Joint Assessment of Commodity Chemicals No. 22

HYDROGEN PEROXIDE

CAS No. 7722-84-1

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This report has been produced as part of the ECETOC programme for preparing critical reviews of the toxicology and ecotoxicology of selected existing industrial chemicals.

In the programme, commodity chemicals, that is those produced in large tonnage by several companies and having widespread and multiple uses, are jointly reviewed by experts from a number of companies with knowledge of the chemical. It should be noted that in a JACC review only the chemical itself is considered; products in which it appears as an impurity are not normally taken into account.

ECETOC is not alone in producing such reviews. There are a number of organisations that have produced and are continuing to write reviews with the aim of ensuring that toxicological knowledge and other information are evaluated. Thus a Producer, Government Official or Consumer can be informed on the up-to-date position with regard to safety, information and standards. Within ECETOC we do not aim to duplicate the activities of others. When it is considered that a review is needed every effort is made to discover whether an adequate review exists already; if this is the case the review is checked, its conclusions summarised and the literature published subsequent to the review assessed. To assist ourselves and others working in this field we publish annually a summary of international activities incorporating work planned, in hand, or completed on the review of safety data for commodity chemicals. Interested readers should refer to our Technical Report No. 30 entitled "Existing Chemicals: Literature Reviews and Evaluations".

This document presents a critical assessment of the toxicology and ecotoxicology of Hydrogen Peroxide (CAS No. 7722-84-1).

JACC REPORT NO. 22 - HYDROGEN PEROXIDE

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SECTION 1. SUMMARY AND CONCLUSIONS

Hydrogen peroxide (H_2O_2) and its aqueous solutions (up to 70% concentration) are clear, colourless, weakly acidic liquids with a distinctive and mildly pungent odour, and a low vapour pressure. Pure solutions of H_2O_2 are stable. Small quantities of stabilisers are added to commercial H_2O_2 solutions to prevent catalytic decomposition caused by impurities or contaminants. When decomposition occurs, heat is evolved and large quantities of oxygen are generated. The saturated vapour concentration over a 90% H_2O_2 solution is 3,049mg/m^3 at 25°C.

World-wide about 1,000kt/y H_2O_2 (100% basis) are used, mainly for the production of chemicals, for bleaching of cellulose pulp and textiles and for other purposes such as waste water treatment. Minor quantities are used in applications (food processing, disinfection, drinking water treatment and hair bleaching) in which there is direct contact with human beings.

H_2O_2 occurs naturally as a consequence of photochemical processes and may also be formed through oxygen reduction by iron and copper. Emissions of H_2O_2 from industrial and domestic sources are limited due to its rapid decomposition in the waste water.

Concentrations H_2O_2 found in the atmosphere vary with the intensity of solar radiation, humidity and temperature and the presence of precursors and scavangers of free radicals. The tropospheric lifetime of H_2O_2 is 10-20h. During day-time H_2O_2 concentrations range from 0.4-4µg/m^3, falling to below 0.01µg/m^3 at night. H_2O_2 in the atmosphere tends to concentrate into cloud droplets (where concentrations can reach 8,000µg/l) and is removed by rain-out. During fog and severe smog, atmospheric concentrations may rise to 4,000µg/m^3.

H_2O_2 is produced in water and soil, the amount depending upon the intensity of light, concentration of promoters and dissolved oxygen. Decomposition in water and soil takes minutes or several hours depending on the mineral content and the concentration of micro-organisms. Surface waters generally contain less than 100µg/l, dropping to below 1µg/l at 100m depth. Levels up to 3µg/l are found in ground water.

Human beings may be exposed to H_2O_2 via food. In certain vegetables naturally occurring levels of up to 8,000µg/l have been found. Environmental exposure by inhalation is less than the levels of H_2O_2 normally found in exhaled air; environmental exposure becomes significant only in foggy conditions. No exposure data are available from the uses of H_2O_2.

The acute toxicity of H_2O_2 to aquatic organisms is low. Effects have been observed only in certain marine algae below 1mg/l. Concentrations below 200mg/l affect freshwater algae, microcrustaceans, snails, bacteria, some fish species and certain aquatic plants, whilst aquatic insects, vegetative growth,

seed germination and early seed growth of terrestrial plants are not affected. The H_2O_2 present in cloud water and fog may be harmful to spruce and beech trees.

H_2O_2 is produced during normal aerobic cell metabolism, it involves a number of enzymatic reactions, especially superoxide dismutase. H_2O_2 is decomposed to oxygen and water by enzymes such as catalase, peroxidase and selenium dependent glutathione peroxidase. The activity of these enzymes varies between tissues and between different animal species or strains. For example, duodenal catalase levels in rats are orders of magnitude greater than those found in mice. In human jejunum, catalase levels were found to be several times higher than in mice. H_2O_2 exposure induces catalase activity in bacteria. This induction is less apparent in tissues of rats and mice. H_2O_2 can also be decomposed by transition metals (e.g. iron, copper) to highly reactive hydroxyl radicals capable of inducing various toxic effects.

After contact with organs and tissues, H_2O_2 undergoes decomposition to water and oxygen. This may lead to small gas embolies and reversible blanching of the exposed tissue area. Larger volumes of gaseous oxygen can lead to detachment of cell layers and the rupture of tissues and organs. The increase in oxygen content in the blood results in a hyperbaric response.

The acute oral toxicity of H_2O_2 in experimental animals varies with the strength of its solution, the lethal dose varying from 75 to 2,000mg/kg body weight. In human beings, death has resulted from the accidental ingestion of unknown quantities of 30-40% solutions. Toxic effects were generally related to the corrosive action on the gastrointestinal tract and the generation of large volumes of oxygen. There was complete recovery within 2-3 weeks even in near fatal cases.

Dermal toxicity is low. H_2O_2 solutions of less than 35% are not classified as irritant to the rabbit skin; solutions of 50% and higher are corrosive. Effects on the mucosa of the gingiva and tongue of dogs were found after direct contact with a 1% solution, whilst in human beings mouth washes with up to 3% neutralised H_2O_2 did not cause mucosal irritation. Therapeutical/ clinical use of 1-3% solutions in contact with the intestinal mucosa induced colitis and inflammation.

H_2O_2 solutions of 10% or more cause irreversible damage to the eye, including blindness. Solutions of less than 5% are not classified as irritant to the rabbit eye. The first effects on the rabbit cornea are observed with a 1% solution. In human beings 1-3% solutions have been used for eye treatment without significant injury. However, solutions containing more than 200ppm cause hyperaemia and down to 100ppm pain and stinging.

Acute exposure to saturated vapour induces only minor clinical signs in rodents. Brief exposure to aerosols at 9,400mg/m^3 H_2O_2 was lethal to mice with effects limited to the respiratory tract and the eyes. After repeated exposure, mortality in mice was observed at 80mg/m^3. In rats and dogs,

respiratory irritation and transient skin thickening have been observed at 1-10mg/m^3 after prolonged exposure.

In human beings exposed for 4h the irritation threshold for the respiratory tract was 10mg/m^3 and for the skin 20mg/m^3. At these concentrations eye and throat irritation as well as gradual bleaching of hair have been reported. Under conditions of occupational exposure, when respecting a limit value of 1.4mg/m^3, no adverse effects have been reported.

Exposure to H_2O_2 at concentrations above 1% in drinking water was lethal to mice and rats within weeks. After prolonged exposure, decreased body weight gain was observed in mice with concentrations of 0.4% and in rats with 0.25% and above. In mice, 0.1% induced inflammation of the gastro-intestinal tract. In certain studies, hydropic changes in the liver, haemosiderin deposition in the spleen and epithelial degeneration of kidney tubuli have been reported, but it is unclear whether these changes were treatment-related.

H_2O_2, at concentrations which induce significant inflammation of the exposed tissue, has been shown to be a weak tumour promoter, the tumours being localised in the exposed organs. H_2O_2 itself induced an increase of duodenal tumours in mice exposed to 0.1-0.4% in drinking water. The tumour incidence correlated with a specific inflammatory response in this tissue and was more pronounced in those mice which have a low catalase activity. Rats exposed to near lethal concentrations in their drinking water developed forestomach papilloma but no tumours of the glandular stomach and duodenum. The absence of tumours in these organs correlates well with the high catalase levels found and demonstrates the protective role of this enzyme. Repeated topical application of 15% H_2O_2 to Sencar mice did not induce skin tumours.

In *in vitro* tests without metabolic activation, H_2O_2 induces gene mutations in bacteria sensitive to oxidative stress, in yeast and in mammalian cells. Primary DNA damage was observed in bacteria and mammalian cells. H_2O_2 induced chromosome aberrations and micronuclei in mammalian cells and morphological cell transformations. In general, the addition of exogenous metabolic activation or catalase reduced or abolished the genotoxic response.

Little information is available concerning the *in vivo* genotoxic potential of H_2O_2. No chromosomal aberrations or micronuclei were observed in the bone marrow of rats and mice after oral administration. In contrast, gene mutations in bacteria and chromosomal aberrations in tumour cells were observed in a host-mediated assay with mice. Overall, H_2O_2 has been shown to be genotoxic only to the cells with which it comes in direct contact.

Reproductive toxicity data are limited. Spermatozoa in mice and rabbits were unaffected and male mice were fertile after treatment with 1-3% H_2O_2 in drinking water. Data on the teratogenic potential of H_2O_2 are too limited to allow any evaluation.

SECTION 2. IDENTITY, PHYSICAL AND CHEMICAL PROPERTIES, ANALYTICAL METHODS

2.1 IDENTITY

Name:	Hydrogen peroxide (H_2O_2)
IUPAC name:	Hydrogen peroxide
Synonyms:	Hydrogen dioxide Hydrogen superoxide
D	Wasserstoffperoxid
F	Eau oxygenée Peroxyde d'hydrogène
I	Perossido di idrogeno
J	過酸化水素
NL	Waterstofperoxyde
S	Vaeteperoxid
CAS name:	Hydrogen peroxide
CAS registry No.	7722-84-1
EEC No.	008-003-00-9 (<5% not classified)
EINECS No.	231-765-0
MITI No.	GN 1-149 SN 1-334
TSCA inventory:	Listed
Formula:	H_2O_2
Molecular weight:	34.0
Structural formula:	H-O-O-H

2.2 PHYSICAL AND CHEMICAL PROPERTIES

H_2O_2 is normally handled as an aqueous solution, in concentrations ranging from dilute (<5%) to >90% by weight. Commercial grade solutions up to 70% H_2O_2 by weight are generally available; solutions over 70% are produced for specific applications such as the manufacture of organic peroxides and caprolactone; there are also some military applications.

Physical and chemical properties of H_2O_2 solutions are given in Table 1.

H_2O_2 and its aqueous solutions are clear, colourless liquids with a low viscosity and low vapour pressure. The odour is distinctive and mildly pungent. The partial vapour pressure of H_2O_2 increases as its concentration in water increases; the total vapour pressure of a solution is due to both H_2O_2 and water and decreases with the concentration of H_2O_2.

2.2.1 Stability

A pure solution of H_2O_2 is slightly acidic (pH 3.5-4.5). It is relatively stable if stored in the dark in a clean, inert container. At a fixed pH more concentrated solutions appear to be more stable than diluted solutions. The decomposition reaction is strongly exothermic (Delta H = -98.3kJ/mol), and large quantities of oxygen gas are evolved (e.g. one litre 70% solution of H_2O_2 yields about 250 litres oxygen at 0°C and 1,013hPa) (Becewa, 1984). The decomposition of H_2O_2 is generally a catalysed reaction. Common catalysts are dissolved transition metals (e.g. copper, iron, manganese), solid metals (e.g. platinium, osmium or silver), solid metal oxides and hydroxides (e.g. manganese, iron, copper oxides), activated carbon and enzymes (Schumb *et al*, 1955; Goor *et al*, 1989). H_2O_2 is also decomposed by alkaline impurities sufficient to raise the pH to 7 or above. Heat and sunlight can induce photochemical decomposition with the formation of free radicals.

Stabilisers are added to the commercial product to prevent its decomposition into oxygen and water (section 2.3.1). In the gas phase, H_2O_2 at high vapour concentrations (≥26vol.%) may be exploded by an electric spark or by heating to ≥150°C (Schumb *et al*, 1955).

H_2O_2 is miscible with many polar organic solvents, e.g. low molecular weight alcohols, glycols, ketones. Concentrated aqueous H_2O_2 solutions may become explosive with these solvents (Merrifield, 1988).

2.2.2 Chemical Reactivity

H_2O_2 oxidises compounds such as nitrites, cyanides, sulphites and hydrogen sulphide. The oxidising ability of H_2O_2 is greater at low pH. H_2O_2 can also reduce compounds such as hypochlorites, permanganates and cerium salts (Ce^{4+}).

TABLE 1

PHYSICAL AND CHEMICAL PROPERTIES OF H_2O_2 SOLUTIONS

Property	Concentration of H_2O_2 (by weight)					Reference
	10%	45%	50%	70%	90%	
Density at 20°C (kg/m^3)	1.034	1.113	1.195	1.288	1.387	MCA, 1969
Freezing Point (°C)	-6	-33	-52	-40	-11	Schumb *et al*, 1955
Boiling Point (°C)	102	108	114	125	141	MCA, 1969
Flash Point (°C)	Not applicable					
Ignition Point (°C)	Not applicable					
Solubility in Water	Miscible at any ratio					
Viscosity at 20°C (10^3kg/m.s)	1.01	1.11	1.17	1.24	1.26	MCA, 1969
Surface tension at 20°C (N/m)	0.0731	0.0746	0.0757	0.0773	0.0792	Schumb *et al*, 1955
Vapour Pressure (Pa)						
Total (H_2O_2+H_2O) at 20°C	-	1.700	1.300	800	-	Atochem 1989a-e, 1990
Total (H_2O_2 + H2o) at 22°C	-	-	1.486	873	375	Solvay, 1991
Partial (H_2O_2) at 20°C	-	-	46	98	178	Solvay, 1991
Vapour pressure at 30°C (Pa).						
Total (H_2O_2+H_2O)	-	3.070	2.400	1.470	667	MCA, 1969
Partial (H_2O_2)	-	48	99	200	333	MCA, 1969
Vapour saturation in air at 25°C (mg/m^3)	-	-	787	1.685	3.049	Solvay, 1991
Henry's Law Constant at 20°C ($Pa.m^3/mol$)	-	-	$1x10^{-3}$	-	-	Hwang and Dasgupta, 1985

H_2O_2 oxidises organic compounds such as alcohols, olefins and amines and also forms organic peroxides (Schirmann and Delavarenne, 1979).

2.3 ADDITIVES AND IMPURITIES

Commercial H_2O_2 solutions normally contain added stabilisers and some impurities from production process.

2.3.1 Stabilisers

Common stabilisers are phosphoric or other mineral acids (to keep the product acidic), sodium pyrophosphate (a complexing agent to inhibit metal-catalysed decomposition), sodium stannate (a colloid-forming inhibitor) and organic stabilisers such as 8-hydroxyquinoline, pyridine carboxylic acids, tartaric acid and benzoic acids (mainly complexing agents and radical inhibitors) (Schumb *et al*, 1955). Stabilised H_2O_2 solutions normally lose <2%/y of their H_2O_2 content, when stored at ambient temperature.

Nitrate salts can be added as passivators to improve the chemical resistance of stainless steel and aluminium against H_2O_2.

2.3.2 Impurities

Commercial H_2O_2 solutions contain 0.005-0.10% organic impurities (total organic carbon). These impurities are aromatic hydrocarbons and other organic compounds used during purification of the crude product (section 3).

The amounts of inorganic impurities are low; the total concentration does not normally exceed 10ppm (total) with total heavy metals usually <2ppm.

2.3.3 Grades of Hydrogen Peroxide

In technical and chemical grades, the total stabilisers and impurities (total non-volatile compounds) range from of 0.01-0.25%; chemical grades having the lower and technical grades the higher levels.

"Food Grade" H_2O_2 (30-50%) meets the US Food Chemical Codex requirements and contains ≤0.006% non-volatile compounds (FCC, 1981). "Electronic Grade" H_2O_2 (30%) is extremely pure. Typically, the total concentration of non-volatile impurities is 0.001% (L'Air Liquide, 1990a). "Cosmetic Grade" H_2O_2 (35, 50 and 70%) contains more stabilisers (≤0.5%) added by individual customers. This is because the product is diluted with water before use in cosmetics, e.g. to a 12% solution in hair care products, 4% in skin care and 2% for nail hardening. In general, the cosmetic user industry complies with the European Specification for 3% and 30% H_2O_2 (Pharmacopée Européenne, 1985; COLIPA, 1991).

2.4 CONVERSION FACTORS

Conversion factors at 20°C and 1,013hPa:

1ppm = 1.414mg/m^3
1mg/m^3 = 0.707ppm

2.5 ANALYTICAL METHODS

2.5.1 Product Analysis

Titration with permanganate (ISO, 1984) is normally used during the production of H_2O_2. Density measurement is an alternative method. Aqueous solutions which contain components in addition to H_2O_2, such as bleaching liquids and waste water, are analysed preferably by an iodometric method (Kolthoff *et al*, 1969).

