			
Bleaching agent	Good manufacturing practice	21 CFR 182.1366	
In dry food packaging	Cotton and cotton fabrics	21 CFR 182.70	
To produce bleached lecithin	No residual unreacted hydrogen peroxide		Unpublished GRAS (5)
<u>Treatment of wine:</u>			
To reduce aldehydes in distilling materials	Not to exceed 200 ppm	27 CFR 240.1051	Deemed GRAS for these uses by Department of the Treasury (6)
To facilitate secondary fermentation in production of sparkling wines	Not to exceed 3 ppm, with no residual amount		
<u>OTHER USES</u>			
<u>A. Standards of identity:</u>			
<u>Cheese production</u>			
Cheddar	Not to exceed 0.05 percent by weight and must not contain preservative	21 CFR 133.113	Excess H ₂ O ₂ must be destroyed by catalase
Colby		21 CFR 133.118	
Washed and soaked curd		21 CFR 133.136	
Swiss and Emmentaler		21 CFR 133.195	
Dried egg treatment			
Whole eggs	H ₂ O ₂ must comply with USP specifications	21 CFR 160.105	Treated with glucose oxidase-catalase preparation to remove glucose
Egg whites		21 CFR 160.145	
Egg yolks		21 CFR 160.185	
Bleaching of tripe	H ₂ O ₂ must be removed after use, by rinsing with water	9 CFR 318.7	(7)
<u>B. Food additives, direct:</u>			
Modification of food starch	0.45 percent	21 CFR 172.892	Bleaching agent
Production of hydroxylated lecithin	"...separated fatty acid fractions...has an acetyl value of 30 to 38."	21 CFR 172.814	
<u>C. Food additives, indirect:</u>			
Component of adhesives		21 CFR 175.105	

Because of these uses, the FDA requested that the Select Committee evaluate the health aspects of employing hydrogen peroxide as an antimicrobial agent in cheese and whey processing, as well as using it as a bleaching agent (12).

III. CONSUMER EXPOSURE DATA

On the basis of surveys conducted by the National Research Council (NRC), the amount of hydrogen peroxide used in foods in 1975 was reported to be 630,000 kg (98). This was approximately sixty percent more than the amount used in 1970 (13), based on reports from those respondents providing information for both years. The quantity reported in 1975 is equivalent to a per capita daily "intake" of 8 mg. However, this is an illusory value, for only traces, if any, of the added hydrogen peroxide would survive food processing. The ingestion of hydrogen peroxide from foods is almost certainly miniscule. Man's exposure to hydrogen peroxide from other sources is also very slight under normal conditions. Small amounts may be absorbed from common pharmaceutical preparations (dentifrices, mouthwashes, deodorants) and from contaminated atmospheres. From 40 to 180 parts per billion of hydrogen peroxide have been reported in the atmosphere during smog formation, corresponding to a potential daily respiratory intake of 0.5 to 2.5 mg (14).

IV. BIOLOGICAL STUDIES

Absorption

Hydrogen peroxide in the absence of a stabilizing agent gradually decomposes to oxygen and water. Decomposition is rapid in the presence of catalase. Ingested hydrogen peroxide presumably undergoes such degradation in the gastrointestinal tract, leaving little of the intact compound available for absorption. Perfusion of dog intestine with dilute hydrogen peroxide raised significantly the oxygen saturation of blood (15). No attempt was made to determine if the peroxide breakdown occurred before or after absorption.

The rapid release of oxygen into the tissues and vascular network can readily be observed after administering hydrogen peroxide by various routes. The characteristic whitening of the skin after topical application has been attributed to an avascularity produced by oxygen bubbles acting as microemboli in the tissues and capillaries. Hauschild *et al.* (16) have demonstrated this reaction in a variety of tissues including human skin, cat and dog tongues, rat paws, and animal hearts (species not stated). When 3 or 30 percent hydrogen peroxide was applied sublingually in rabbits, cats, and dogs, gas bubbles rapidly appeared in the jugular vein (17). With the higher concentration, these bubbles were observed within a few seconds and in sufficient quantity to cause pulmonary gas embolisms. Using ^{18}O -labeled-hydrogen peroxide, Ludewig (18) confirmed its rapid absorption after sublingual administration and the passage of liberated oxygen to the lungs. Approximately 7 percent of the oxygen theoretically available from the administered hydrogen peroxide was detected in the expired air within 18 minutes and 30 percent within 34 minutes. Traces of the labeled oxygen were also detectable in the arterial blood.