2.5.2 Environmental Media

Sakugawa *et al* (1990) reviewed a large number of studies of the H_2O_2 content of air and rain/cloud water and stated that the different analytical procedures used to measure atmospheric concentrations gave different results. Further improvements in H_2O_2 sampling and analysis would be needed. Sampling presents a special problem at low concentrations in air. Thus, Lee *et al* (1991) found that for low atmospheric levels (2ppb) as much as 90% of the gaseous H_2O_2 could be lost by surface reactions at the inlet of the sampling line.

Examples of methods for the determination of H_2O_2 in air are summarised in Table 2. In ambient air, a sensitive, direct fluorescence method is available (Lazrus *et al*, 1985, 1986). Other methods involve trapping H_2O_2 in wash traps or on filters, followed by analytical determination. The lowest detection limit, using a colorimetric method, is approximately 3ppt (v/v) (Ferm, 1988).

Methods for the determination of H_2O_2 in water are summarised in Table 3. The most sensitive method is used for ground-water (Holm *et al*, 1987).

2.5.3 Biological Media

A summary of methods for the determination of H_2O_2 in biological media including the analysis of plant tissues, animal tissues, blood, food and milk, is given in Table 4.

TABLE 2

ANALYSIS OF AIR

Sample Preparation	Analytical measurement	Limit of detection (ppb)	Reference
Ambient Air			
Collect in impingers containing distilled water; react with luminol using alkaline Cu^{2+} catalyst	Chemilumi-nescence	0.5 (0.7ng/l)	Kok *et al*, 1978b Das *et al*, 1983
Collect in aqueous gas washing traps; react with scopoletin and horseradish peroxidase	Flourescence decay at 365nm and 490nm	At least 70ng/l in trap water	Zika and Saltzman, 1982
Decomposition of H_2O_2 by catalase	Automatic flourometry	0.010-0.100	Lazrus *et al*, 1986
Trap in Ti^{4+} + H_2SO_4 on glass filter	Colourimetry at 475nm	0.003	Ferm, 1988
Workplace Air			
Absorption in water containing $TiCl_4$/HCl	Colourimetry at 415nm	10	Pilz and Johann, 1974
Absorption in acidic potassium titanium oxalate	Colourimetry at 400nm	140-2,800	Interox, 1991

TABLE 3

ANALYSIS OF WATER

Sample preparation	Analytical measurement	Limit of detection (µg/l)	Reference
Surface Water			
Add leuco crystal violet, horseradish peroxidase and acetate buffer	Spectro-photometry at 596nm	50	Draper and Crosby, 1983
Boil, cool, filter and irradiate	Thin-layer chromatography with peroxidase-catalysed leuco crytsal violet spray	50	Draper and Crosby, 1983
Rain and Cload Water			
React with alkaline luminol	Chemilumi-nesence	1	Kok, 1980
Acidify, cool to 5°C	Liberation of O_2, conversion of O_2 to CO_2	A few	Holt and Kumar, 1986
Sea Water			
Unknown	Flourescence decay	≤1	Kieber and Helz, 1986
Ground Water			
Add scopoletin, measure flourescence; add horseradish peroxidase, measure flourescence	Flourescence decay	A few (0.034)	Holm *et al*, 1987
Cooling Water			
Mix with phenolphtalein leuco base	Photometry	Unknown	Zabelin and Karbovnichii, 1983

TABLE 4

ANALYSIS OF BIOLOGICAL MEDIA

Sample preparation	Analytical measurement	Limit of detection (µg/l)	Reference
Plant Tissue			
Transfer frozen sliced tissue into 5% thyrocalcitonin; homogenise; centrifuge; pass over anion exchange resin; add to ammoniacal luminol; add potassium ferri-cyanide	Chemilumines-cence	At least 1ng (corresponding to 0.1-1g fresh tissue)	Warm and Laties, 1982
Animal Cells and Tissue			
Mix tissue homogenates or subcellular fractions with catalase	Spectrophotometry (640-660nm)	Unknown	Sies, 1981
Blood Serum			
Add sodium azide, ascorbate oxidase, catalase and 1,4-piperazine-diethane-sulphonic acid or phosphate buffer	Hydrogen peroxide-selective electrode with oxidase meter	Unknown	Nakane and Kosaka, 1980
Plasma			
Blood centrifuged, deproteinated	HPLC and spectrometry	Unknown	Nahum *et al*, 1989
Food			
Extract with 0.5% $KBrO_3$ at pH 7.0	O_2 release by catalase, oxygen electrode	0.01mg/l (liquid) 0.1mg/kg (solid)	Toyoda *et al*, 1982
Milk			
Treat with trichloro-acetic acid; filter	Colourimetry ($TiCl_4$/HCl) 415nm	2mg/l 0.4mg/l	Matz and Dietze, 1971 Gupta *et al*, 1977

SECTION 3. PRODUCTION, STORAGE, HANDLING, TRANSPORT AND USE

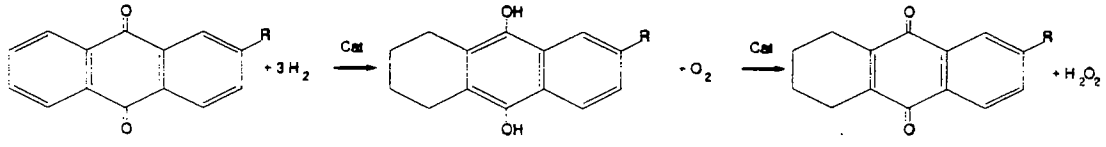
3.1 PRODUCTION METHODS

3.1.1 Anthraquinone Autoxidation

The predominant industrial method of H_2O_2 manufacture is by anthraquinone autoxidation involving two main steps (SRI, 1988; Goor *et al*, 1989; Figure 1). The first step is catalytic hydrogenation of 2-alkyl-9,10-anthraquinone or other anthraquinone derivatives to corresponding anthrahydroquinones using a palladium or nickel catalyst and separation of the solid catalyst from the "working solution". The second main step is oxidation of anthrahydroquinones by bubbling air or oxygen through the solution, when the anthrahydroquinones are oxidised back to anthraquinones and H_2O_2 is formed. The crude H_2O_2 is extracted with water from the organic solution which is returned to the first hydrogenation step producing a cyclic process.

FIGURE 1

ANTHRAQUINONE AUTOXIDATION METHOD (Goor *et al*, 1989)



R = alkyl -

The extracted crude aqueous solution contains about 20-40% H_2O_2 and is normally purified in two or three stages by extraction with organic solvents, such as xylene and/or methyl cyclohexanol acetate (Goor *et al*, 1989). An optional treatment with activated carbon or absorbent resin can be applied to reduce the organic carbon content. Finally, the aqueous solution is distilled to give 50-70% H_2O_2 solutions.

3.1.2 Other Production Methods

Small quantities are produced by older methods using electrolysis of aqueous ammonium sulphate or sulphuric acid solution in water (Goor *et al*, 1989). An organic process based on 2-propanol is in use in the Soviet Union (Goor *et al*, 1989).

3.2 HANDLING, STORAGE AND TRANSPORT

To prevent the decomposition of H_2O_2 solutions, measures are taken to avoid contamination during storage and transport. Tanks of aluminium (≥99.5%) or stainless steel (AISI 316L or 304L) are used for the bulk storage of ≤70% H_2O_2 solution. Before use the metal surfaces must be cleaned and passivated (the metal oxide layer on the surface improved). High density polyethylene tanks are also used for ≤60% H_2O_2. Aluminium (min 99.5%) or stainless steel is mainly used for concentrations >70%. Smaller quantities (concentration ≤60%) are mainly stored in drums or bottles of high density polyethylene.

Storage vessels are vented to the atmosphere in order to avoid pressure build-up resulting from decomposition.

Regulations for rail, road and sea transport are in force for the transport and packaging of >8% H_2O_2 solutions (Table 5).

TABLE 5

CLASSIFICATIONS FOR TRANSPORT AND PACKAGING

Regulation	Concentration (wt %)		
	8-20	20-60	>60
RID/ADR	8, No. 62c	8, No. 62b	5.1, No. 1
IMDG code (sea)	5.1	5.1	5.1
DOT (rail, road, sea)	Corrosive	Corrosive + oxidiser	Corrosive + oxidiser
UN No.	2984 pack. group III	2014 pack. group II	2015 pack. group I

RID European Rules for International transport of Dangerous goods (by rail).

ADR Agreement concerning international carriage of Dangerous goods by Road (class 8, Corrosive; class 5.1, Oxidising). Classes marked with an * have been reclassified to class 5.1, No. 1c and 1b respectively (effective in Europe on 1/1/1993).

IMDG International Maritime Dangerous Goods, vol III (1989).

DOT US Department of Transportation (<8% not regulated).

3.3 USES

3.3.1 Quantities Used and Produced

The estimated world consumption of H_2O_2 in 1989 was 1,023kt/y, distributed between Europe 49%, North America 23%, South America 5%, Asia 20% and Africa/Middle East 3% (Interox, 1990).

H_2O_2 is produced at approximately 40 production sites world-wide, each site having a production capacity in the range of 2-90kt/y (100% basis). In Western Europe, there are about 20 production sites.

3.3.2 Usage

There are three main uses [percent total world consumption in 1987 (ECN, 1988)]:

Production of chemicals (39%). Chemical or technical grade 35-70% H_2O_2 is used for the production of chemicals such as detergent raw materials (sodium perborate and sodium percarbonate), epoxidised soybean oil (stabiliser for PVC), cathecol and hydroquinone, hydrazine, organic peroxides (hardeners and initiators for the polymer industry), peracetic acid (a disinfectant and oxidising agent), caprolactone (a polyester raw material) and fatty amine oxides (detergent chemicals).

Bleaching of cellulose pulp (29%). Technical grade 50-70% H_2O_2 is used for bleaching mechanical pulp, chemi-thermo-mechanical pulp (CTMP) and chemical (kraft and sulphite) pulp and for de-inking waste paper. This use is expected to continue to increase.

Bleaching of textiles (19%). Technical grade 35-70% H_2O_2 is used for the bleaching of textile and cotton.

The remaining 13% of the world consumption is used for applications such as environmental control (waste water, waste gas and ground water treatment), metal etching (printed circuit boards), mining (gold ore leaching) and semiconductor chips manufacturing (cleaning). Most of these applications require a technical grade H_2O_2 (35-70%), except for semiconductor chips for which a special, highly pure electronic grade (30%) has been developed.

A relatively small quantity (≤2%) is used in applications which are particularly relevant to human exposure (section 5.2); namely: disinfectant in aseptic packaging of juice, milk, etc. (food grade), disinfection of drinking water (food grade), bleaching of certain foodstuffs, e.g. tripe, herring (food grade), sanitisation of chemical instruments, disinfectant for eye contact lenses, disinfection of wounds, mouthwashing and hair bleaching (cosmetic grade).

SECTION 4. ENVIRONMENTAL DISTRIBUTION AND TRANSFORMATION

4.1 SOURCES

There are natural, industrial and domestic sources of H_2O_2.

4.1.1 Natural Sources

H_2O_2 is produced naturally in each environmental compartment.

Surface water. H_2O_2 is generally produced in surface water by a photochemical process involving dissolved light-absorbing organic matter and molecular oxygen (Schumb *et al*, 1955; Cooper and Zika, 1983; Zika *et al*, 1985a).

$$Org + h\nu \rightarrow Org^{\cdot} \quad (290\ nm < \lambda < 385\ nm)$$

$$Org^{\cdot} + O_2 \rightarrow Org^{\cdot -} + \cdot O_2^-$$

$$2 \cdot O_2^- + 2H^- \rightarrow H_2O_2 + O_2$$

A large number of organic compounds such as glycerol, glucose, acetic acid, benzoic acid, aniline, quinone, tryptophan, humic acid and tyrosine can serve as promoters (Draper and Crosby, 1983). In river and lake waters, terrestrial humic promoters are responsible for the photo-sensitisation process, while in sea-water the photo-reactive chromophores have not been identified.

In sea-water, the Weiss mechanism may also be responsible for the production of H_2O_2 due to the oxidation of iron and copper ions (Moffet and Zika, 1987):

$$Fe^{2-} + O_2 \rightarrow Fe^{3-} + \cdot O_2^-$$

$$Fe^{2-} + \cdot O_2^- + 2H^- \rightarrow Fe^{3-} + H_2O_2$$

Laboratory experiments with deep water (250m) and surface water samples from the Mediterranean showed similar H_2O_2 production rates of between 1-10nmol/l/h after illumination. In deep water no H_2O_2 is found under natural conditions (section 5.1.1) (Johnson *et al*, 1989).

Concentrations in surface water are also influenced by precipitation of rain, snow or fog, which have a relatively high H_2O_2 content (section 5.1.3). The higher concentrations at the surface are attributed to input by precipitation rather than dry deposition of gaseous H_2O_2

(Cooper and Zika, 1983). As an example, in the case of France, with an average precipitation of 630mm/y, containing 10µg/l H_2O_2 (Table 9 -Section 5.1.3.), the annual input of H_2O_2 would be 3,500t/y (Chemoxal, 1992).

Ground water. Ground water samples taken from wells in a shallow sand and gravel aquifer at depth of 11, 15, 21 and 32m were examined. The H_2O_2 concentration was related to the content of dissolved oxygen through the O_2:H_2O_2 redox couple activity, indicating the possibility of a Weiss type oxidation mechanism in the soil (Holm *et al*, 1987).

Atmosphere. The formation of H_2O_2 in ambient air requires the presence of one or more free radical species.

In an unpolluted atmosphere, the formation of H_2O_2 is ultimately due to the photolysis of ozone (O_3) to give oxygen atoms. The latter are converted to free radical species, including ·OH, ·RO, ·HO_2 and ·RO_2, which initiate the breakdown of organic species and give rise to ·HO_2 radicals which are precursors of H_2O_2 (Wayne, 1988):

$$O_3 + h\nu \rightarrow O(^1D) + O_2 \qquad (\lambda \leq 310nm)$$

$$O(^1D) + H_2O \rightarrow 2{\cdot}OH$$

$$\cdot OH + CH_4 \rightarrow \cdot CH_3 + H_2O$$

$$\cdot CH_3 + O_2 + M \rightarrow \cdot CH_3O_2 + M$$

$$\cdot CH_3O_2 + NO \rightarrow \cdot CH_3O + NO_2$$

$$\cdot CH_3O + O_2 \rightarrow HCHO + \cdot HO_2$$

$$\cdot HO_2 + \cdot HO_2 \rightarrow H_2O_2 + O_2$$

($k=3.1 \times 10^{-12} cm^3 molecule^{-1} s^{-1}$; Jacob *et al*, 1987)

In polluted air, the most important source of free radicals, especially ·HO_2, is by photolysis of aldehydes, mainly formaldehyde (Calvert and Stockwell, 1983).

$$HCHO + h\nu \rightarrow \cdot H + \cdot HCO \qquad (\lambda \leq 370nm)$$

$$\cdot HCO + O_2 \rightarrow CO + \cdot HO_2$$

$$\cdot H + O_2 + M \rightarrow \cdot HO_2 + M \qquad (M \text{ is a molecule acting as a catalyst})$$

$$2{\cdot}HO_2 \rightarrow H_2O_2 + O_2$$

The reaction of ·OH and ozone with olefins such as isoprene and terpenes, emitted by trees can result in the formation of H_2O_2. Measurements of gaseous H_2O_2 in a conifer forest showed considerably higher concentrations within the forest than outside.

Levels of H_2O_2 were raised by 70% when the air temperature increased from 10-30°C with other meteorological factors and air pollutant concentrations remaining constant (Sakugawa *et al*, 1990).

High solar radiation intensity enhances peroxide production, which is consequently higher during the day than at night, higher in summer than in winter and higher at low latitudes than at high latitudes. Because the rate of H_2O_2 production from $\cdot HO_2$ is second order and the generation of ozone is only first order, larger amounts of H_2O_2 will be generated photochemically, especially with high solar UV irradiation. Unlike ozone, photolysis of H_2O_2 is slow and of minor importance. As a consequence, the evolution and concentration profiles of ozone and H_2O_2 are not parallel and the compounds have different tropospheric half-lives: 10-20h (Kleinman, 1986) for H_2O_2 and 10-100d for ozone (Liu *et al*, 1987) have been reported.

Thompson *et al* (1989) suggested that a 10% decrease in stratospheric ozone content would result in a >100% increase of H_2O_2 in the troposphere.

Water vapour is involved in another process of H_2O_2 formation, namely, the production of hydrated hydroperoxyl radicals ($HO_2 \cdot H_2O$), which rapidly react with $\cdot HO_2$ radicals to generate H_2O_2. About twice as much H_2O_2 is formed at 100% relative humidity than at 50% humidity (ambient temperature 25°C) (Calvert and Stockwell, 1983).

H_2O_2 accumulates in the atmosphere during extended dry periods (Jacob and Klockow, 1987). High concentrations of volatile organic compounds (VOC) in the atmosphere aid H_2O_2 generation, because of greater photochemical production of free radical species.

In an unpolluted atmosphere, about 70% of ·OH radicals react with CO and 30% with CH_4 to form ·H radicals, and yield H_2O_2.

$$\cdot OH + CO \rightarrow \cdot H + CO_2$$

$$\cdot H + O_2 + M \rightarrow \cdot HO_2 + M \quad \text{(M is a molecule acting as a catalyst)}$$

$$2 \cdot HO_2 \rightarrow H_2O_2 + O_2$$

The reaction with methane (CH_4) has been given above (Jacob *et al*, 1987).

A doubling of the emission rate of CO or CH_4 caused the concentration of H_2O_2 to increase by 30% (for CO), or 15% (for CH_4), in an unpolluted continental mid-latitude atmosphere (Thompson *et al*, 1989).

H_2O_2 formation may be inhibited in various ways, particularly by SO_2 and NO_x which are scavengers for H_2O_2.

A high concentration of NO_x inhibits H_2O_2 formation by scavenging $\cdot HO_2$ and its free radical precusors from the air.

$$NO + \cdot HO_2 \rightarrow \cdot OH + NO_2$$

H_2O_2 levels were inversely related to NO levels in polluted air. The production rate of H_2O_2 was extremely sensitive to the reaction of NO_2 with the $\cdot$OH radical, because this removes both NO_x and $\cdot$OH radicals from the pool of photochemical reactants. NO levels were relatively low in marine air, giving enhanced H_2O_2 production compared to continental air. It should also be noted that the concentration ratio of VOC/NO_x together with their absolute concentrations, influences the formation of H_2O_2 (Stockwell, 1986).

Living organisms. H_2O_2 is produced naturally by many living organisms (Section 7), either by the organism itself or by its surrounding medium. Examples of organisms capable of producing H_2O_2 are blue-green algae under light irradiation, at a rate of 1-50µg H_2O_2/l/h (Zepp *et al*, 1987); for *Nostoc muscorum* the rate was 0.9nM/µl cells (Stevens *et al*, 1973). *Hymenomonas carterae* (phytoplankton) formed H_2O_2 at 0.034-0.068µg/l/h (Johnson *et al*, 1989). The bombardier beetle (*Brachinus crepitans* (L.)) has been shown to produce H_2O_2 in solution at concentrations up to 28.5% (Schildknecht and Holubek, 1961). H_2O_2 has been detected in human exhaled breath at relatively high concentrations (300-1,000µg/m^3) (Williams *et al*, 1982).

H_2O_2 may also be formed in the medium surrounding an organism where certain metabolites from the organism act as promoters, e.g. riboflavin, excreted by a number of marine organisms. Laboratory experiments showed a H_2O_2 production of 0.34µg/l for every 1nM/l of riboflavin added to seawater (Moffet and Zika, 1987).

4.1.2 Industrial Sources

Plants manufacturing or using H_2O_2. Total losses of H_2O_2 during production are estimated to be 0.3%, the main release being in works waste water where the concentration can be 200mg/l. Following biological or Fenton based treatment, the maximum concentration in the final effluent is normallly in the range of 0-50mg/l or 0-30mg/l respectively for a plant using

H_2O_2 (Chemoxal, 1992) and 0-10mg/l for a manufacturing plant (Interox, 1990). The emission of H_2O_2 to the atmosphere from manufacturing plants is limited by its high Henry's Law constant and because it is processed in closed systems. In all, it is estimated that 0.1% of the produced quantity of H_2O_2 is released to the (aquatic) environment.

In the pulp industry, the amount of H_2O_2 lost to waste water is 2-10kg/t pulp for plants using mechanical processes, 0-2kg/t pulp for chemical processing plants and <1kg/t pulp from de-inking. H_2O_2 could not be detected in the effluent leaving these plants because of its rapid decomposition (Eka Nobel, 1990).

Nuclear power plant cooling water contains traces of H_2O_2 formed by radiochemical processes (Giguère, 1975; IARC, 1985).

Sterilisation of drinking water and food packaging. A potential source of H_2O_2 is from drinking water which has been treated with ozone and UV radiation. The authorised residual concentration of H_2O_2 in potable water is 0.1mg/l in the USSR (Antonova, 1974) and Germany (Bundesminister, 1990) and 0.5mg/l in France (Ministère de la Solidarité, de la Santé et de la Protection Sociale, 1990).

4.1.3 Domestic Sources

The domestic release of H_2O_2 is mainly from the use of sodium perborate (tetrahydrate and monohydrate) and sodium carbonate peroxyhydrate for laundering. The H_2O_2 concentration in the outlet of a washing machine ranges from 0-5mg/l, assuming that 4kg clothing, 80l water and 120g washing powder containing 15% tetrahydrate perborate (with 10% unreacted H_2O_2) are used in the whole washing cycle. In the case of France, with a perborate consumption of 80,000t/y, the amount of H_2O_2 released would be 1,700t/y (Chemoxal, 1992). The decomposition resulting from the mixing of washing effluents with other domestic waste water greatly reduces the H_2O_2 concentration in the inflow to municipal sewage treatment works.

4.1.4 Evaluation

H_2O_2 occurs naturally as a result of photochemical processes involving free radicals, organic matter and molecular oxygen.

H_2O_2 production in fresh and marine water depends upon the intensity of daylight and the concentration of promoters and dissolved oxygen. In the absence of light, H_2O_2 may be formed through the oxidation of iron and copper. Its concentration in soil depends on the presence of these metals and the dissolved oxygen content.

H_2O_2 concentrations in the atmosphere vary with temperature, solar radiation, humidity and the presence of precursors (CH_4, CO, volatile organic compounds) and inhibitors (SO_2, NO_x).

H_2O_2 is produced naturally by living organisms.

H_2O_2 emissions to the environment from industrial and domestic sources are limited due to its rapid decomposition in the waste water.

The amount of H_2O_2 deposited by rain-water into surface waters is greater than that from industrial and domestic sources together.

4.2 TRANSFORMATION

4.2.1 Aquatic Fate

H_2O_2 is subject to various reduction or oxidation processes in the environment and decomposes into water and oxygen at rates which depend on contact with catalytic materials and other factors (section 2.2.1). The actual concentration of H_2O_2 in the environment results from a dynamic equilibrium between its production and degradation (Figure 2). As a first approximation, degradation kinetics are generally assumed to be of a first order.

At ambient temperatures and concentrations, the rate of formation and degradation of H_2O_2 in sea-water varies widely, from 0.34-17µg/l/h (Johnson *et al*, 1987). Similar rates, 2-12µg/l/h, were found in freshwater (Cooper and Lean, 1989).

In sea-water, the oxidation of organic substrates by H_2O_2 catalysed by metals, under certain conditions, may be an important factor in the increase its degradation rate.

The catalytic action of iron and copper salts on the degradation of H_2O_2 is unlikely to affect the H_2O_2 content because at the same time it is formed by the oxidation of iron and copper (Weiss Mechanism) (Moffet and Zika, 1987).

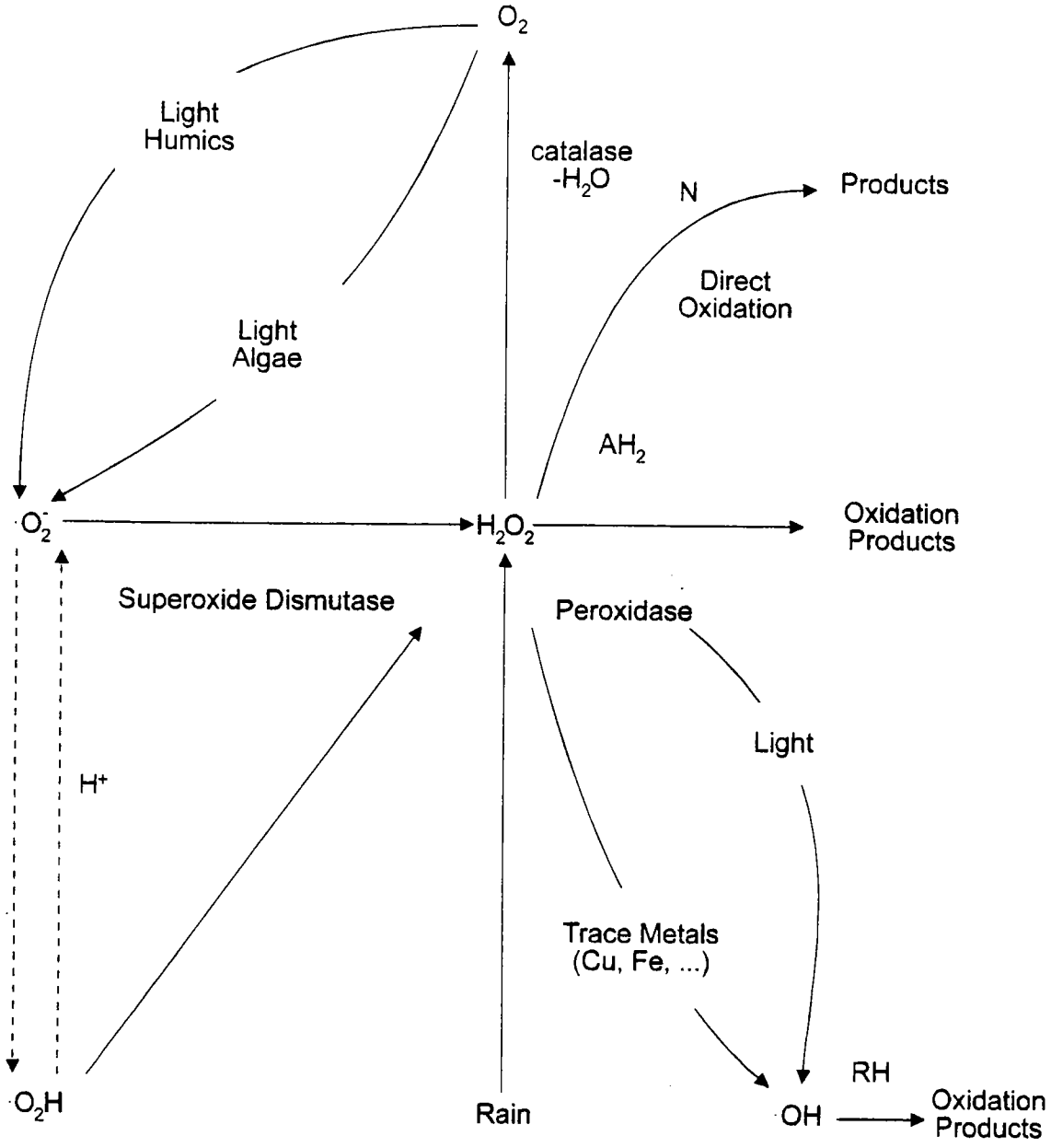
The half-life of H_2O_2 in sea-water samples from the Bay of Biscay (filtered 0.2µm) was 60h (Petasne and Zika, 1987).

The degradation of H_2O_2 in freshwater has been studied in Jacks Lake (Ontario). The half-life of H_2O_2 (initial concentration of 3.4µg/l) was:

7.8h (unfiltered lake water)
8.6h (filtered, 5µm)
31h (filtered, 1µm)
>24h (filtered, 0.45µm).

FIGURE 2

FORMATION AND DECAY IN AQUATIC ENVIRONMENTS (Zepp *et al*, 1987)



N Nucleophile that reacts with H_2O_2;

AH_2 Substrate capable of oxidation by peroxidases (horseradish peroxidase) and H_2O_2, e.g. phenols, anilines;

RH Organic chemical

These results indicate that the fraction containing picoplankton (defined as 0.2-2μm) contains the major proportion of the biological agent responsible for the degradation of H_2O_2. The fraction <1μm contained roughly 90% of the bacterial and <5% of the phytoplankton biomass (Cooper and Lean, 1989).

Laboratory studies on water from the River Saône showed that H_2O_2 degradation kinetics were of a first order and that half-lives were dependent on the initial H_2O_2 concentrations (Table 6). Filtering out particles over 0.2μm had little effect. Similar studies with de-ionised water containing 500mg/l H_2O_2 showed an increase in concentration, probably due to the influence of daylight. The decay of H_2O_2 appeared to be slower at initial concentrations <500mg/l (L'Air Liquide, 1991).

The half-life of H_2O_2 decreases with increasing size of the microbial population in water (Table 7).

TABLE 6

DEGRADATION IN THE RIVER SAÔNE

(L'Air Liquide, 1991)

Initial concentration (mg/l)	$t_{½}$ (days)	
10,000	2.5	
1,000	8.1	
500	8.2	±2*
250	15.2	±2.5*
100	20.1	

* Higher values for filtered samples

TABLE 7

DEGRADATION AND SIZE OF MICROBIAL POPULATION

(Degussa, 1991)

	Fresh Water	Process Water	Waste Water	Sludge
Cells/ml	$\leq 10^3$	$\leq 10^6$	$>10^6$	10^8-10^{10}
$t_{½}$	h-d	h-d	min-few h	few s

Catalase is a powerful decomposing agent: when 5μg of a 1μg/ml solution of catalase was added to 20ml of sun-lit water from the Patuxent River (saline, estuarine water), the degradation rate of H_2O_2 was sharply increased and the half-life reduced from >24h to 20min; in freshwater (River Paint Branch) without photolysis but with catalase, the half-life was 5min (Kieber and Helz, 1986). Similar results have been obtained in waste waters containing catalase, where H_2O_2 half-lives of 0.3-9min were measured (Barenschee, 1990).

Algae can photoproduce and decompose H_2O_2 (Zepp *et al*, 1987). For example, H_2O_2 in the dark was decomposed in the supernatant of an algal culture, the rate of decay being first order:

$$-\frac{d[H_2O_2]}{dt} = k_{bio}\, C_a\, [H_2O_2]$$

where C_a is the concentration of chlorophyl-*a* (Chl-*a*) and the median values of k_{bio} is $4.4 \times 10^{-3} m^3/mg$ Chl-*a*/h. Assuming that C_a is $100 mg/m^3$, the half-life of H_2O_2 in an euphotic zone of a eutrophic lake in the dark is estimated to be about 1.5h.

After exposure of a eutrophic water sample to sunlight for 3h, H_2O_2 concentrations (50-250μg/l) reached a plateau, indicating a steady state. In the dark, the half-life of H_2O_2 was measured as 1h with approximately first order kinetics (Draper and Crosby, 1983).

Ground-water. The half-life of H_2O_2 in ground-water, taken from wells in a shallow sand and gravel aquifer at 11-32m below ground level was <1h (Holm *et al*, 1987).

4.2.2 Atmospheric Fate

The tropospheric half-life of H_2O_2 is normally 10-20h (section 4.1.1). The reaction of H_2O_2 with ·OH radicals in the gas phase and subsequent photolysis are thought to be major degradation pathways in air (Sakugawa *et al*, 1990).

Photolysis. Photolysis of H_2O_2 leads to the formation of ·OH radicals. The photodecomposition of H_2O_2 in air occurs within a light spectrum of 380nm >λ >280nm. The extinction coefficient is independent of H_2O_2 concentration in air. The rate of decomposition for moderate light intensities (<1,017quanta/ls) is directly proportional to the concentration of H_2O_2 and to the square root of light intensity. The mechanism may be summarised (Schumb *et al*, 1955) as:

$$2H_2O_2 + h\nu \rightarrow H_2O + \cdot OH + \cdot HO_2$$

The ·OH radical is a scavenger for $\cdot HO_2$ according to:

$$\cdot OH + \cdot HO_2 \rightarrow H_2O + O_2$$

Reactions.. Formation of organic hydroperoxides (R-OOH) appears to be a pathway for the decomposition of H_2O_2 in the atmosphere. They are formed by ·OH-induced oxidation of hydrocarbons or by direct oxidation of aldehydes by H_2O_2 under acidic conditions (e.g. in clouds).

$$\text{aldehydes} + H_2O_2 \xrightarrow{\textit{acidic}} \text{hydroxyalkyl hyproperoxides}$$

Conversely, hydroperoxides can also produce H_2O_2 (section 4.1.1). The hydroperoxide content is 1-10% of the atmospheric H_2O_2 concentration (Hellpointner and Gaeb, 1989).

In clouds and aerosols the fate of H_2O_2 as an oxidising agent depends strongly on the pH of atmospheric water (Heikes, 1987). Measurements in cloud water made at the top of Whiteface Mountain (USA) showed that the degradation rate of H_2O_2 was 5.8%/h at a pH of 5.3-5.9 (Lazrus *et al*, 1985). Under more acidic conditions (pH <4.5) H_2O_2 may act as a replacement for ozone and oxidises sulphite to sulphate in the liquid phase; the oxidation rate of S^{4+} can be 100%/h. In humid air, the atmospheric level of sulphur dioxide varies inversely with the level of H_2O_2, especially during haze and fog, suggesting that H_2O_2 also contributes to the oxidation of sulphur dioxide. The rate of oxidation (a few percent of S^{4+}/h) in the gaseous phase is slower than in the liquid phase (Sagukawa *et al*, 1990).

A marked depletion of H_2O_2 has been shown to occur inside a cloud, both in the vapour and the liquid phase. For example, gaseous H_2O_2 concentrations were 0.4-0.5ppb inside a cloud and 1-1.25ppb outside, with SO_2 concentrations of 2-3ppb (Heikes *et al*, 1987). In a cloud, liquid phase H_2O_2 concentrations were 2-4 times lower than those resulting from the equilibrium with gaseous concentrations according to Henry's Law. The reactions are thought to be controlled by mixing of H_2O_2 from above the cloud with SO_2 from below (Barth *et al*, 1989).