Metabolism

Hydrogen peroxide is a normal product of aerobic metabolism and may result from a number of oxidase-catalyzed reactions (e.g., d-amino acid oxidase, urate oxidase, glycolate oxidase) or by the breakdown of superoxide by superoxide dismutase. The hydrogen peroxide thus formed is rapidly decomposed by tissue catalase or peroxidase (19). Enzymes metabolizing hydrogen peroxide are localized largely in specialized vesicles or organelles, known as peroxisomes. The peroxisomes comprise about 2 percent of the liver volume, and are especially rich in catalase (20), which efficiently and rapidly decomposes endogenous hydrogen peroxide. Sies calculated that 1 g of rat liver produces approximately 50nM ($1.7 \mu\text{g}$) of hydrogen peroxide per minute. Boveris *et al.* (21) arrived at a higher figure for the normal production of hydrogen peroxide under physiological conditions: 90nM ($3.1 \mu\text{g}$) per min per g

wet weight of liver. Extrapolating to man, this would be roughly equivalent to the production of 150 to 270 mg hydrogen peroxide per hour by the human liver under normal conditions and even larger amounts under the stimulus of appropriate substrates. Breakdown by catalase is so efficient, however, that the steady-state concentration in the liver is 10^{-9} molar (30 ng per kg). Even with maximal stimulation of hydrogen peroxide production, the concentration increases only to 10^{-7} molar (3 μ g per kg) (20).

Despite its rapid destruction, hydrogen peroxide may have an important role in certain localized metabolic reactions. For example, it has been shown to exert an insulin-like effect in fat, and perhaps in other tissues (22). When rat adipocytes were incubated with hydrogen peroxide in the presence of glucose, glycogen synthase I was activated, which stimulated the incorporation of glucose into glycogen. It has been suggested also, that hydrogen peroxide may be involved in the body's defense against bacterial infection (23). When microorganisms are phagocytized, hydrogen peroxide or activated forms of oxygen are generated, which may account at least in part for the bactericidal effectiveness of the phagocytic cells.

Acute toxicity

No reports were available to the Select Committee on the acute toxicity in animals of orally administered hydrogen peroxide. Several cases of accidental poisoning in man have been described (24,25) including the death by respiratory failure of a 1-year-old infant within 1 hour after ingesting an unknown quantity of concentrated hydrogen peroxide solution (24). Five non-fatal poisonings were reported of persons who had consumed 25 to 100 ml of 30 percent hydrogen peroxide (25). The victims experienced sharp pains in the abdomen and behind the sternum, foaming from the mouth, vomiting, fleeting loss of consciousness, transitory motor and sensory impairment, rise in temperature, microhemorrhaging in the skin and conjunctiva, and a moderate leukocytosis. One patient, who had swallowed 100 ml of the hydrogen peroxide solution, displayed for several days marked visual and neurological symptoms which the authors attributed to oxygen microembolisms.

Aoki and Tani (26) referred without elaboration to a "mass poisoning in Japan...caused by hydrogen peroxide treated noodles." The authors did not specifically incriminate the residual peroxide, but pointed out that approximately 20 percent of commercial products tested contained hydrogen peroxide in excess of the Japanese permissible limits of 100 ppm.

Acute toxicity data on animals are available only for percutaneous and intravenous administration (Table II). Death generally has been attributed to embolic phenomena resulting from liberated oxygen. As is evident from Table II, there is a marked

TABLE II
LD₅₀ of Hydrogen Peroxide

Animal	Route	Concentration (percent)	mg/kg	Reference number
Rabbit	Percutaneous	90	630	27
Pig	"	90	2500	27
Cat	"	90	>4000	27
Rat (white)	"	90	4800	27
Rat (black)	"	90	>7500	27
Rat (white)	"	N.S.*	700	28
Rabbit	IV	90	19	27
	"	58.6	16	27
	"	36.0	10	27
	"	14.4	5	27
	"	3.6	3	27
Rat	IV	N.S.*	21	28

*N.S. = not stated.

species difference in the susceptibility to percutaneous application. There is also an inverse relationship between the concentration of hydrogen peroxide injected intravenously and its toxicity. Hrubetz et al. (27) explained this paradox by pointing out that intravascular oxygen bubbles appear at the site of injection and mechanically hinder further access of hydrogen peroxide to the general circulation. The more concentrated the solution, the more marked is this effect and the less peroxide actually reaches the systemic circulation.

Intraperitoneal injection of hydrogen peroxide (from 0.5 ml of 5 percent to 1.0 ml of 10 percent) into adult mice had a radiation-like effect (29). It produced pyknotic nuclei in the intestine and thymus within 2 hours which, with the strongest dose, persisted for up to 24 hours. Toxic signs were produced in horses, rabbits, and dogs by intravenous injection of relatively dilute hydrogen peroxide (1 to 2 ml of 0.4 to 3 percent solutions per kg) (30). Shortness of breath was evident in all species after injection. The rabbits, in addition, responded with a characteristic backward tossing of the head while the horses and dogs displayed increased peristalsis and repeated defecation. Rabbits and cats dying after intravenously administered hydrogen peroxide or oxygen had pale, emphysematous lungs with considerable amounts of gas in the great veins and in the right side of the heart (31).