Under strongly acidic conditions (pH <2), airborne H_2O_2 oxidised nitrite (HNO_2) to nitrate (HNO_3), which is also a substantial component of acid precipitation. At pH 2 and a H_2O_2 level of about 1ppb, 1% of the total amount of nitrous acid (gas and liquid) was converted within one hour; 1,5% was converted at pH 1 (Damschen and Martin, 1983). The reaction is:

$$HNO_2 + H_2O_2 \rightarrow HOONO + H_2O$$

$$HOONO \rightarrow H^- + NO_3^-$$

In conclusion, H_2O_2 plays a role in the generation of sulphate and, to a lesser extent, of nitrate in the atmosphere, either in clouds and aerosols or in dry air. Some researchers claim that ≥40% of sulphate acidity of precipitation derives from the reaction of sulphur dioxide with H_2O_2.

Deposition. Airborne H_2O_2 shows a strong tendency to dissolve in the aqueous phase. Henry's Law constant is H = 10^{-3}Pa.m^3/mol at 20°C. This is valid for H_2O_2 concentrations of 5.1-5,100mg/l (Hwang and Dasgupta, 1985). On the other hand, evaporating clouds can release considerable amounts of gaseous H_2O_2, a phenomenon which occurs even at night. The appearance of clouds lowers the gas-phase H_2O_2 mixing ratio. During wet deposition, H_2O_2 is efficiently removed from the atmosphere.

The above-mentioned reactions have been summarised in a one dimensional model, in which altitude is the only dimension (Thompson *et al*, 1988):

$$[H_2O_2] = \frac{k[\cdot HO_2]^2}{J\text{-}H_2O_2 + k[\cdot OH] + RO}$$

where J-H_2O_2, k[·OH], and RO are first order coefficients for H_2O_2 photolysis, reactions with the hydroxyl radical and rain-out phenomena respectively.

Tropospheric H_2O_2 concentrations vary by two orders of magnitude, depending on the geographical latitude (intensity and duration of light irradiation), altitude (intensity of UV radiation), water vapour content and the presence of precursors (CH_4, CO, VOC) and inhibitors (NO_x, SO_2). Deposition by rain-out and transport by wind naturally influence local H_2O_2 concentrations.

4.2.3 Fate in Soil

The fate of H_2O_2 in soil is determined by the following decomposition reaction:

$$H_2O_2 \xrightarrow{k_1} H_2O + \tfrac{1}{2}O_2$$

The rate of decomposition of H_2O_2 injected into, or spilled on soil depends on the microbiological flora and mineral composition of that soil.

Half-lives of H_2O_2 in soil have been shown to vary from 15h in soils without microbiological activity and few minerals, to several minutes in soils with 10^8-10^9cells/g total solids and in the presence of iron and manganese (Barenschee, 1990).

Studies have been made of the clean-up of polluted soils by enhancing the oxidation processes using H_2O_2 as a source of oxygen by injection into the infiltration water. When a 30% solution of H_2O_2 was added to recirculated soil water, the half-life was only 25 minutes (Spain *et al*, 1989).

A k_1 in the range of 0.1-0.01/min is expected for decomposition rates of H_2O_2 concentrations of about 300mg/l in ground-water (Hinchee and Downey, 1988). The half-life of H_2O_2 varied from 7-70min (Barenschee, 1990).

When k_1 is <0.01/min, only 1.3% of the available oxygen is used for microbial respiration. Thus, more than 98% of the oxygen produced by decomposition is present in the form of bubbles which escape to the surface either directly or dissolved in percolation water.

4.2.4 Bioaccumulation

H_2O_2 is decomposed by enzymatic action (section 7) and does not accumulate in cell systems.

4.2.5 Effects on Biological Treatment Plants

At concentrations ≤200mg/l in waste water, H_2O_2 does not affect the performance of biological treatment works (activated sludge process) (Solyom, 1973). It is partly degraded and has even been shown to stimulate biodegradation in waste water treatment works (activated sludge process) (Interox, 1990). At higher concentrations (>200mg/l) H_2O_2 becomes toxic to micro-organisms (section 6).

4.2.6 Evaluation

H_2O_2 formed in the atmosphere tends to condense and is removed by wet deposition. Reactions with hydroxy radicals and subsequent photolysis give a troposphere life of 10-20h.

Decomposition in water and soil takes minutes to several hours, depending on the mineral content and the concentrations of micro-organisms.

SECTION 5. ENVIRONMENTAL LEVELS AND HUMAN EXPOSURE

5.1 ENVIRONMENTAL LEVELS

5.1.1 Natural Waters

Sea-water. H_2O_2 has been detected in sea-water at concentrations ranging from 0.14-58µg/l (Table 8). The concentration of H_2O_2 is determined by several factors (section 4.1.1).

TABLE 8

LEVELS IN NATURAL WATERS

Location	Concentration (µg/l)	Reference
Sea Water		
Western Mediterranean Sea	> 3.4	Johnson *et al*, 1989
Gulf of Mexico	3.4 - 9.5	Zika *et al*, 1985a
Gulf of Peru	0.27 - 0.17	Zika *et al*,1985b
Chesapeake Bay	1.0 - 58	Helz and Kieber, 1985
	1.8 - 2.9	Kieber and Helz, 1986
Texan coastal waters	0.5 - 5.8	Van Baalen and Marler, 1966
North Atlantic	0.14 - 5.1	Zika, 1978*
Biscayne Bay/Florida	2.7 - 7.1	Zika, 1980*
Bahama bank	1.7 - 6.5	Zika, 1980*
Fresh Water		
Lakes Ontario, Erie, Jacks	0.34 - 27.2	Cooper and Lean, 1989 Cooper *et al*, 1989
Patuxent River	1.0 - 16	Kieber and Helz, 1986
Volga River	44.0 - 109	Sinel'nikov, 1971*
reservoir, Russia	24 - 44	Sinel'nikov, 1971*

* Quoted in Cooper *et al*, 1988

Spatial and temporal variations of H_2O_2 are principally due to changes in its photochemical production. For example, the concentration level dropped from 9.9µg/l at the surface to 1.1µg/l at 30m depth at a coastal station and from 5µg/l to 0.3µg/l at 100m depth at an ocean station. Variation of H_2O_2 concentrations occurred throughout the day, with mid to late afternoon maxima (9.9µg/l at 6p.m.) and pre-dawn minima (6.2µg/l at 6a.m.) at a coastal station. In oligotrophic open oceanic waters, photochemical generation still occurred at a depth of 75m, where the H_2O_2 concentration was 1.7µg/l; vertical mixing probably contributed to this. In coastal waters, where light attenuation is relatively greater, the rate of

photochemical formation of H_2O_2 decreases sharply with depth, resulting in concentrations dropping from 8.5-9.9µg/l at the surface to 0.7-1.1µg/l at 30m. The lifetime of H_2O_2 in coastal waters was relatively short, probably due to higher concentrations of transition metals and organic compounds. Thus, diel variations are more pronounced in coastal surface waters than in oligotrophic waters (Zika *et al*, 1985a).

Similar profiles and diel cycles have been observed in the western Mediterranean (Johnson *et al*, 1989). In waters off the coast of Peru, depth profiles and diel cycles were less pronounced and H_2O_2 levels did not increase nearer to the coast as in the Gulf of Mexico. The authors related this phenomenon to a lack of coastal vegetation (Zika *et al*, 1985b).

Freshwater. Surface levels of H_2O_2 in freshwater ranged from 0.34-109µg/l (Table 8). H_2O_2 concentrations declined with increasing depth (6µg/l at the surface to <0.7µg/l at 20m in lake Erie) and followed attenuation of light intensity. It has been suggested that bacteria and algae may also have had a role. Diel fluctuations in H_2O_2 levels indicate that photochemical processes could be involved in its formation (Cooper *et al*, 1989; Cooper and Lean, 1989).

5.1.2 Sewage - Waste Water

The concentration of H_2O_2 in a sample of raw sewage exposed to sunlight was 85µg/l. When samples from sewage stabilisation ponds were exposed to sunlight, concentrations of 420-1,100µg/l were encountered (Draper and Crosby, 1983).

5.1.3 Atmosphere (Table 9)

It should be noted that the analytical methods reported in the literature need further development (section 2.4).

Ambient air. In rural air, H_2O_2 concentrations ranged from 0.3-3ppb during day-time but could not be detected at night (<0.01ppb) (Das *et al*, 1983). Other results from a variety of locations ranged from 0.02-2.4ppb (Sakugawa and Kaplan, 1987; Slemr *et al*, 1986; Tanner *et al*, 1986; Barth *et al*, 1989). At the summit of Whiteface mountain (NY), Mohnen and Kadlecek (1989) found <2.7 to <6.1ppb H_2O_2. H_2O_2 concentrations were highest in the afternoon (Dollard *et al*, 1988; Possanzini *et al* 1988; Sakugawa and Kaplan, 1989).

Latitude dependency has been found with a concentration increase from north to south of 0.04-0.05ppb per degree of latitude (Van Valin *et al*, 1987). Values ranging from 0.2-3.9ppb in Brazil have been compared with those obtained using similar techniques in West Germany ranging from 0.01-0.6ppb (Jacob *et al*, 1987; Jacob *et al*, 1990). Heikes *et al* (1987) have also noted an increase of H_2O_2 concentration with latitude decrease near the surface layer (0.05ppb/degree of latitude) and above cloud top (0.1ppb/degree of latitude), but with an uniform distribution above 3,000m.

TABLE 9

LEVELS IN THE ATMOSPHERE

Sampling site, time, height	Concentration (ppb)	Reference
Ambient Air		
Rural air, day, ground	0.3-3	Das *et al*, 1983
night, ground	< 0.01	
Canada, Ontario, summer 1984-85, ground	< 0.3-2.1	Slemr *et al*, 1986
Upton, NY, summer-autumn 1985, ground	< 0.1-1.2	Tanner *et al*, 1986
Carolina coast, Jan.-Mar. 1986, cloud	< 0.2-2.4	Barth *et al*, 1989
Whiteface Mountain, NY, (1,500m), summer 1986 (463 samples)	< 2.7 (mean 0.6)	Mohnen and Kadlecek, 1989
summer 1987 (673 samples)	< 6.1 (mean 0.8)	
Southern England, Apr. 1987, ground	< 1.8	Dollard *et al*, 1988
Rome, Italy, Jan.-Mar. 1988, ground	< 0.2	Possanzini *et al*, 1988
Southern California, summer-autumn, 1985, ground	0.03-2.04	Sakugawa and Kaplan, 1987
1985-88, ground	0.02-1.2	Sakugawa and Kaplan, 1989
Dortmund, Germany, Oct. 1984-Jul. 1985, ground	0.03 (mean)	Jacob *et al*, 1987
Brazil, Mar.-Apr. 1988, ground	0.2-3.9	Jacob *et al*, 1990
Eastern USA, autumn 1984, cloud (150-3,700m)	0.2-4.1	Heikes *et al*, 1987
South central USA, Feb. 1987, cloud (1,700-2,600m)	< 0.1-1	Van Valin *et al*, 1987
Central USA, Jun. 1987, ground to 5.5km	< 0.2-7	Daum *et al*, 1990
Northeastern USA, Jun. 1987, ground to 4km	0.6-3.6	Van Valin *et al*, 1990
Summit of Whitetop Mountain, VA, (1,689m), summer 1986	< 0.02-2.6	Olszyna *et al*, 1988
fall 1986	< 0.02-0.57	
Central USA, 1987, (1,450-2,450m)	< 0.3-4.8	Boatman *et al*, 1989
Riverside, CA Hoboken, NJ, summer 1970, ground (smog)	40-180	Bufalini *et al*, 1972
California, south coast, summer 1970, ground (smog)	10-30	Kok *et al*, 1978a

TABLE 9 (continued)

Sampling site, time, height	Concentration (µg/l)	Reference
Rainwater		
Strasbourg, France	2-38	Lagrange and Lagrange, 1990
Dortmund, Germany, 1983-84, summer	25-2,200	Klockow and Jacob, 1986
winter	0-290	
Netherlands, Apr.-Jun. 1986	< 279	Keuken *et al*, 1987
North Sea, summer 1982	17-2,414	Roemer *et al*, 1985
winter 1981	0.34-6.8	
Florida and Bahama Islands, 1981	340-2,380	Zika *et al*, 1982
California, LA basin, 1978-79	1-159.8	Kok, 1980
Gulf of Mexico	390-2,800	Cooper *et al*,1987
Florida	130	
Summit of Whitetop Mountain, VA (1,689m), spring-autumn 1986	< 1.36-1,353	Olszyna *et al*, 1988
Eastern USA, summer 1982-83	3.4-2,142	Kelly *et al*, 1985
Ontario, Canada, Jan.-Feb. 1984	< 17-170	Daum, 1990
Brazil, Mar.-Apr. 1988	578-6,766	Jacob *et al*, 1990
Tokyo, Japan, 1981-82	6.8-1,064	Yoshizumi *et al*, 1984
Cloudwater		
Cumbria, Great Dun Fell, autumn	< 34	Dollard *et al*, 1988
spring	> 34	
North Sea, summer 1982, (150-3,000m)	< 41-3,000	Roemer *et al*, 1985
Eastern US, summer 1982-83, (< 3,000m)	3.4-3,400	Kelly *et al*, 1985
Eastern US, 1982-83, (450-1,500m)	< 2,550	Daum *et al*, 1984
California, LA basin, May 1982	31-2,992	Richards *et al*, 1983
California, LA basin, May-Jun. 1985	408-5,678	Richards, 1989
Carolina coast, US, Jan.-Mar. 1986	10.2-3,808	Barth *et al*, 1989
Summit of Whitetop Mountain, VA, spring-autumn 1986, (1,689m)	< 1.36-8,398 (mean: 880.6)	Olszyna *et al*, 1988

An increase of H_2O_2 concentration with increasing altitude has been observed by several workers (Heikes *et al*, 1987; Van Valin *et al*, 1987; Daum *et al*, 1990; Van Valin *et al*, 1990).

Seasonal differences in H_2O_2 concentration have been studied by Olszyna et al (1988) and Boatman *et al* (1989). The latter observed that the average H_2O_2 concentration varied by a factor of 16 (0.3-4.8ppb) between winter and summer.

Ice cores, drilled in Greenland and West-Antarctica, give an archive of solid precipitation over the past 50,000-100,000 years. H_2O_2 was one of the dominant trace components of the ice, indicating that it was the main trace species in clouds over remote and unpolluted areas. In the Greenland ice cores, the H_2O_2 level decreases from 92 ±76 to <0.5μg/kg ice with increasing depth from 26 to 25,000m and was extremely low during the last glaciation. In Antarctic cores, a H_2O_2 concentration was observed in the ice deposited 6,000-12,000 years ago, decreasing from 17.3 ±1.0μg/kg ice at 650m to 2.8 ±0.24μg/kg ice at 22,000m below the surface. The annual deposition rate of H_2O_2 at Greenland was calculated to be 30 times higher than at Antarctica (Neftel *et al*, 1984, 1986). Seasonal variations can be detected for both recent and ancient times.

H_2O_2 is a significant, active component of photochemical smog. Levels as high as 0.18ppm (0.25 mg/m^3) have been reported in severe smog (Bufalini *et al*, 1972). Concentrations during periods of moderate smog ranged from 10-30ppb (Kok *et al*, 1978a).

Stratosphere. At an altitude of 38.3km, the H_2O_2 level was 0.68 ±0.21ppb, while at 29.3km, the level was about 0.08ppb. Measured increases in H_2O_2 concentration with altitude were in disagreement with profiles obtained by mathematical modelling. The discrepancies were believed to be due to incomplete current knowledge of the stratospheric chemistry of H_2O_2 (Chance and Traub, 1987).

Rain-water and cloud-water. H_2O_2 is present in rain-water and cloud-water at a wide range of concentrations. In rain-water, levels ranged from 0-2,414μg/l in Europe, 1-2,800μg/l in the USA/Canada, 578-6,766μg/l in Brazil and 6.8-1,064μg/l in Japan (Table 9). The concentrations in summer were often much higher than those found in winter (Roemer *et al*, 1985; Klockow and Jacob, 1986). Concentrations in cloud-water in the USA ranged from 3.4-8,398μg/l. Measurements in cloud water 150-3,000m above sea level in background or barely polluted air masses from the North Sea, gave values from <41-3,000μg/l H_2O_2 (Table 9).

5.1.4 Soil

H_2O_2 was found in concentrations of 0.034-2.2µg/l in ground-water taken from wells 11, 15, 21 and 32m deep in a shallow sand and gravel aquifer (Holm *et al*, 1987).

5.2 HUMAN EXPOSURE

5.2.1 Non-Occupational Exposure

Human beings may be exposed to H_2O_2 as a result of its use in non-prescriptive pharmaceuticals (antiseptics, cleaning agents), cosmetics (hair bleaches, dentifrices, deodorants, mouthwashes), food, drinking water and because of its use as a sterilising agent for polymeric food packaging and for juice and wine processing.

Data on the quantity used per capita from preparations containing H_2O_2 are not available. It is generally only permitted for food processing if residual quantities are removed by appropriate physical and chemical means or if the residual level in food directly after aseptic packaging is 0.05% (500ppm) (US FDA, 1990).

Recent studies or other information on human exposure to H_2O_2 via foodstuffs are not available.

It should be noted that endogenous H_2O_2 levels in plant tissues are relatively high. For example, the concentration in tomatoes was 3.1-3.5ppm, in castor beans 4.7ppm and potato tubers 7.6ppm (Warm and Laties, 1982).

In Japan, H_2O_2 has been used as an antiseptic and bleaching agent for food manufacturing for several decades and was designated as a food additive with the enforcement of the Food Sanitation Act (1948). Since 1969, the residue level of H_2O_2 has been limited to 100ppm in noodles, kamaboko (fish sausage) and chikuwa (fish paste cooked in a bamboo-like shape) and 30ppm in other foods. During a survey in 1977-78, H_2O_2 was present in 1% of fish paste products, and in 30-50% of boiled noodles. In another study in 1977, high levels of H_2O_2 (≥30ppm) were found in 32% of boiled wheat-noodles. The intake per capita from these residues was calculated to be 4.3-13.6µg/kg (Koseishyo, 1980a). From October 1980, H_2O_2 should be decomposed or eliminated completely from the final food products (Koseishyo, 1980b).

Evaluation. Exposure of the general population from ambient air is usually <4µg/m^3. However, exhaled air contains 300-1,000µg/m^3 (section 4.1.1). Therefore ambient air is not a significant source of exposure, except in foggy conditions. Total human exposure cannot

be assessed due to a lack of data on foodstuffs, drinking water, non-prescriptive pharmaceuticals and cosmetics.

5.2.2 Occupational Exposure

The national regulations and guidelines on occupational exposure limits for H_2O_2 are listed in Table 10. In general, the time-weighted average concentration over an 8h working day which should not be exceeded is 1ppm (1.414mg/m^3); this level should not be higher than 2-3ppm (2.8-4.2mg/m^3) for any short period of exposure (5-15min).

The maximum concentration from which one could safely escape within 30 minutes without a respirator (or in the event of respirator failure) has been estimated to be 75ppm; this concentration is thought not to cause any impairment or irreversible health effects "immediately dangerous to life and health" (NIOSH, 1990).

The US-National Institute for Occupational Safety and Health estimated that 52,800 US-workers were potentially exposed to H_2O_2 in 18 different industry sectors in 1982 (OSHA, 1989a).

Limited data on work exposure levels are reported in Table 11.

TABLE 10

NATIONAL OCCUPATIONAL EXPOSURE LIMITS

Country, year of enforcement	TWA (8h) concentration (mg/m^3)	Short-term exposure or excursions (mg/m^3)	Ceiling value (mg/m^3)	Legal status*	Reference
Australia, 1990	1.4	-	-	R	ILO, 1991
Belgium, 1990	1.4	-	-	R	Cardinaels, 1990
Bulgaria, 1971	-	-	1	R	IARC, 1985
Canada, 1989	1.4	3	-	R	Québec, 1989
Denmark, 1988	1.4	-	-		Arbejdstilsynet, 1988; ILO, 1991
Finland, 1990	1.4	4.2 (15 min)	-	G	ILO, 1991
France, 1988	1.4	-	-	G	INRS, 1987
Germany, 1991	1.4	3 (5 min)	-	G	DFG, 1991
Italy, 1990	1.4	-	-	G	ACGIH, 1991
Netherlands, 1989	1.4	-	-	G	Arbeidsinspectie, 1989
Norway, 1990	1.4	2.8 (15 min)	-	G	Arbeidstilsynet, 1989
Switzerland, 1990	1.4	-	-	R	CNA, 1987; ILO, 1991
UK, 1991	1.4	3 (10 min)	-	R	UK HSE, 1991
USA,					
- OSHA, 1989	1.4	-	-	R	OSHA, 1989b
- ACGIH, 1990-91	1.4	-	-	G	ACGIH, 1986, 1991
USSR, 1977	-	-	2	R	IARC, 1985; INRS, 1990
Yugoslavia, 1971	-	-	1.4	R	IARC, 1985
Sweden, 1991	1.4	3 (15 min)	-	R	AFS, 1990
Thailand	1.4	-	-	Unknown	Hasle, 1987

* R, regulatory; G, guideline.

TABLE 11

OCCUPATIONAL EXPOSURE LEVELS

Process, location	Year of measurement	Concentration (mg/m^3)	Reference
Production plant			
general work area	1987	0.85	ECETOC, 1991
	1989	0.07-0.14	
	1989	5.7[a]	
drum filling station (during filling)	1987	2.8	ECETOC, 1991
	1989	2.8	
	1979	0.3	
	1982, 1990	0.3 - 2.0	
storage/shipping tank car loading near storage tank	1983	< 0.01	ECETOC, 1991
	1979	0.3	
	1982	1-2	
pump house (unmanned)	1982	10, 2[b]	ECETOC, 1991
Packaging machine			
coffee cream, during startup	Unknown	< 0.20	Suenaka *et al*, 1984
	Unknown	0.21 - 1.2	
milk	1987	12-42	Kaelin *et al*, 1988
		1.5, 4.5[c]	
fruit juice	1983-1988	0.2-0.66	Tetra Pak, 1991

a Over opened vessel with 70% solution.

b Before and after reparation of leaking seal box.

c Before and after installation of ventilation.

SECTION 6. EFFECTS ON ORGANISMS IN THE ENVIRONMENT

6.1 MICRO-ORGANISMS

Several authors studied the bactericidal efficiency of H_2O_2 in sterilisation of milk and food material packaging. The rate and extent of destruction varied with the organisms tested, the contact time and temperature (Table 12).

H_2O_2 has been shown to be more effective as a sporicide than as a bactericide. H_2O_2 was bacteriostatic at concentrations >5.1mg/l (Baldry, 1983).

The 16-18h EC_{10} to *Pseudomonas putida* was 11mg/l (Knie *et al*, 1983).

Of bacteria indigenous in dental plaque, *Actinobacillus actinomycetem* - comitans was more resistant to H_2O_2 (not completely killed at 34mg/l H_2O_2) than *Haemophilus aphrophilus*, which was destroyed after 1h exposure to 1.7mg/l H_2O_2 (Miyasaki *et al*, 1985, 1987).

6.2 AQUATIC ORGANISMS

6.2.1 Fish (Table 13)

Studies with several fish species showed no effects following exposure to H_2O_2 concentrations ranging from 2 to 40mg/l. The 96hLC_{50} was 37.4mg/l for Channel catfish (*Ictalurus punctatus*), and 16.4mg/l for fathead minnow (*Pimephales promelas*) with a NOEC of 5mg/l.

H_2O_2 did not affect glutamic oxalacetic transaminase activity, but inhibited lactic dehydrogenase activity in blood plasma of white sucker (*Catostomus commersoni*) exposed to 2,000mg/l for 2 weeks (Christensen, 1971/72). H_2O_2 (340mg/l) did not affect acetylcholinesterase activity prepared from muscle of a fathead minnow (*Pimephales promelas*, Rafinesque) (Olson and Christensen, 1980).

There was no evident effect on the dispersal of schooling of *Kuhlia sandvicenis*, a saltwater fish, at a concentration of 20mg/l H_2O_2 (Hiatt *et al*, 1953).

The Task Force is aware of, but unable to obtain data on acute toxicity studies with 35% H_2O_2 in Bluegill sunfish (*Lepomis macrochirus*) and Rainbow trout (*Salmo gairdneri*), submitted confidentially to the US EPA (McAllister and Cohle, 1984a,b).

TABLE 12

TOXICITY TO MICRO-ORGANISMS

Species	Duration (h)	Temperature (°C)	Lethal concentration (mg/l)	Reference
Aerobic, non-pathogenic				
Enterobacter aerogenes	4	Ambient	EC_{10} : 50	Lal *et al*, 1985
	2	Ambient	EC_{100} : 300	
pathogenic				
Salmonella typhosa	4	Ambient	EC_5 : 50	Lal *et al*, 1985
	4		EC_{13} : 300	
	30 min	54	EC_{100} : 600	Naguib, 1972
Staphylococcus aureus	4	Ambient	EC_7 : 50	Lal *et al*, 1985
	4	Ambient	EC_{26} : 300	
	30 min	54	EC_{100} : 600	Naguib, 1972
	2	Ambient	EC_{100} : 90	Yosphe *et al*, 1968
Salmonella typhimurium	15 min	54	EC_{100} : 1,000	Mahmoud *et al*, 1984
	1	5	EC_{99} : 5,000	Uenluetuerk and Turantas, 1987
Anaerobic, non-pathogenic				
Streptococcus lactis	4	Ambient	EC_{10} : 50	Lal *et al*, 1985
	4	Ambient	EC_{20} : 300	
	15 min	54	EC_{100} : 1,000	Mahmoud *et al*, 1984
Streptococcus faecalis	15 min	54	EC_{100} : 1,000	Mahmoud *et al*, 1984
non-pathogenic sporformer				
Bacillus subtilis	4	Ambient	EC_7 : 50	Lal *et al*, 1985
	4	Ambient	EC_9 : 300	
Streptococcus thermophilus	15 min	54	EC_{100} : 1,000	Mahmoud *et al*, 1984

TABLE 12 (cntd)

TOXICITY TO MICRO-ORGANISMS

Species	Duration (h)	Temperature (°C)	Lethal concentration (mg/l)	Reference
Anaerobic, pathogenic				
Escherichia coli	2	Ambient	EC_{52} : 30	Yosphe *et al*, 1986
	5	Ambient	EC_{70} : 30	
	4	Ambient	EC_{20} : 50	Lal *et al*, 1985
	5	Ambient	EC_{94} : 90	
	2	Ambient	EC_{100} : 200-300	
Clostridium botulinum	6 min	55	EC_{100} : 50	Ito *et al*, 1973
	2 min	55	EC_{100} : 200	
pathogenic sporformer				
Clostridium perfringens	15 min	54	EC_{100} : 1,000	Mahmoud *et al*, 1984
Clostridium butyricum	15 min	54	EC_{100} : 1,000	Mahmoud *et al*, 1984
	30 min	54	EC_{100} : 600	Naguib, 1972
Aerobic/anaerobic, non-pathogenic				
Lactobacillus vulgaricus	4	Ambient	EC_{16} : 50	Lal *et al*, 1985
	4	Ambient	EC_{22} : 300	
non-pathogenic sporformer				
Bacillus cereus	4	Ambient	EC_{5} : 50	Lal *et al*, 1985
	4	Ambient	EC_{9} : 300	
	15 min	54	EC_{100} : 1,000	Mahmoud *et al*, 1984
pathogenic				
Listeria monocytogenes	9-24	15	EC_{100} : 710	Dominguez *et al*, 1987

TABLE 13

TOXICITY TO FISH

Species	Effect	Concentration µg/l	Reference
Freshwater Fish			
Rainbow trout, fingerling *(Salmo gairdneri)*	Lethality, 48h	>40	Southgate, 1950; McKee, 1963
Squawfish *(Ptychocheilus oregonensis)* Coho salmon *(Oncorhynchus kisutch)*	No mortality LC_0, 24h	10	MacPhee and Ruelle, 1969
Channel catfish *(Ictalurus punctatus)*	LC_{50}, 96h	37.4	Kay *et al*, 1982
Mosquito fish *(Gambusia affinis)*	Unharmed (field study)	2.38-9.86	Kay *et al*, 1982
Guppy *(Lebistes reticulatus)*	Unharmed	34	Quimby, 1981
Golden orfe *(Leuciscus idus melanotus)*	LC_{50}, 24h	35	Degussa, 1977
Fathead minnow *(Pimephales promelas)*	LC_{50}, 96h NOEC	16.4 5	Shurtleff, 1989
Marine Fish			
Kuhlia sandvicenis	Behaviour not affected	20	Hiatt *et al*, 1953

6.2.2 Crustacea (Table 14)

Immobilisation by H_2O_2 of a variety of crustaceans was seen at concentrations between 2.4-7.7mg/l. Crayfish (*Procambarus clarkii*) were not affected by 64.6mg/l H_2O_2 (Kay *et al*, 1982).

6.2.3 Molluscs (Table 14)

The 96h LC_{50} for *Physa* sp., a freshwater snail, was 17.7mg/l (Kay *et al*, 1982).

In the marine environment, H_2O_2 at a concentration of 170mg/l caused synchronous spawning in male and female red abalones (*Haliotis rufescens*). The authors suggested that H_2O_2 activates the prostaglandin endoperoxide-forming cyclo-oxygenases (Morse *et al*, 1976).

6.2.4 Insects (Table 14)

Aquatic insects were unharmed at H_2O_2 concentrations of 170-218mg/l (Kay *et al*, 1982).

6.2.5 Algae (Table 15)

H_2O_2 was tested as a potential algicide for fresh aquaculture, where effects on several species were measured in terms of chlorophyll reduction at concentrations of 1.7mg/l and above (Kay *et al*, 1982).

It was highly toxic in one species of marine algae and may be a natural growth-inhibiting factor (Florence and Stauber, 1986).

6.2.6 Aquatic Plants (Table 15)

H_2O_2 is toxic to hydrilla (*Hydrilla verticillata*) and coontail (*Ceratophyllum demersum*) at concentrations ranging from 34-136mg/l, but does not affect alligator weed or water hyacinths (*Eichhornia crassipes*) (Quimby, 1981).

TABLE 14

TOXICITY TO AQUATIC INVERTEBRATES

Species	Effect	Concentration (mg/l)	Reference
Freshwater Crustacea			
Gammarus	LC_{50}, 96h mortality	4.42	Kay *et al*, 1982
Daphnia magna	$EC(I)_{50}$, 24h Immobilisation	7.7	Bringmann and Kuehn, 1982
	EC_0	3.8	
Daphnia pulex	Immobilisation 5min.	4.2	Gannon and Gannon, 1975
	LC_{50}, 48h	2.4	Shurtleff, 1989
	NOEC	1.0	
Crayfish *(Procambarus clarkii)*	LC_0 No mortality	64.6	Kay *et al*, 1982
Freshwater Snail			
Physa sp.	LC_{50}, 96h	17.7	Kay *et al*, 1982
Marine Mollusc			
Red abalones *(Haliotis rufescens)*	Induced spawning	170	Morse *et al*, 1976
Insects			
Stratiomyd fly *(Statiomis* sp.)	No effect	217.6	Kay *et al*, 1982
Dragonfly naiads *(Pachydiplax longipennis)*	No effect	170	Morse *et al*, 1976

TABLE 15

TOXICITY TO AQUATIC ALGAE AND PLANTS

Species	Effect	Concentration (mg/l)	Reference
Freshwater Algae			
Anabaena	Chlorophyll reduced to 5% after 24h	9.86	Kay *et al*, 1982
Ankistrodesmus	Chlorophyll reduced to <5% after 24h	17	Kay *et al*, 1982
Raphidiopsis	Chlorophyll reduced to <5% after 24h	6.8	Kay *et al*, 1982
Microcystis	Chlorophyll reduced to <6% after 48h	1.7	Kay *et al*, 1982
Marine Algae			
Nitzchia closterium	Cell count reduced to 50%	0.85	Florence and Stauber, 1986
Freshwater Plants			
Ceratophyllum demersum	80% necrosis continuous exposure	34	Quimby, 1981
Hydrilla verticillata	30% necrosis 1h exposure	34	Quimby, 1981
	80% necrosis	136	Quimby, 1981

6.3 TERRESTRIAL PLANTS

There were no significant effects on growth, seed germination and early seed growth of rice, corn, soybeans, tomatoes or barnyard grass when irrigated with water containing H_2O_2 at concentrations ranging from 3.4-217.6mg/l (Kay *et al*, 1982).

Severe changes have been observed in the internal structure of needles of young Norwegian spruces (*Picea abies*) and leaves of red beeches (*Fagus sylvatica*) following exposure to acidic fog (pH 4) containing H_2O_2 (0.7-5ppm, 3h/d) for 6-8 weeks, e.g. a decrease in all histological parameters studied and an increased accumulation of phenols in the needle cells. The changes were similar to those observed during forest decline and may eventually reduce the transport capacity of assimilation products and water (Masuch and Kettrup, 1986; Mallant *et al*, 1988; Masuch *et al*, 1989).

6.4 SOIL ORGANISMS

Apart from data on micro-organisms (section 6.1), no data are available.

6.5 WILDLIFE

The Task Force is aware of, but unable to obtain data on oral toxicity studies with 35% H_2O_2 in Mallard duck and Bobwhite quail, submitted confidentially to EPA (Roberts and Phillips, 1985a,b,c).

6.6 ECOSYSTEMS

Lake Morillon was treated with H_2O_2 to oxidise sulphur compounds and to improve amounts of dissolved oxygen. Due to the physical impact of the large volume of gas produced, the biological equilibrium was strongly disturbed, with a decreased chlorophyll content and phytoplankton biomass. These disturbances were transient (Balvay, 1981).

6.7 EVALUATION

The first signs of growth inhibition of bacteria are seen from a concentration of 5mg/l of H_2O_2. Lethality is observed from 10mg/l, depending on the type of micro-organism.

Several fish species are unharmed at concentrations up to 40mg/l H_2O_2. The 96h LC_{50} in certain fish species was 16.4-37.4mg/l.

Freshwater algae, microcrustacea and freshwater molluscs are affected by H_2O_2 concentrations from 2-20mg/l, whilst 1mg/l affects certain marine algae.

H_2O_2 is toxic to certain aquatic plants at 34-136mg/l, but has no effect on terrestrial plants at concentrations up to 218mg/l in irrigation water.

Experiments on spruce needles and beech leaves exposed to acidic fog containing 0.7-5ppm H_2O_2 have confirmed that a role for H_2O_2 in forest decline cannot be excluded.