Aerosols of hydrogen peroxide containing 3 to 5 mg per liter produced pulmonary irritation and congestion in mice within 5 minutes (32). Exposure to higher concentrations (10 to 40 mg per l) for 15 minutes caused death of a high percentage of mice, usually within an hour, apparently from pulmonary edema.

Short-term studies

Three-week-old dd mice drinking 0.15 percent hydrogen peroxide ad libitum (about 150 mg per kg per day) grew normally and developed no visible abnormalities during a 35-week test period (26). Upon necropsy, degenerative changes were observed in the liver and kidney. The stomach wall was slightly necrotic, inflamed, and irregular and the lymphatic tissue of the small intestinal wall was hypertrophic. Solutions in excess of 1 percent (more than 1 g per kg per day) caused pronounced weight loss and death of the mice within 2 weeks.

Romanowski et al. (33) replaced the drinking water of rats with solutions of 0.25 to 10 percent hydrogen peroxide. All animals receiving 2.5 percent solutions or higher died within 43 days. Nine of ten rats fed the 0.25 percent solution and eight of ten fed the 0.50 percent survived the test period of 146 days, although the weight gain of each group was less than that of the controls. The daily hydrogen peroxide intakes for these two groups were approximately 250 and 500 mg per kg, respectively.

A group of weanling male Osborne-Mendel rats given a solution of 0.45 percent hydrogen peroxide to drink ad libitum for 3 weeks was compared with a similar group receiving tap water (34). Both fluid intake and weight gain of the peroxide group were significantly less than those of the controls. However, when fluid intake of the control rats was limited to that of the experimental animals, there were no significant differences in body or organ weights. The daily consumption of hydrogen peroxide during the test period was approximately 500 mg per kg body weight. Three weanling female rats were given 0.45 percent hydrogen peroxide for 5 months, then switched to tap water and mated with normal males. Normal litters were produced. Male rats from these litters were then given 0.45 percent hydrogen peroxide for 9 months. The only noticeable difference between these rats and male littermates receiving tap water was a decreased weight gain in the peroxide group.

In a similar experiment, young, male Holtzman rats were provided with solutions of 0, 0.5, 1.0, and 1.5 percent hydrogen peroxide (approximately 0, 500, 1000 and 1500 mg per kg per day) as their source of drinking fluid for 8 weeks (35). Growth was significantly retarded in all groups receiving hydrogen peroxide and the degree of retardation was proportional to the peroxide concentration. Seven of 24 rats receiving 1.5 percent hydrogen peroxide died during the course of the experiment. All surviving animals in this group and 15 of 24 rats receiving 1 percent had extensive carious lesions and pathological changes in the periodontium. Seven of 24 rats on the 0.5 percent solution also had mild caries, but no periodontal changes.

In none of the experiments described above was any mention made of the use of stabilizers or of other measures to prevent the decomposition of hydrogen peroxide in drinking water.

Kawasaki et al. (36) reported no adverse effects in male Wistar rats receiving up to 30 mg hydrogen peroxide per kg per day by gastric intubation. Rats given twice this dose showed significantly decreased growth rates after 20 days and decreased hematocrit, plasma protein, and plasma catalase activities after 100 days. When the same amount of hydrogen peroxide was administered in the feed, no harmful effects could be detected.

Young mice were injected subcutaneously twice daily for 2 weeks with 0.1 ml of 1.5 percent hydrogen peroxide, roughly equivalent to 100 mg per kg body weight per injection (31). Several animals showed brief embolic signs immediately after the injection and 5 of 30 mice died with embolic signs during the experimental period. A few mice exhibited brief generalized convulsions. Local subcutaneous emphysema was invariably present after injection and small ulcers eventually developed at the injection sites in about 25 percent of the animals.

Rats have proved to be highly resistant to hydrogen peroxide vapor (37). Only 1 of 10 rats died after exposure to 93 mg per m³ (67 ppm) for 6 hours daily, 5 days per week for 6 weeks, whereas more than half of the mice under approximately the same conditions died within 2 weeks. However, this greater sensitivity of the mouse to inhaled hydrogen peroxide may be more apparent than real. Because of its small size and rapid respiration, the mouse would inhale more hydrogen peroxide per unit time and weight than the rat. Dogs and rabbits exposed to 7 and 22 ppm hydrogen peroxide on a similar schedule for 6 and 3 months, respectively, showed only minor respiratory damage.

Long-term studies

Molnár (38) injected female mice subcutaneously with 0.5 ml of 0.01 percent hydrogen peroxide (about 2.5 mg per kg body weight) two or three times weekly from the age of 6 weeks to 14 months for a total of 95 injections. The average life-span decreased from 595 days in mice receiving similar injections of physiological saline to 446 days among those receiving the hydrogen peroxide. A weight loss in the peroxide-treated group was noted after about 6 months.