SECTION 7. KINETICS AND METABOLISM

It is difficult to obtain information about the toxicokinetic processes involving exogenous H_2O_2 in mammals because of the ubiquitous presence of an active defence mechanism against oxidising agents. It will quickly decompose to oxygen and water at the absorption site before it can reach other tissues and organs via the blood circulation.

7.1 ABSORPTION

There is limited information on the absorption of H_2O_2. After local application of H_2O_2 solutions (1-30%) to human skin, tongues of cats and dogs, rat's foot pads and hearts, a characteristic blanching of the exposed tissue area was observed (Hauschild *et al*, 1958; Ludewig, 1959). Oxygen bubbles occurred in the tongue and jugular veins of dogs, cats and rabbits after sublingual administration of H_2O_2 solutions (3% or 30%) (Ludewig, 1959).

Following sublingual administration of ^{18}O-labelled H_2O_2 (19% aqueous solutions) to cats, rapid absorption occurred, the decomposition product ($^{18}O_2$) being transported to the lungs. After 18 minutes 7% and after 34 minutes 30% of $^{18}O_2$ was detected in the expired air, with no increase in ^{18}O-carbon dioxide (Ludewig, 1964).

The perfusion of the large intestine of dogs with diluted H_2O_2 solutions raised the oxygen saturation of blood in the portal vein. No attempt was made to determine if H_2O_2 decomposition occurred before or after absorption (Urschel, 1967).

7.1.1 Mechanism of Absorption

The permeability constant of erythrocyte membrane for H_2O_2 is approximately 0.04cm/min (Nicholls, 1972), and for peroxisomal membrane 0.2cm/min (De Duve, 1965). The permeability of biological membranes to H_2O_2 is comparable to that of water (Dick, 1964). The permeability of the erythrocyte membrane to oxygen is higher than to H_2O_2 (Nicholls, 1972).

Significant amounts of topically applied H_2O_2 can penetrate the epidermis or mucous membranes followed by rapid spontaneous or enzyme-catalysed decomposition to oxygen and water in the underlying tissue. The formation of gaseous oxygen causes capillary microembolism and prevents irrigation of tissues by blood resulting in a visible, reversible bleaching of the exposed tissue area (Hauschild *et al*, 1958). Large volumes of gaseous oxygen (1ml of 30% H_2O_2 yields approximately 100ml oxygen) within tissues can lead to the detachment of epithelial cell masses and mechanical rupture of tissues causing haemorrhage

or even the rupture of whole organs, e.g. the large intestine (Sheehan and Brynjolfson, 1960; Ludewig, 1965; Urschel, 1967).

Locally formed oxygen is removed by the blood. Toxicity is enhanced by i.v. administration which causes symptoms of gas embolism (Ludewig, 1959).

The i.v. toxicity of highly concentrated H_2O_2 solutions (90%) is related to the degree of decomposition at the site of administration as well as the administered dose. The higher the concentration of H_2O_2, the greater the local destruction and breakdown so that less H_2O_2 is available for producing systemic toxic effects (Hrubetz *et al*, 1951).

Administration by other routes also shows decreased absorption rates with higher concentrations of H_2O_2 (Hrubetz *et al*, 1951). Dieter (1988) explained this by the local, massive liberation of oxygen bubbles which obstructed blood flow, thus preventing H_2O_2 entering the general circulation and exerting systemic effects.

Thus, little is known about the mechanisms of absorption of exogenous H_2O_2 because it is difficult for the substance to enter body tissues intact. There is, however, ample information on the endogenous formation and fate of H_2O_2 in body tissues.

7.2 ENDOGENOUS FORMATION

H_2O_2 is normally found in each aerobic cell as an endogenous metabolite. It is generated during cell respiration by various metabolic process (e.g. oxidase-catalysed reactions), by oxidative stress (i.e. super-oxide anion degradation by superoxide dismutase) and by patho-physiological reactions such as those involving activated phagocytes (Fridovich, 1978; Chance *et al*, 1979; Sies, 1985).

Most of the molecular oxygen consumed in mammalian organisms is reduced to water via oxidative phosphorylation in mitochondria (cytochrome oxidase), without the formation of oxygen intermediates. A small proportion is decomposed via specific pathways yielding reactive oxygen species including singlet oxygen ($^1O_2$), superoxide radical anion ($\cdot O_2^-$), H_2O_2 (H_2O_2) and hydroxyl radical ($\cdot OH$):

$$O_2 \xrightarrow{e^-} \cdot O_2 \xrightarrow[2H^-]{e^-} H_2O_2 \xrightarrow[H^-]{e^-} H_2O + \cdot OH$$

These reactive oxygen species are formed during enzymatic and spontaneous redox reactions such as the reduction and oxidation of hydroquinone, xanthin, haemoglobin or catecholamines

(Vuillaume, 1987). A characteristic of these enzyme reactions is the role of transition metals, especially copper, manganese, iron and selenium (Sies, 1985).

Mitochondria, microsomes, peroxisomes and cytosol contain a number of H_2O_2 generating enzymes including superoxide dismutase (SOD), several oxidases (e.g. glycolate oxidase, urate oxidase, fatty acyl CoA-oxidase) and several peroxidases, cyctochrome P-450 dependent mono-oxygenases and flavin dehydrogenases (Hemmerich *et al*, 1970; Dixon, 1971; Boveris *et al*, 1972; Bors *et al*, 1974). During substrate oxidation oxygen is reduced by these enzymes in an univalent or divalent step to superoxide anion or to H_2O_2 (Sies and Chance, 1970; Misra and Fridovich, 1972; Chance *et al*, 1979; Fridovich, 1983):

$$O_2 + e^- \rightarrow \cdot O_2^-$$

$$O_2 + 2e^- + 2H^- \rightarrow H_2O_2$$

The reduction of oxygen to superoxide anion is preferred because of an electron spin restriction (Fridovich, 1983). The superoxide anion is considered to be a precursor of H_2O_2 (Loschen *et al*, 1974; Boveris, 1977). The formation rate of superoxide anion in mammalian liver is estimated to be 24nmol $\cdot O_2^-$/min.mg protein and the intramitochondrial steady state concentration 10^{-11}mol $\cdot O_2^-$/mg tissue (Fridovich, 1983).

The predominant reaction of superoxide anions is dismutation to H_2O_2 and oxygen. The reaction can occur spontaneously or is catalysed by SOD (Fridovich, 1983):

$$\cdot O_2^- + \cdot O_2^- + 2H^- \rightarrow H_2O_2 + O_2$$

In an aqueous solution, spontaneous dismutation is a second-order reaction and the half-life of $\cdot O_2^-$ is an inverse function of its initial concentration, whereas the SOD-catalysed reaction is first order and the $\cdot O_2^-$ half-life is independent of its initial concentration. Assuming typical concentrations of 10^{-5}mol SOD/mg tissue and 10^{-11}mol $\cdot O_2^-$/mg tissue (steady state), Fridovich (1983) calculated the rate of catalysed elimination of $\cdot O_2^-$, and subsequent formation of H_2O_2, to be 10^{10} times higher than spontaneous dismutation.

SOD is specific for $\cdot O_2^-$ substrate and is present in several subcellular compartments such as cytosols, mitochondria, microsomes and nuclei, where $\cdot O_2^-$ formation may occur. Induction of higher concentrations of SOD in cells can be induced by increased $\cdot O_2^-$ levels in rat lung and rat liver (section 7.3.2), in endothelial cells and also in bacteria (Fridovich, 1983).

In perfused rat liver, H_2O_2 is dependent on the substrate used and a great variation in the results is obtained. Uric acid led to a production rate of 100-300nmol H_2O_2/min/g liver (wet weight)

(Boveris *et al*, 1972). Sies (1981) measured a formation of 80nmol H_2O_2/min/g liver (wet-weight) during the oxidation of decanoate by perfused rat liver isolated from normally fed rats. Succinate added to rat liver mitochondria produced approximately 0.5µmol H_2O_2/min/mg protein (Boveris and Chance, 1973; Nohl and Hegner, 1978). The maximum rate of induced H_2O_2 formation was 11-15µmol H_2O_2/min/g liver (Tamura *et al*, 1990).

Boveris *et al* (1972) estimated the total rate of H_2O_2-production in rat liver to be 90nmol (3.1µg) H_2O_2/min/g liver (wet weight) under physiological conditions.

Using the value reported by Boveris *et al* (1972), H_2O_2 production in human liver was estimated to be 270mg/h under normal conditions and even greater when stimulated by appropriate substrates (US FDA, 1983).

The neutrophils in patients with chronic granulomatosis have a defect in their NADPH oxidase-superoxide generating system and these cells generate less endogenous H_2O_2 (Baehner *et al*, 1982).

7.3 METABOLISM (Figure 3)

7.3.1 Enzymatic Metabolism

In aerobic cells, the catabolic pathways of H_2O_2 are determined by catalase, peroxidases and glutathione peroxidase enzymes.

Catalase. The decomposition of H_2O_2 by catalases is shown as follows:

$$H_2O_2 + H_2O_2 \rightarrow 2H_2O + O_2$$

Catalase is present at a wide range of concentrations in nearly all mammalian cells and is particularly efficient in dealing with large amounts of H_2O_2 (Chance *et al*, 1979). The enzyme is located in subcellular compartments, mainly in peroxisomes (De Duve and Baudhuin, 1966). Catalase was found in a soluble state only in erythrocytes (Saito *et al*, 1984).

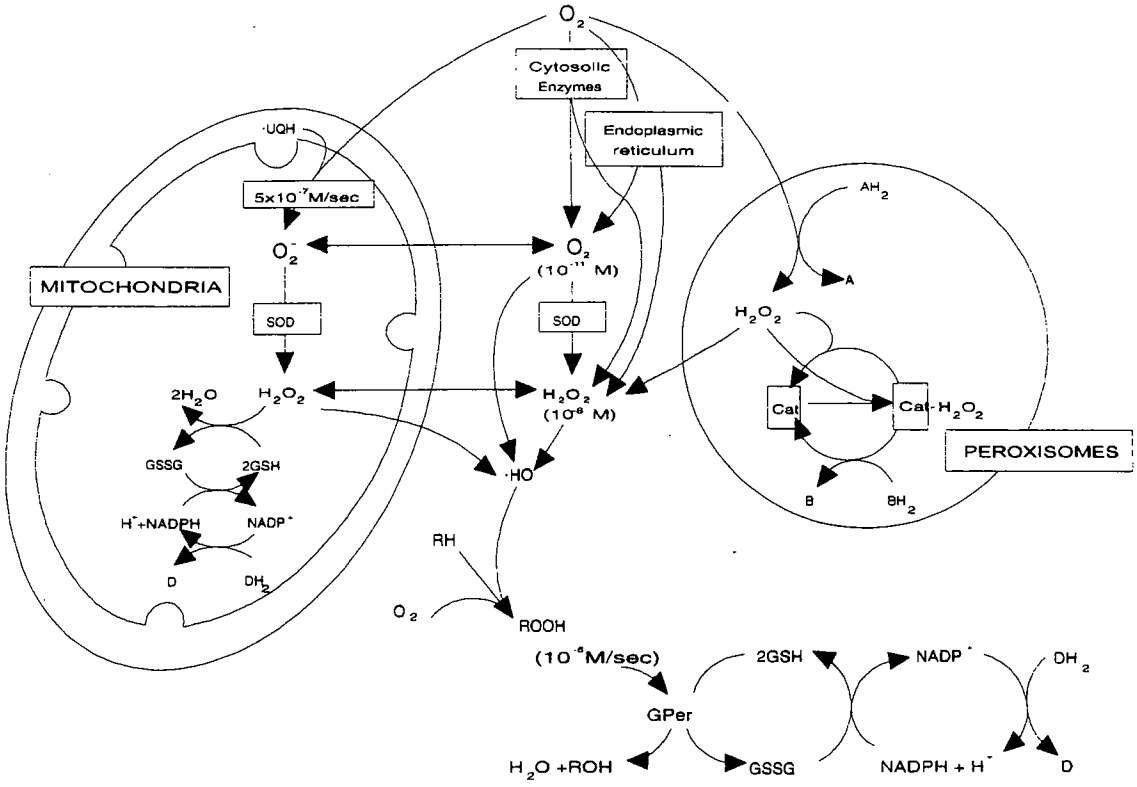
In mammalian tissue, the highest catalase activity is observed in the duodenum, liver, spleen, kidney, blood, mucous membranes and other highly vascularised tissues; the lowest catalase activity occurs in the brain, thyroid, testes and connective tissues (e.g. Table 20, controls) (Matkovics and Novak, 1977).

Ito *et al* (1984) measured catalase activity in duodenal mucosa, blood and liver in four strains of mice. Activity in the liver was 8-10 times that in blood and 14-83 times that in duodenal mucosa. Differences in catalase activity as high as 20-fold were seen in the same organs

FIGURE 3

METABOLISM OF HYDROGEN PEROXIDE

(Chance *et al*, 1979)



Concentrations and formation rates of oxygen metabolites are estimated.

·UQH, ubiquinone radical; GSSG, oxidised glutathione; GSH, reduced glutathione; DH_2 and D, a non-specified NADP-reducing system; SOD, superoxide dismutase; NADPH and NADP, nicotinamide adenine dinucleotide phosphate; O^-_2, superoxide anion; ·OH, hydroxyl radical; ROOH, an alkyl hyperoxide; GPer, glutathione peroxidase; Cat, catalase; B and BH_2, hydrogen donors of a specificity appropriate to catalase, such as ethanol.

of the different strains (Table 16). In gastro-intestinal tissue catalase activity varied among different strains of mice. In rats, duodenal catalase levels are orders of magnitude greater than those in mice strains (Table 17). This difference may explain the different responses of these two species to exogenous H_2O_2 in drinking water (section 8.4.2). Data on catalase levels in human beings are limited. Catalase levels in human jejunum biopsies were several times higher than those in mice (Table 17). However, the data on different species are not completely comparable due to differences in assay conditions, tissue preparation and analytical sensitivities of the different studies.

TABLE 16

CATALASE ACTIVITY IN 4 STRAINS OF MICE AT 6-8 WEEKS OF AGE

(Ito *et al*, 1984)

Organ or tissue	Catalase activity (10^{-6}mol/min/mg protein) in strain:			
	C3H/HeN[a]	B6C3F1[b]	C57BL/6N[c]	C3H/C[d]
Duodenal mucosa	5.3	1.7	0.7	0.4
Blood	7.8	7.7	5.1	0.4
Liver	75.3	62.8	40.7	33.3

a C3H/HeN, high catalase activity;
b B6C3F1, F_1 hybrid of C3H and C57BL, with "normal" catalase activity;
c C57BL/6N, low catalase activity;
d C3H/C, mice with hypocatalasemia.

TABLE 17

GASTRO-INTESTINAL CATALASE LEVELS IN VARIOUS SPECIES

Species, strain	Tissue	Catalase Level (U/mg protein)	Reference
Human	Jejunum	0.065 ±0.0047 (n=3)	Dawson *et al*, 1981
	Gastric antrum	0.048 ±0.0007 (n=5)	
Mouse, C3H/HeN	Duodenum	0.0317 ±0.0084 (n=11)	Ito *et al*, 1984
Mouse, B6C3F_1	Duodenum	0.0102 ±0.0012 (n=12)	
Mouse, C57BL/6N	Duodenum	0.0042 ±0.0018 (n=8)	
Mouse, C3H/C^{b}g	Duodenum	0.0024 ±0.0006 (n=7)	
Rat, Wistar	Duodenum	2.42 ±0.6*	Manohar and
	Jejunum	1.60 ±0.1*	Balasubramanian, 1986

* Number of samples used to calculate the mean not stated.

In human serum, catalase activity was 3,600 times lower than in erythrocytes (Goth *et al*, 1983). Plasma activity may increase during certain disease states, especially in haemolytic and pernicious anaemia (Goth *et al*, 1983; Winterbourn and Stern, 1987). In human plasma, the decomposition is 0.01-0.05mol H_2O_2/l/min (Yamagata and Seino, 1953).

Erythrocytes can degrade gram quantities of H_2O_2 in several minutes (Yamagata *et al*, 1952; Winterbourn and Stern, 1987). During and after i.v. infusion of 1.0M H_2O_2 in a 0.9% saline solution for 60min at a rate of $3x10^{-3}$mol H_2O_2/min, H_2O_2 could not be detected in dog plasma (Nahum *et al*, 1989).

A rare genetic defect in red cell catalase activity (acatalasemia) is thought to be inherited as an incomplete autosomal recessive trait. Heterozygous individuals carrying the acatalasemic gene have blood catalase activity levels below normal (hypocatalasemia). The blood catalase activity levels in normal, hypocatalasemic and acatalasemic individuals in Japan were found to be 3,380 ±180 (n=5), 1,520 ±350 (n=4) and 5.5 ±0.8 (n=5) U/g haemoglobin, respectively. In the Orient the mean frequency of hypocatalasemia lies between 0.2% and 0.4% of the population. In Japan the prevalence of the recessive gene was estimated to be 0.0087% (Ogata, 1991).

The red blood cells of acatalasemic patients show greater sensitivity to oxidative stress and are particularly sensitive to lipid peroxidation by H_2O_2 *in vitro* (Aebi and Suter, 1972). In addition, approximately half the Japanese acatalasemic patients develop progressive gangrene of the mouth, called Takahara's disease. This condition is characterised by small, painful ulcers in the gingival crevices and tonsillar lacunae, attributed to excess levels of H_2O_2 generated by various micro-organisms in the mouth without normal destruction by catalase. The incidence of Takahara's disease among Japanese acatalasemic individuals has been declining, probably due to improved dietary and hygiene factors. Thus, although 46% of acatalasemics born between 1876 and 1935 showed symptoms of the disease, only 25% of those born between 1946 and 1965 were affected (Ogata, 1991). The remainder of the acatalasemic individuals show no signs of tissue damage. These individuals have higher residual red cell catalase activity levels. For both types of individuals, the defect is compensated for by an increase of hepatic glutathione peroxidase and catalase and by increased blood glutathione peroxidase (Chance *et al*, 1979; Vuillaume, 1987 and Ogata, 1991). Apart from the extremely rare occurence of occasional oral lesions, acatalasemic patients lead normal lives, suggesting alternate degradation mechanisms for H_2O_2 (Ogata, 1991).

Increased sensitivity of isolated red cells to H_2O_2 *in vitro* has also been noted among individuals with deficiency in other enzymes involved in the degradation of H_2O_2 (Chiu *et al*, 1982). The sensitivity of these individuals to endogenous and exogenous H_2O_2 will depend on the degree of compensation by the alternative pathways for destruction of H_2O_2.

Peroxidases. Peroxidases decompose H_2O_2 through the reaction:

$$H_2O_2 + 2RH \rightarrow 2H_2O + R\text{-}R$$

Peroxidases require as an electron donor a co-substrate such as alcohol, nitrite or formate for metabolising H_2O_2 (Little, 1972; Vuillaume, 1987). When the H_2O_2 concentration is low and a cosubstrate is present, catalase can also act as a peroxidase (Halliwell, 1974; Oshino *et al*, 1974).

Relatively high peroxidase activities occur in human adrenal medulla, liver, kidney and leucocytes and saliva (Fridovich, 1978; Marklund *et al*, 1982). Inside the cell, peroxidases are located in peroxisomes.

Glutathione peroxidase. Glutathione peroxidase (GSHPx) is an enzyme which is specific for glutathione (GSH) but not for H_2O_2; as a consequence, it can react with both H_2O_2 and organic hydroperoxides (R-OOH) (Guenzler *et al*, 1974). Glutathione peroxidase is more efficient at low concentrations of H_2O_2 compared to catalase (Halliwell, 1974). Glutathione reduces H_2O_2 to water with formation of oxidised glutathione (GSSG), which is generated by GSSG-reductase by consuming NADPH.

There are two kinds of glutathione peroxidase. One is Se-dependent; selenium is necessary for decomposing activity and uses H_2O_2 as a substrate. The other is Se-independent and cannot use H_2O_2 but can use organic hydroperoxides such as phosphatidylcholine-hydroperoxide or physiological peroxides (prostaglandin G_2) (Ursini *et al*, 1985).

A high glutathione peroxidase activity is found in the liver and erythrocytes, moderate levels are found in the heart and lungs and a low activity is present in muscle (Mills, 1960; Chow and Tappel, 1972). The subcellular distribution of glutathione peroxidase in rat liver is complementary to that of catalase. About two thirds of the enzyme activity is present in cytosols and the other third is located in mitochondria. Glutathione peroxidase is not found in peroxisomes (Flohé and Schlegel, 1971).

The rate constant of reduction of H_2O_2 with GSH is approximately 10^7mol/s, similar to that of catalase. Glutathione peroxidase located in erythrocyte membranes decomposed low H_2O_2 concentrations; catalase degraded higher concentrations (Nicholls, 1972).

In glucose-6-phosphate dehydrogenase (G6PD) deficiency, the NADPH levels in erythrocytes are not sufficient for the reduction of oxidised glutathione and this results in inadequate detoxification of H_2O_2 by glutathione peroxidase. As a result, erythrocyte membranes are damaged and sequestration by phagocytes leads to haemolytic anaemia (Hochstein, 1988).

The regeneration or "bursts" of H_2O_2 at low concentrations of oxidised glutathione (GSSG) to give glutathione (GSH) is controlled by a NADPH-dependent GSSG-reductase (Mannervik, 1980) (Figure 3).

Distribution in the body of enzymes that form and eliminate H_2O_2

The distribution of glutathione peroxidase, catalase and SOD has been studied in various regions of the gastro-intestinal tract of fasted Wistar rats, in the villus and crypt cells of the small intestine (Table 18), and in its subcellular fractions. The specific activity of glutathione peroxidase and CuZn-superoxide dismutase was maximal in the stomach. Catalase activity was uniform in all regions of the gastro-intestinal tract. Villus cells in the small intestine had higher glutathione peroxidase and superoxide dismutase activities than crypt cells. Among subcellular fractions, cytosol had the maximum activity of all these enzymes except for Mn-superoxide dismutase which was mainly associated with the mitochondrial fractions. Age dependent distribution studies showed that the specific activity of glutathione peroxidase and catalase was uniform from weaning to adulthood in the rat while there was a gradual increase in the specific activity of superoxide dismutase with development (Manohar and Balasubramanian, 1986).

To characterise lung antioxidant enzyme activities in different species, Bryan and Jenkinson (1987) measured the glutathione peroxidase, SOD and catalase activity in the Sprague-Dawley rat, Syrian Gold hamster, baboon (*Papio cycocephalas*) and human lung. SOD activity was similar for all four species (Table 19). GSH-Px activity was higher in rat than baboon or hamster lung (Figure 4). Lung catalase activity was variable with the highest activity present in the baboon, which was 10 times higher than in the rat (Figure 5). Non-Se-dependent GSH-Px was present in rat but absent in hamster, baboon and human lung. Bryan and Jenkinson (1987) concluded that the hamster was the best model for mimicking human lung antioxidant enzyme activity. Rat lung antioxidant enzyme activities were markedly different from the other species examined.

7.3.2 Hydrogen Peroxide Intake and Enzyme Activity

Bacteria. Pretreatment of bacteria, e.g. *Salmonella typhimurium* and *Rhodopseudomonas spheroides* with small doses of H_2O_2 rendered them resistant to higher doses, and the resistance was proportional to the amount of induced catalase activity (Chance *et al*, 1979; Winquist *et al*, 1984). In case of *Bacillus subtilis*, 4 electrophoretically distinct catalases were identified; 2 of which increased with this treatment (Ishida and Sasaki, 1981).

Mammals. In CFY inbred rats receiving 0.5% H_2O_2 in their drinking water for 2 months, the SOD activity in several organs and tissues was increased, except in the spleen (Table 20).

TABLE 18

REGIONAL DISTRIBUTION OF GLUTATHIONE PEROXIDASE, CATALASE AND SUPEROXIDE DISMUTASE IN RAT GASTRO-INTESTINAL TRACT (Manohar and Balasubramanian, 1986)

Region	Concentration (U/mg protein)				
	Se-glutathione peroxidase	Non-Se glutathione peroxidase	Catalase	CuZn superoxide dismutase	Mn superoxide dismutase
Stomach	35.28 ±3.6	58.85 ±6.0	2.42 ±0.6	32.72 ±0.4	6.57 ±0.6
Duodenum	10.55 ±2.4	7.29 ±0.8	2.42 ±0.8	11.87 ±0.4	3.05 ±0.1
Jejunum	17.39 ±0.6	6.30 ±0.1	1.60 ±0.1	3.78 ±0.3	2.99 ±0.7
Ileum	33.43 ±6.2	5.48 ±0.1	4.95 ±0.7	9.08 ±0.4	5.59 ±0.3
Colon	36.30 ±14.2	16.36 ±5.0	3.98 ±1.2	12.99 ±1.8	ND
Rectum	ND*	ND	1.75 ±0.6	13.22 ±1.4	1.94 ±0.3

* Not detectable.

TABLE 19

LUNG SUPEROXIDE DISMUTASE ACTIVITY IN VARIOUS SPECIES (Bryan *et al*, 1987)

Superoxide dismutase[a]	Activity (U/mg protein)			
	Human (n=5)	Baboon (n=6)	Hamster (n=6)	Rat (n=6)
CuZn	8.60 ±3.0	4.94 ±1.6[b]	8.73 ±2.0	9.56 ±3.3
Mn	3.14 ±1.5	2.36 ±0.33	2.69 ±0.24	2.91 ±0.96

a CuZn, cyanide-sensitive; Mn, cyanide-resistant.

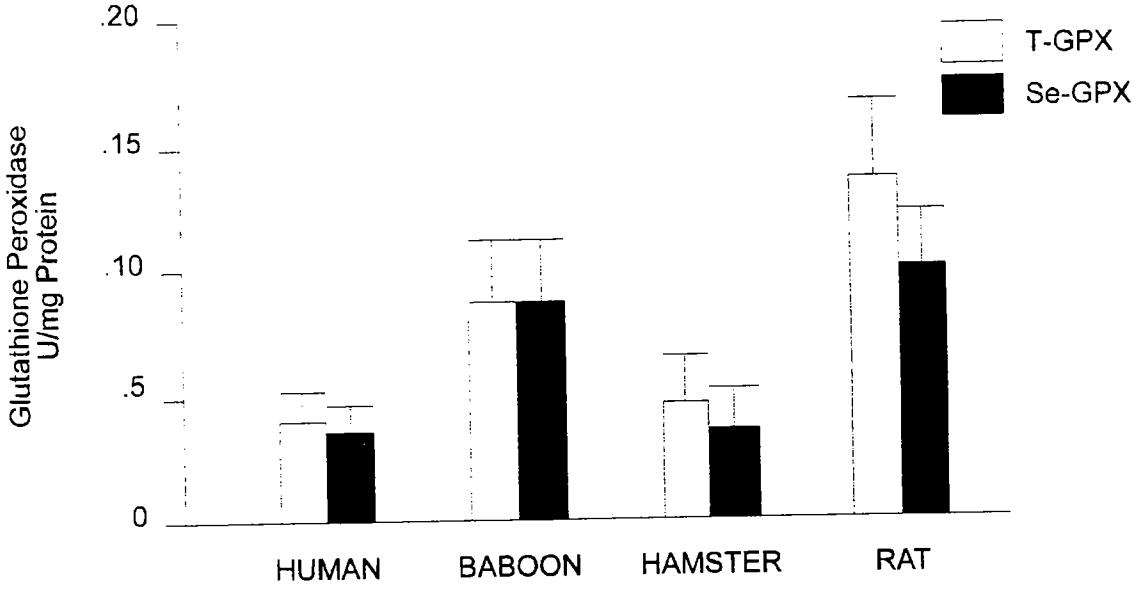
b Significantly different from other species, $P < 0.05$.

The peroxidase activities were also increased with the exception of heart muscle. Catalase activity increased in the liver and the kidney, but decreased in spleen, testes, brain and skeletal muscle (Matkovics and Novak, 1977).

FIGURE 4

LUNG GLUTATHIONE PEROXIDASE (GSH-Px) ACTIVITY

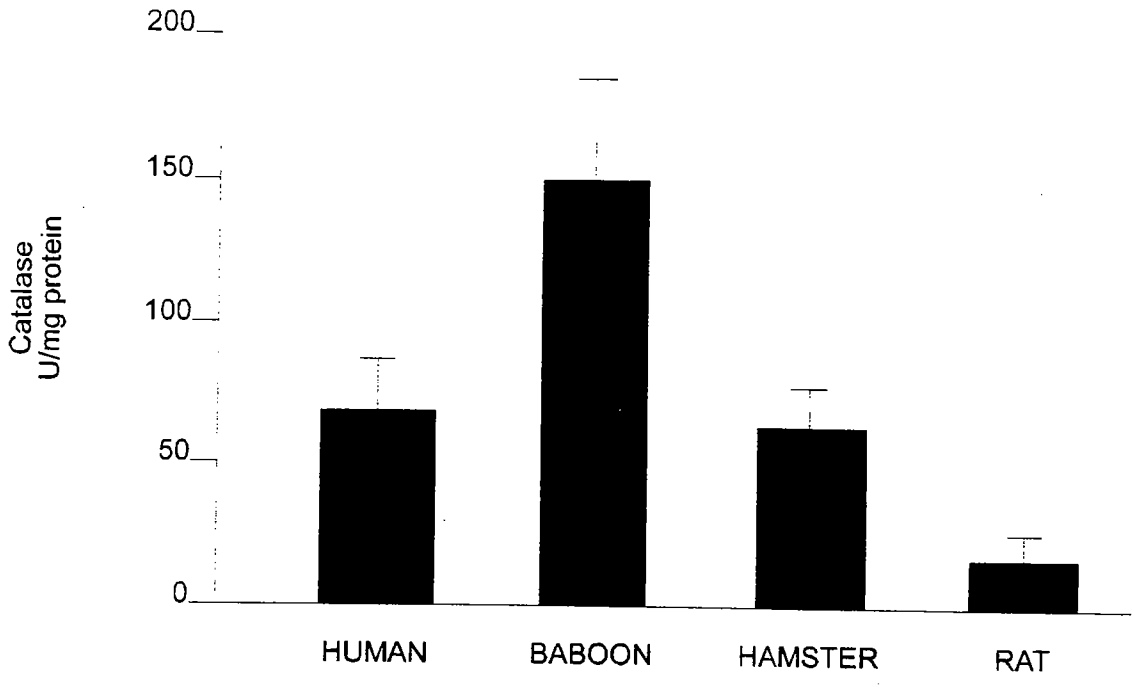
(Bryan and Jenkinson, 1987)



Units are µmol of reduced nicotinamide adenine dinucleotide phosphate oxidised /min/mg protein. Values are means ± SD for 6 animals/group and 5 humans/group. T-GPx is total GSH-Px activity and was measured using cumene hydroperoxide as substrate. Se-GPx is selenium-dependent GSPx acivity and was measured using hydrogen peroxide as substrate.

Oral intake of 0.5% H_2O_2 in drinking water in Wistar rats decreased the Se-dependent glutathione peroxidase activity in skeletal muscle, kidney and liver but not in the heart. The non-Se-glutathione peroxidase activity was decreased only in the kidney. Administration of H_2O_2 reduced the water intake; after water deprivation, corresponding to that of the H_2O_2 group, the Se-glutathione peroxidase activity in rat kidney, but not other tissues, was decreased. The activity of the non-Se dependent activity decreased in the kidney and liver but not in muscle tissue. The catalase activity in skeletal muscle, but not in other tissues, was substantially lower than in the control. In NMRI mice, neither exogenous H_2O_2 nor water deprivation changed the activities of both enzymes in the tissues (Kihlstrom *et al*, 1986).

FIGURE 5
CATALASE (CAT) ACTIVITY IN LUNG
(Bryan and Jenkinson, 1987)



Units are first-order rate-constant units. Values are means ±SD for 6 animals/group and 5 humans/group

It should be noted that the findings of catalase activity by Matkovics and Novak contradict those of Kihlstrom. Furthermore, Antonova (1974) reported a decrease of about 70% of hepatic catalase activity in a subacute study with H_2O_2, while Kawasaki *et al* (1969) found changes in the catalase activity of the liver which were not dose-dependent.

7.3.3 Non-enzymatic Metabolism

The reactivity of H_2O_2 with biological molecules such as carbohydrates, proteins, fatty acids or nucleic acids is not pronounced in the absence of transition metals, except for a few nucleophilic reactions.

In the presence of transition metals, particularly ferrous ions (Fe^{2+}), H_2O_2 can be reduced to hydroxyl radicals. This corresponds to the metal catalysed Fenton reaction:

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

Another possibility is reduction of the iron ion by the superoxide anion:

$$\cdot O_2^- + Fe^{3-} \rightarrow Fe^{2-} + O_2 \quad (\cdot O_2^-)$$

The overall reaction gives the so-called Haber-Weiss reaction:

$$\cdot O_2^- + H_2O_2 \rightarrow \cdot OH + OH^- + O_2$$

The generation of hydroxyl radicals depends on the availability of H_2O_2 and iron catalyst. Normally, the rate constant of the Haber-Weiss reaction is nearly zero due to the low steady state concentration of H_2O_2 in cells and tissues. Only in the presence of ferric ions (Fe^{3+}) can hydroxyl radical formation occur (Halliwell and Gutteridge, 1984).

The hydroxyl radical is highly reactive and will attack most molecules in living cells. Because of its short half-life (10^{-9}s) and the short diffusion radius (2.3nm), it will only react with other compounds when they are present close to the site where the radical is generated (Roots and Okada, 1975; Kappus, 1987). It is not clear whether sufficient free ferric ions are present in biological systems to catalyse the formation of hydroxyl radicals (Aust and White, 1985) and, additionally, there is no conclusive proof of hydroxyl radical involvement in the toxicity of superoxide anions and H_2O_2 (Birnboim, 1982; Troll and Wiesner, 1985).

7.4 TISSUE AND BODY FLUID LEVELS

Endogenous H_2O_2 levels depend on the balance between its formation and decomposition. Assay conditions may affect the H_2O_2 levels found in subcellular fractions (Chance *et al*, 1979).

In principle, quantitative measurement of H_2O_2 in biological fluids is difficult to perform in the presence of metabolic enzymes, because any blocking of H_2O_2 degradation will alter the steady state between its formation and decomposition (Nahum *et al*, 1989).