Special studies

Reproduction. As noted above, female rats receiving 0.45 percent hydrogen peroxide (about 500 mg per kg per day) as the sole drinking fluid for 5 weeks produced normal litters when mated with normal males (37). Because hydrogen peroxide is reportedly toxic to spermatozoa in vitro (39), the effect of its oral administration on fertility is of interest. Three-month-old male albino mice were given 0.33 and 1.0 percent hydrogen peroxide (about 330 and 1000 mg per kg per day) as their sole drinking fluid for 7 to 28 days before placing them with normal females. All females became pregnant within a few days and in each case, healthy offspring were born in litters of normal size.

Carcinogenicity. The recognition of the radiomimetic (40) and mutagenic (41,42) properties of hydrogen peroxide, as well as its detection in tumors (43), prompted the direct testing of this compound for carcinogenic or cocarcinogenic activity. Schmidt (44) gave groups of newborn mice consisting of strains AB, C57BL, C57BL/6, A/JAX and X a single subcutaneous injection of 0.1 ml of 0.6 percent hydrogen peroxide (about 300 mg per kg) and another group (strain AB) three such injections. The intervals between injections were not stated. Only about 10 percent of the mice survived for 6 months. Six of 30 survivors (20 percent) in the single injection groups developed tumors (3 leukemias, 3 breast cancers, 1 lymphosarcoma of the thymus). In the triply injected group, 14 of 42 (33 percent) were affected (6 breast

cancers, 3 lung adenomas, 4 leukemias, 1 ovarian tumor, 1 hemangiosarcoma of the liver). No tumors arose at the sites of injection. Although no statistical analysis was reported, Schmidt claimed the incidence of tumors among the injected mice was significantly higher than that normally observed in his colony (2 to 5 percent).

Shamberger (45) reported no cocarcinogenic action of hydrogen peroxide in mouse skin initiated with 7,12 dimethylbenz(α)-anthracene (DMBA). He applied 0.25 ml of 3 percent hydrogen peroxide in acetone daily for 40 weeks to the skin of 30 ICR Swiss female mice previously treated with DMBA. No tumors were present after 40 weeks. These findings were confirmed by Bock *et al.* (46) who painted the dorsal skins of 33 female ICR Swiss mice with DMBA followed by treatment five times weekly for 56 weeks with 3 percent hydrogen peroxide. No skin tumors were produced. Nagata and colleagues (47) found that injections of hydrogen peroxide in female ddN mice significantly reduced the incidence and delayed the appearance of benzo[α]pyrene-induced tumors, suggesting a possible antitumor effect.

Mutagenicity. Since hydrogen peroxide is a radiolytic product of ionizing radiation, its possible role in radiation-induced mutagenesis has been studied extensively. Its effects on isolated DNA, microbial cells, and tumor cells have been explored in numerous reports. Hydrogen peroxide has been shown to be mutagenic to various microorganisms (41,42,48,49) and to mouse ascites tumors (50). DNA degradation, cell damage, and increase in mutants were reported under the specific conditions of the experiments, usually involving the addition of hydrogen peroxide to the media. Although there is a similarity of effect between radiation and hydrogen peroxide, the concentration of hydrogen peroxide produced by radiation must be increased tenfold to achieve the same effects resulting from exogenously-added hydrogen peroxide. Treatment of DNA solutions or bacterial cells with hydrogen peroxide or with X-rays has caused DNA strand breakage (41). Both treatments generated hydroxyl radicals, which are thought to be the active agents.

Inactivation and mutation leading to respiratory deficiency were induced in yeast cells by hydrogen peroxide. This effect was probably due to selection of preexisting mutants in log phase populations, although a small increase in forward mutations of nuclear genes also was reported (48).

Schöneich (50) studied the chromatid aberrations in several lines of mouse ascites tumors after hydrogen peroxide injection. The S2 sarcoma, Erlich ascites carcinoma, and sarcoma 180 were grown in the inbred mouse strain ABJena Gat. One ml of 0.1 M hydrogen peroxide (about 170 mg per kg) was injected intraperitoneally 48 hours after implantation of the tumor. Less than 1 percent of the cells of untreated tumors contained spontaneous chromatid aberrations whereas 4 to 44.7 percent of the examined tumor

cells in the hydrogen peroxide treated animals showed chromosomal changes. The frequency of aberrations varied considerably from animal to animal, but there was a consistent increase with increasing hydrogen peroxide concentrations.

Destruction of essential nutrients. In evaluating the health aspects of hydrogen peroxide, one must consider not only its intrinsic toxicity, but also any secondary deleterious effects which may result from its addition to foods; e.g., the possible destruction of essential nutrients or the production of toxic substances.

As indicated earlier, hydrogen peroxide is authorized as a bactericidal agent in the manufacture of certain cheeses [21 CFR 133.113 et seq.]. Clarified raw milk is treated with hydrogen peroxide equivalent to 0.02 to 0.05 percent by weight of the milk, heated to 52°C for 25 seconds and cooled to setting temperature (30° to 34.5°C). Residual hydrogen peroxide is destroyed by the addition of a small amount of catalase. Hydrogen peroxide has a selective action on bacteria in milk, destroying most of the facultative anaerobic types that are associated with common defects in cheese, while the desirable aerobic acid-forming species are more resistant to the peroxide treatment (51). This treatment produces a higher quality cheese than would result with pasteurized milk. Inasmuch as this process is not equivalent to pasteurization, cheese thus prepared must be held for 60 days before sale, just as if raw milk had been used.

Jasewicz and Porges (52) found that a concentration of 0.02 percent hydrogen peroxide exerted a preservative effect on freshly obtained cheese whey for as long as 10 days. With grossly contaminated whey (2.8×10^7 microorganisms per ml) this concentration of hydrogen peroxide effected 97 percent destruction within 1 hour and 99 percent after 24 hours.

The nutritional quality and wholesomeness of peroxide-treated milk have been studied by various investigators. In a commercial fractionation of whey, a concentration of 0.02 to 0.04 percent hydrogen peroxide is employed to control bacterial growth. Demineralization is achieved by electrodialysis and normally requires 8 to 12 hours at 30° to 38°C. An additional 22 hours are required for lactose crystallization at 50° to 70°C. Any residual hydrogen peroxide is removed by catalase treatment upon completion of the fractionation and prior to spray drying (53,54).

Tepley et al. (55) added hydrogen peroxide to milk at 49°C in amounts sufficient to produce levels of 0.1, 0.2, or 0.5 percent. The milk was held at this temperature for 10 minutes and then cooled to 32°C. Any remaining peroxide was destroyed by the addition of catalase. The amount of added hydrogen peroxide was 2 to 25 times that normally employed in the manufacture of cheese or the processing of whey. There was no significant effect in milk

or whey on levels of thiamin, riboflavin, niacin, pyridoxine, pantothenic acid, folic acid, vitamin B₁₂, vitamin A, or β -carotene. Ascorbic acid was not determined. Treatment of milk with the highest concentration of hydrogen peroxide (0.5 percent) employed lowered the cystine and methionine content of the corresponding cheese by 10 to 25 percent although this effect was not noted in the milk and whey samples. The amounts of tryptophan and lysine were not affected by any of the treatments. Little, if any, reduction in protein efficiency ratios was noted when treated milk, whey, or cheese was fed for 6 weeks to weanling Sprague-Dawley rats as the sole source of protein in an otherwise complete ration. All animals remained in good health and no abnormalities were detected at necropsy. These findings were essentially confirmed by Gregory *et al.* (56) who treated milk for a much longer period (8 hours) but at a lower temperature (24°C) and hydrogen peroxide concentration (0.05 percent). No vitamin destruction was detected but the nutritive value of the milk proteins was slightly reduced, probably resulting from a slight reduction in the methionine content. Lück and Schillinger (57,58) treated milk with 0.3 percent hydrogen peroxide for 24 hours at 30°C or for 30 minutes at 51°C with no effect on the fat-soluble vitamins A, D₃, and β -carotene or on the water-soluble vitamins thiamin, riboflavin, and pyridoxine. Ascorbic acid, however, was almost completely destroyed by this treatment (58).

Methionine appears to be the only essential amino acid sensitive to hydrogen peroxide treatment. As suggested above, concentrations considerably greater than those employed in cheese making or whey processing are necessary for significant destruction (55,56). Thus, no reduction of methionine content in fish protein concentrates was noted upon treatment at 50°C for 20 minutes with 1.25 percent hydrogen peroxide and only a slight reduction (8 percent) after treatment with 5 percent hydrogen peroxide (59). To ensure the complete oxidation of methionine in casein, Slump and Schreuder (60) heated each kg of the protein with approximately 750 ml of 30 percent hydrogen peroxide (about 200 g) at 30°C for 2 hours. Under these conditions about 75 percent of the methionine in casein was oxidized to methionine sulfoxide, a nutritionally available derivative, and the remaining 25 percent to methionine sulfone, which has no nutritional value (60,61).

Milk exposed to high concentrations of hydrogen peroxide or to lengthy treatment produces cheese with a relatively high moisture content and a soft body (51). These textural changes are presumably manifestations of milk protein alterations. Fox and Kosikowski (62) detected changes in the casein induced by hydrogen peroxide treatment. Sufficient 33 percent hydrogen peroxide was added to a casein solution to give a final concentration of 1 percent. The solution was heated to 85°C and the excess hydrogen peroxide destroyed with catalase. The treated casein was more susceptible to proteolysis, especially by rennin, and formed

a more soluble calcium caseinate than the protein from untreated milk. Other changes in milk and whey proteins have also been reported (63,64). Grindrod and Nickerson (63) treated individual proteins for 30 minutes at 49.9°C or 20 minutes at 26°C with 1 percent hydrogen peroxide. The electrophoretic mobilities of β -casein and of bovine serum albumin were increased while those of α_s -casein and β -lactoglobulin were decreased. Treatment of skim milk with 1.0 percent hydrogen peroxide at 49.9°C for 2 hours reduced the whey protein nitrogen from 115 to 100.9 mg per 100 ml, with a corresponding increase in the non-protein nitrogen. Cooney and Morr (64) reported that minimal whey protein denaturation and aggregation occurred at room temperatures with 0.5 percent hydrogen peroxide but that extensive whey protein alteration resulted when higher temperatures (55°C) and peroxide concentrations (1 to 2 percent) were used.

Girolitti (65) reported no change in the lactose and butterfat content of milk treated with 400 mg hydrogen peroxide per liter.

Production of toxic compounds

Lipids. In exploring possible adverse consequences of treating foods with hydrogen peroxide, the Select Committee also considered the possible formation of toxic oxidation products. As an oxidizing agent, hydrogen peroxide theoretically can form a number of reaction products with food constituents, whose nature and significance are largely speculative. Of special interest are the unsaturated fatty acids and the sterols that may be present in the treated foods. Both groups of compounds are vulnerable to oxidation and may yield products with putative carcinogenic or other toxic properties.

A number of vegetable oils and animal fats, oxidized by aeration, have proved toxic when fed to rats, with a rough correlation between toxicity and the extent of oxidation (66-69). Since oleic and linoleic acids are the most prevalent unsaturated acids in edible fats and oils, most studies have focused on their oxidation products in search for the toxic principles. Both peroxides and epoxides of these acids have been investigated. Holman and Greenberg (70) reported LD₅₀ values of 6 and 12 mg per mouse after intraperitoneal injection of the peroxides of methyl oleate and ethyl linoleate, respectively, (about 300 and 600 mg per kg body weight). Given by mouth, however, as much as 200 mg of either compound (10 g per kg) failed to kill a single mouse during a 48 hour observation period. Similarly, no deaths resulted among rats fed a daily dose of 75 mg of either peroxide (about 300 mg per kg) for 6 weeks. A single intragastric administration of about 1300 mg per kg methyl linoleate hydroperoxide in rats produced no toxic signs beyond a slight loss in weight (71). The rats died when approximately 5 g per kg of the hydroperoxide were given by stomach tube.

Approximately 80 percent of the fatty acids in soybean lecithin is unsaturated, the bulk coming from linoleic acid (72). This lecithin is commonly treated with hydrogen peroxide to produce bleached preparations widely used commercially. The so-called "bleached lecithin," "double bleached lecithin," and "hydroxylated lecithin" all employ hydrogen peroxide in their preparation. The "double bleached" and "hydroxylated" lecithins utilize benzoyl peroxide as an additional bleaching agent. For preparation of hydrogen peroxide bleached lecithin, 0.05 to 2.0 percent of 35 percent hydrogen peroxide is added to a lecithin emulsion (20 to 50 percent water) at 60° to 93°C and the moisture removed by heating under reduced pressure. Typically, 1.2 percent of 35 percent hydrogen peroxide is added, based on lecithin dry weight. Bleached lecithins have peroxide values ranging from 30 to 90 meq per kg (1.0 to 3.0 g per kg) (73). The estimated intake of lecithins modified by hydrogen peroxide (based on poundage data) is 4 mg daily (13) or a daily intake from this source of less than 0.1 mg peroxide. Hydroxylated lecithin with peroxide values of 52 meq per kg was fed to weanling Sprague-Dawley rats at levels of 5 and 10 percent of their diet. The 10 percent group received about 17.0 mg peroxide per kg body weight daily and was maintained on this diet for 8 weeks. The rats on the 5 percent levels (8.5 mg per kg) were kept for 52 weeks. Weight gains were normal for both groups and no abnormalities were detected in the vital tissues (74). Verrett (75) found no increase in teratogenic effects in the developing chick embryo when 10 to 200 mg per kg egg weight of double bleached lecithin were administered in the air cell at 0 and 96 hours or via the yolk at the same time intervals.

The effect of feeding epoxidized oil was investigated by Kieckebusch *et al.* (76). Soybean oil was epoxidized with hydrogen peroxide and fed to rats at various levels. The toxicity of the treated oil increased with increasing epoxidation, presumably due to the action of diepoxystearic acid produced from linoleic acid, which comprises the bulk of the unsaturated fatty acids in soybean oil. Male rats receiving the equivalent of about 250 mg per kg diepoxystearic acid daily for 8 weeks gained 8 percent less than the control animals. When this amount was tripled (750 mg diepoxystearic acid per kg daily) 80 percent of the rats died within 8 days. The investigators estimated a tolerance level for diepoxystearic acid of 380 mg per kg per day. The greatest changes in the rats receiving the larger dose were a marked increase in water consumption per unit body weight, and testicular atrophy. The investigators speculated that the observed signs might be manifestations, at least in part, of an essential fatty acid deficiency rather than direct epoxide toxicity.

Epoxy acids are natural constituents of some seed oils, including 9:10 epoxystearic acid (77), cis 12:13 epoxyoleic acid (78), cis 9:10 epoxy octadec-12-enoic acid (79), and cis 15:16 epoxylinoleic acid (80). Thus, the monoepoxides of oleic, linoleic, and linolenic acids are all natural constituents of seed

oils. Smith et al. (79) speculated that epoxy acids may be widely distributed in the plant kingdom, derived biogenetically through the action of specific epoxidases. The most abundant natural source of epoxy acids appears to be Vernonia anthelmintica, whose oil contains about 69 percent of 12:13 epoxyoleic acid. Chalvardjian and coworkers (81) fed male, weanling Sprague-Dawley rats a diet containing 10 percent of the Vernonia oil, representing an estimated epoxy acid consumption of approximately 7 g per kg body weight per day. The growth curves of these rats were not significantly different from those of control rats receiving olive oil. The animals were killed after 28 days and the tissues examined. No gross or histological damage was detected.

The epoxides of some of the fatty acids have been tested for carcinogenicity. Van Duuren (82) reported 9:10 epoxystearic acid (from oleic acid) and 9:10, 12:13 diepoxystearic acid (from linoleic acid) to be noncarcinogenic when applied on the skin of mice. The former compound was also shown noncarcinogenic after subcutaneous injection in the mouse and rat (83). Swern et al. (84), however, reported the production of six sarcomas at the injection sites of 70 mice (BALB/C and Swiss) receiving epoxy-stearic acid subcutaneously. They considered the compound to be "marginally active" as a carcinogen. No reports are available on the carcinogenicity of those epoxides that might conceivably be produced from the small amounts of other unsaturated fatty acids in the diet (palmitoleic, linolenic, arachidonic).

Van Duuren et al. (83) points out that even those epoxides found to be carcinogenic are effective only at large doses, and that none has induced gastric tumors when given by mouth or gavage. Seelkopf and Salfelder (85) fed epoxy- and diepoxystearic acid to Holtzman rats and to C57BL/6 mice for 3 to 5 months at daily dose levels varying from about 50 to 150 mg per kg body weight. None of the 45 rats or 41 mice on the monoepoxide diet which survived the test period developed gastric tumors, nor did any of the 31 mice surviving the diepoxystearic acid regimen. One rat (of 46) receiving diepoxystearic acid developed a gastric carcinoma and one of 29 control mice had an early "pregastric" carcinoma.

Van Duuren et al. (83) attributed the relative tolerance of animals to orally administered epoxides to their rapid degradation in vivo, especially in the stomach where acid-catalyzed hydrolysis would be expected. The extent of such epoxide hydrolysis in the stomach is unknown, but the study by Chalvardjian et al. (81) indicates that complete destruction is unlikely when large amounts are fed. These investigators detected epoxyoleic acid in the tissues and feces of rats fed Vernonia oil. No quantitative analyses were performed, but some of the epoxy acid from the oil obviously survived contact with the hydrochloric acid of the stomach. Little is known of the action of the intestinal contents upon the epoxide fraction surviving hydrolysis in the

stomach. Ivie (86) recently reported that epoxides are reduced to their corresponding olefins in ruminal fluids and suggested that such bacterial action in the intestine may represent a significant detoxifying mechanism in mammals. Once absorbed, epoxides are subject to enzymatic action in the liver with conversion to their vicinal glycols (87).

Lipid peroxides are also poorly absorbed from the intestinal tract. Andrews *et al.* (68) and Glavind and Tryding (88) failed to detect peroxides in the lymph of rats after feeding lipoperoxides and only very small amounts could be recovered in the feces. Since pancreatic juice, bile, and lymph had little effect on the peroxides, the latter investigators suggested that the essential site of peroxide destruction was the intestinal mucosa.

In addition to acting upon unsaturated fatty acids, hydrogen peroxide might be expected also to form oxidation products with sterols. The structure, concentration, and significance of such oxidation products remain largely unknown because of the complexity of the resulting mixture. In a recent symposium, it was stated that about 50 oxidation products of cholesterol had been identified, but an additional 50 compounds were possible theoretically (89). Claims that the oxidized derivatives of cholesterol are carcinogenic have focused chiefly on 5,6 α -epoxy-5-cholesten-3 β -ol (cholesterol-(α)-oxide) as the putative agent (90). Bryson and Bischoff (91) reported this compound to be carcinogenic in Evans rats and in Marsh mice following subcutaneous injection, but inactive upon intraperitoneal injection. Seelkopf and Salfelder (85), however, found no increase in tumor frequency above that of control groups among 45 Holtzman white rats of both sexes or in an equal number of C57BL/6 mice fed 50 to 150 mg per kg body weight of cholesterol-(α)-oxide daily for 3 months. Thirty-three rats and 27 mice survived the test period. Smith and Kulig (92) obtained a yield of 0.2 percent of cholesterol-(α)-oxide upon treatment of cholesterol (1 mg per ml) for 6 hours at 50°C with 0.015 percent hydrogen peroxide. From the cholesterol content of milk (0.15 percent) about 0.3 mg cholesterol-(α)-oxide per liter of milk theoretically could be produced by this treatment.

Recent evidence has indicated that some oxidative products of cholesterol may also have angiotoxic or atherogenic effects (93,94). Preliminary experiments suggest that large amounts of the active products are necessary to induce these changes. A concentrate of products of cholesterol oxidation given by gavage to rabbits at levels of 250 mg per kg body weight increased the frequency of dead smooth muscle cells of the aorta (93).

Carbohydrates. The Subcommittee is not aware of any toxic substance produced by the treatment of carbohydrates with hydrogen peroxide. Hydrogen peroxide is known to oxidize simple aldehydes to the corresponding acids and is employed in wine production for this purpose (6). In the presence of ferrous salts

it will oxidize aldoses and ketoses to their corresponding osones (95) and with high concentrations, it will degrade carbohydrates stepwise to products containing one less carbon atom (96).

Proteins. Modification of protein structure by hydrogen peroxide treatment has been discussed in an earlier section. There is no evidence that the altered protein is toxic (56). Treatment of fish protein with 20 to 80 g hydrogen peroxide per kg dry weight at 50°C for 2 hours oxidized small amounts of methionine and cystine to methionine sulfoxide, methionine sulfone, and cysteic acid (61). Methionine sulfoxide was as effective nutritionally as methionine in rat feeding experiments. Methionine sulfone and cysteic acid were ineffective nutritionally but had no apparent toxic effect.

Alarcon (97) demonstrated that acrolein, a volatile toxic unsaturated aldehyde, can be produced by vigorous treatment of various amino acids and polyamines with hydrogen peroxide. The reaction was carried out at 100°C for 10 to 60 minutes with 20 mmoles hydrogen peroxide and 5 mmoles of amino acid. Less drastic treatment was not reported.

V. OPINION

Hydrogen peroxide is GRAS when used as a bleaching agent in foods and in cotton and cotton fabrics for dry food packaging. It is considered GRAS by the Department of the Treasury in the treatment of wines. It is used as an antimicrobial agent in cheese manufacturing under standards of identity and also in whey processing.

Consumer exposure data indicate only 8 mg per capita per day are used by food manufacturers and much of this would be destroyed or dissipated during processing. Toxic effects in animals by all routes studied occurred only at levels several orders of magnitude greater than man's possible exposure from food sources or packaging materials. There is no evidence that hydrogen peroxide is carcinogenic, teratogenic, or mutagenic at levels present in foods treated with hydrogen peroxide during processing.

Vigorous treatment of foods with hydrogen peroxide may cause some destruction of ascorbic acid, methionine, and cystine. Under the conditions normally employed, the Select Committee believes their loss to be nutritionally insignificant.

Various oxidation products of normal food constituents are formed by the action of hydrogen peroxide. It is possible that such products might include epoxides or peroxides of unsaturated fatty acids and sterols, some of which are suspected of being carcinogenic or atherogenic under specialized conditions. However, none of the oxidation products thus far tested has proved carcinogenic when given by mouth, even at levels many times greater than any reasonable intake in food. Angiotoxicity has been produced only with amounts of sterol oxidation products several orders of magnitude greater than would be produced under conditions currently practiced. There is no evidence that such products are, in fact, produced under current conditions of hydrogen peroxide usage. Because of the vulnerability of epoxides and peroxides to gastrointestinal action, only a small fraction of the amount ingested would be absorbed and this in turn would be subjected to hydrolysis by liver enzymes.

Although there is no evidence that the present usage of hydrogen peroxide in foods poses a hazard to consumers, insufficient data are available to ensure a lack of hazard with all foods or when more rigorous treatments are employed, using higher concentrations, prolonged exposures, or elevated temperatures.

In the light of the foregoing considerations, the Select Committee concludes that:

There is no evidence in the available information on hydrogen peroxide that demonstrates or suggests reasonable grounds to suspect a hazard to the public when it is used at levels that are now current and in the manner now practiced. However, it is not possible to determine, without additional data, whether a significant increase in consumption would constitute a dietary hazard.

There is no evidence in the available information on hydrogen peroxide that demonstrates or suggests reasonable grounds to suspect a hazard to the public when it is used in cotton and cotton fabrics for dry food packaging at levels that are now current or might reasonably be expected in the future.

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