In general, the steady state H_2O_2 concentration in rat liver is about 10^{-3}µmol/l (30ng/l); after maximum stimulation of H_2O_2 production its concentration increases to 0.1µmol/l (3µg/l) (Sies, 1974). This limited variation is attributed to the catalase activity in rats (97,000µmol H_2O_2/min/g liver) (Tamura *et al*, 1990).

In rats, H_2O_2 levels of 43-87mmol/kg kidney (dry weight) and 3.6-8.3mmol/kg liver (dry weight) have been reported. The corresponding values in mice were 74-147mmol/kg kidney (dry weight) or 15-31mmol/kg liver (dry weight) (Rondoni and Cudkowicz, 1953).

England *et al* (1986) and Cavarocchi *et al* (1986) reported an increase in plasma H_2O_2 levels in patients from 65mmol/l before to 125mmol/l immediately after a heart bypass operation. The spectrophotometric analytical method was later criticised by Nahum *et al* (1989).

Using a radio-isotopic technique, an average plasma level of 34µmol/l (range 13-57µmol/l) H_2O_2 was found in human volunteers. The average blood level appeared to be 288µmol/l (range 114-577µmol/l), suggesting that red or white blood cells are rich in H_2O_2. Similar concentrations were found in Sprague-Dawley rats (Varma and Devamanoharan, 1991).

H_2O_2 concentrations can reach 12.2µmol/l in the extracellular pool of phorbol myristate ester stimulated neutrophiles (Test and Weiss, 1984).

In the lens of rabbit eyes, the concentration of H_2O_2 was approximately 59µmol/l (Bhuyan and Bhuyan, 1977) and in bovine eyes approximately 20µmol/l (Pirie *et al*, 1970). The concentration range of H_2O_2 in aqueous humour of the human eye was between 10-660µmol/l H_2O_2, with a mean value of 24µmol/l (Spector and Garner, 1981; see also Chalmers, 1989).

7.5 SUMMARY

H_2O_2 undergoes decomposition to oxygen and water when in contact with mammalian tissues. It has therefore not been possible to estimate the amount of the intact molecule available for absorption. A characteristic blanching of exposed tissue is caused by oxygen bubbles which produce microembolies in the capillaries of the tissues.

H_2O_2 is a normal product of aerobic cell metabolism and results from a number of enzymatic reactions including the enzymatic catalysed dismutation of peroxide anion. Under physiological conditions H_2O_2 production in the liver is about 90nmol/min/g liver.

H_2O_2 is metabolised by catalase and glutathione peroxidase. Catalase is located mainly in peroxisomes and the highest activities are found in the duodenum, liver, kidney, mucous membrane and other highly vascularised tissues. It metabolises H_2O_2 to water and oxygen, the decomposition rate in human plasma being approximately 0.01-0.05mol H_2O_2/l/min. Catalase decomposes high H_2O_2 concentrations whereas glutathione peroxidase is more efficient at lower H_2O_2 concentrations. Glutathione is oxidised to glutathione disulfide and H_2O_2 is reduced to water. Glutathione peroxidase is present in cytosol and mitochondria but not in peroxisomes. The highest activities are found in liver and erythrocytes.

Increased sensitivity of erythrocytes to H_2O_2 is seen among individuals with deficiencies in catalase activity (acatalasemia) and of the glutathione pathway (G6P-dehydrogenase-deficiency).

In some studies, H_2O_2 induced increased enzymatic activities in rodent tissue. In other studies, no changes or decreased activities were reported.

In the presence of transition metals, H_2O_2 can be reduced via the Haber-Weiss reaction to the hydroxyl radical, which is highly reactive and can result in lipid peroxidation.

Under normal, physiological conditions, the range of H_2O_2 tissue levels is 1-100nmol/l depending upon the organ, cell type, oxygen pressure and cell metabolic activity.

SECTION 8. EFFECTS ON EXPERIMENTAL ANIMALS AND *IN VITRO* TEST SYSTEMS

8.1 ACUTE TOXICITY

8.1.1 Oral (Table 21)

Acute oral LD_{50} values have been determined for H_2O_2 solutions ranging in concentration from 9.6-90%. Predominant clinical signs in rats administered 35% H_2O_2 included tremors, decreased motility, prostration and oral, ocular and nasal discharge. Most animals that died had reddened lungs, haemorrhagic and white stomachs, and blood-filled intestines; some had white tongues (FMC, 1983a).

TABLE 21

ACUTE ORAL TOXICITY

Concentration of solution (%)	Species, strain	LD_{50} (mg/kg)	Observation period (d)	Reference
9.6	Rat, Wistar-JCL	1,517♂ 1,617♀	7 7	Ito *et al*, 1976
10	Rat, Sprague-Dawley	>5,000	14	FMC, 1989a
35	Rat, Sprague-Dawley	1,193♂ 1,270♀	14 14	FMC, 1983a
50	Rat, Sprague-Dawley	>225 <1,200	14	FMC, 1986
60	Rat, Wistar	872♂ 801♀	14	Mitsubishi, 1981
70	Rat, Unknown	75♂	Unknown	FMC, 1979a
90	Mouse, Unknown	2,000	Unknown	Liarskii *et al*, 1983

8.1.2 Dermal (Table 22)

The acute, dermal toxicity of H_2O_2 solutions (35-90%) has been determined in various species. The 35% and 70% solutions had a low dermal toxicity in the rabbit, the only clinical signs being lacrimation and nasal discharge. The 90% solution had a low dermal toxicity in the pig, cat and rat, but not in the rabbit. This may reflect species differences in tissue and blood catalase activity. Accumulation of oxygen beneath the skin was noted, particularly in the rat.

TABLE 22

ACUTE DERMAL TOXICITY

Concentration of solution (%)	Species, (strain)	Dose (mg/kg)	Mortality rate	Observation period (d)	Reference
35	Rabbit	2,000	0/10	14	FMC, 1983b
70	Rabbit	6,500	0/4	Unknown	FMC, 1979b
		13,000	4/4	Unknown	
90	Rabbit	690	6/12	Unknown	Hrubetz *et al*, 1951
90	Pig	2,760	2/5	Unknown	
90	Cat	4,361	0/2	Unknown	
90	Rat (white)	4,899	4/12	Unknown	
		5,520	9/12	Unknown	
90	Rat (black)	6,900	0/6	Unknown	
		8,280	2/6	Unknown	
Unknown	Rat	4,060	50%	Unknown	Kondrashov, 1977

8.1.3 Inhalation (Table 23)

In several studies on the acute inhalation toxicity of H_2O_2 to rats, it is not always clear what form of H_2O_2 (vapour or aerosol) to which animals were exposed. Thus in studies of Comstock *et al* (1954) and Oberst *et al* (1954), groups of rats were exposed for 8h to "saturated" vapours of 90% H_2O_2 at a nominal concentration of 4,000mg/m^3 (2,840ppm). There were no signs of toxicity or deaths. Animals killed between days 4 and 14 showed severe pulmonary congestion and emphysema.

In another series, 3 groups of 10 male Wistar rats were exposed for 4 or 8h to "saturated" vapour concentrations of 338-427mg/m^3 (243-307ppm) as determined analytically. The nominal concentration of a "saturated" vapour is 4,670mg/m^3 (3,300ppm). "Scratching" and "licking" occurred but there were no deaths. These studies indicate that rats are affected by acute exposures to vapours from 90% H_2O_2, while death does not occur (Comstock *et al*, 1954; Oberst *et al*, 1954).

Whole body exposure of white rats for 4h to various concentrations of H_2O_2, vaporised from a solution, resulted in a LC_{50} of 2,000mg/m^3. The LOEC for the respiratory mucosa (increase in NAD-diaphorase) was 60mg/m^3 and exposure at 110mg/m^3 produced hyperanaemia and transient thickening of the skin (Kondrashov, 1977).

A 4h inhalation toxicity study was conducted in which the atmosphere was generated by bubbling air flow through a reservoir containing 1,000ml of 50% H_2O_2. Five male and five female rats were exposed to a maximum attainable vapour concentration of 170mg/m^3 (122ppm). The test atmosphere was analysed by colorimetric analysis of impinger samples drawn from the breathing zone of the animals. There were no deaths. Clinical effects noted during the exposure included decreased activity and eye closure. The predominant clinical signs post-exposure included dry, red nasal discharge, dried, red material on the facial area and fur and anogenital staining. The 4h LC_{50} was greater than 170mg/m^3 (122ppm), the maximum attainable vapour concentration (FMC, 1989c).

Groups of 10 mice (strain not given) were exposed to aerosols of 90% H_2O_2 at concentrations varying between 3,600 and 19,000mg/m^3 for 5, 10, or 15 minute intervals (Punte *et al*, 1953), the concentrations being near or above the saturation level of 4,670mg/m^3 (3,300ppm) for gaseous H_2O_2. The average mass median particle size as determined by cascade impaction was approximately 3.5μm. Exposure to concentrations of up to 5,000mg/m^3 (3,535ppm) for 5 minutes produced evidence of mild nasal irritation, blinking and slight gasping. There were no deaths from these exposures but there was evidence of lung congestion at necropsy. Four out of twenty mice exposed to 5,200mg/m^3 (3,676ppm) showed necrosis of the bronchial epithelium. Animals exposed to concentrations of 9,400mg/m^3 (6,645ppm) or more for 5-15min showed similar but more severe signs and 10-50% of animals died within 1h following a short convulsant period. Most of the animals that died showed pulmonary congestion. Animals that survived for several days to 8 weeks showed necrosis of the bronchial epithelium. In addition, animals surviving 9,400mg/m^3 (6,645ppm) or more showed slowly developing corneal damage which appeared at about 5 weeks after exposure (Punte *et al*, 1953).

8.1.4 Evaluation

The rat oral LD_{50} varies from about 1,500 to >5,000mg/kg for 10% solutions and was 1,200mg/kg for a 35% solution. The highest toxicity was shown by a 70% solution with an LD_{50} of 75mg/kg. A value of 2,000mg/kg has been reported for a 90% solution for the mouse but details of the study are not available.

Acute dermal toxicity is low for all concentrations. Acute exposure to saturated vapour produced only slight clinical signs of toxicity. Brief exposure to aerosols (15 minutes) at concentrations of 9,400mg/m^3 (6,645ppm) were lethal to mice with effects limited to the respiratory tract and eyes. Effects on the eye were still apparent and developed slowly 5 weeks after exposure. In rats, the 4h LOEC for respiratory mucosa was 60mg/m^3 and exposure at 110mg/m^3 induced hyperemia and trancient thickening of the skin.

TABLE 23

ACUTE INHALATION TOXICITY

Species	Strain	Exposure level (mg/m^3)	Exposure time (h)	Observations	Reference
Rat	Unknown	338-427	8	Licking/scratching; no deaths; pulmonary oedema and emphysema at necropsy	Comstock *et al*, 1954; Oberst *et al*, 1954
Rat	Wistar	23	4	No mortality	Svirbely *et al*, 1961
Mouse	Swiss	23	4	No mortality	
Rat	Unknown	2,000	4	"LC_{50}"*	Kondrashov, 1977
Rat	Sprague-Dawley	170	4	Nasal discharge; no deaths or necropsy findings	FMC, 1989c

* Combined inhalation exposure and skin application.

8.2 SKIN, MUCOUS MEMBRANE, RESPIRATORY TRACT AND EYE IRRITATION, SENSITISATION

8.2.1 Skin Irritation (Table 24)

H_2O_2 solutions were evaluated in primary skin irritation studies in rabbits at concentrations from 3-70%. Concentrations of 3-8% were non-irritating to intact and abraded skin following exposure for 24h under occlusive dressing (Du Pont, 1953, 1972a, 1973a). Irritation was slight following 4h exposure to 10% H_2O_2 and mild with 35% H_2O_2. Desquamation occurred in two of six animals at day 14 at the latter concentration (Aguinaldo *et al*, 1992). Application of a 35% solution to intact skin for 24h under an occlusive dressing induced mild erythema and moderate to slight oedema at 24h and severe to moderate erythema and slight to very slight oedema at 48h (Du Pont, 1974b).

Solutions of 50% and 70% H_2O_2 were severe skin irritants; studies were carried out on one anaesthetised rabbit in each case. Histopathological examination 48h after a 4h exposure demonstrated severe irritation with the 50% solution and extensive damage to the dermis, epidermis blood vessels, connective tissue and adnexa with the 70% solution after 30 min contact (FMC, 1987b; Aguinaldo *et al*, 1992).

In preliminary studies, 30% and 15% H_2O_2 solutions (0.2ml) were applied to the shaved dorsal skin of Sencar mice. Both solutions had caused epidermal necrosis 24h after application. Marked epidermal hyperplasia and leukocytic infiltration were observed within 6d of application but, by 10d, the epidermis was essentially normal (Klein-Szanto and Slaga, 1982).

TABLE 24

SKIN IRRITATION CLASSIFICATION IN RABBITS

Concentration of solution (%)	Scoring Interval (h)	Irritation Score[a]	Irritation Classification[b]	Reference
3	24	0[c]	Non-irritant	Du Pont, 1953, 1972a
	72	0		
6	24	0[c]	Non-irritant	Du Pont, 1973a
	72	0[c]		
8	24	0[c]	Non-irritant	Du Pont, 1974a
	72	0[c]		
10	4.5	0.3	Non-irritant	FMC, 1989b
	24	0.2		Aguinaldo *et al*, 1992
	48	0		
	72	0		
35	4	2.8	Non-irritant	FMC, 1983c
	24	2.6		Aguinaldo *et al*, 1992
	48	0.58		
	72	0.58		
	96	0.42		
	5-14d	0		
50	-	-[d]	Corrosive	Aguinaldo *et al*, 1992
70	-	-[d]	Corrosive	FMC, 1987b

a Irritation scores were determined by the Draize method with a maximum possible score of 8.0.

b Irritation scores were based on separate calculation for oedema and erythema of the mean 24, 48 and 72 hour scores for all animals divided by number of test sites, as described in the 6th and 7th EEC Amendment (Directive 67/548/EEC and Labelling Guide, July 1991).

c Scores indicated are for intact skin sites.

d Since only one animal was used in the 50% and 70% studies, no scores could be calculated. Evaluation was based on histopathological evaluation of skin sections.

8.2.2 Mucous Membrane Irritation

H_2O_2 (1 or 1.2%) applied to the gingiva or tongues of anaesthetised dogs by continuous drip caused oedema, followed by destruction and sloughing of the cornified epithelial layer of the gingiva (Martin *et al*, 1968; Dorman and Bishop, 1970).

8.2.3 Respiratory Tract Irritation

No specific studies on the respiratory irritancy of H_2O_2 have been performed. Effects on the respiratory tract are described in section 8.1.3.

8.2.4 Eye Irritation (Table 25)

The ocular irritancy of H_2O_2 solutions (5-70%) has been evaluated in rabbits; 0.1ml was instilled into the conjunctival sac and in certain cases, the eyes were washed with 100ml tap water after 20-30s. A 5% solution caused minimal irritation to unwashed eyes (Weiner *et al*, 1990). An 8% solution was moderately irritating to unwashed eyes (exhibiting severe conjunctivitis, slight corneal opacities and iritis), but was extremely irritating to washed eyes (including severe conjunctivitis, vascularisation, severe corneal opacities and severe iritis) (FMC, 1987a). A 10% solution was extremely irritating to both washed and unwashed eyes; severe corneal opacity, iritis and conjunctivitis were observed (Weiner *et al*, 1990). A 35% solution was extremely irritating, producing corneal opacities, iritis and moderate conjunctivitis, blanching of the conjunctiva, haemorrhagic iris, bubbles under the cornea, blanching of the cornea or corneal ulcerations (Weiner *et al*, 1990). Similarly, a 70% solution was extremely irritating and corrosive to the eyes of rabbits (FMC, 1979c).

In other rabbit studies, 3% H_2O_2 had no effect when instilled into eyes; 6% solution caused severe, reversible ocular damage; 8% solution caused mild, reversible injury to the cornea, iris and conjunctiva with washed eyes returning to normal in 2d. 10% and 12% solutions caused severe damage to the cornea, conjunctiva and iris which was irreversible in unwashed eyes and reversible in washed eyes (Du Pont 1972b,c, 1973b).

Grant (1974) concluded that rabbit eyes appear to be more susceptible to injury by H_2O_2 than human eyes. A drop of 5-30% solution applied to rabbit eyes caused severe damage, which was persistent when concentrations were greater than 10%. Even 5% H_2O_2 resulted in severe corneal oedema and vascularisation which only improved partially over 4-5 months (Grant, 1974, 1986). Instillation of a drop of a 1% H_2O_2 solution onto rabbit cornea caused severe conjunctivitis with chemosis and corneal opacities (Miller, 1958).

Several drops of a 2-5% solution induced much clouding of the cornea and inflammation of the conjuctiva of rabbit eyes. A 1% solution applied repeatedly caused conjunctival

TABLE 25

EYE IRRITATION CLASSIFICATION

Concentration of solution (%)	Scoring interval (h)	Irritation index[a]		EEC Score[b]	Reference
		unwashed	washed		
5	1	8.0	6.0	C = 0	Weiner *et al*,
	24	3.0	3.0	I = 0	1990
	48	2.0	1.0	R = 0.83	
	72	0	0	H = 0	
				Non-irritant	
8	1	13.0	12.0	C = 1.66	FMC, 1987a
	24	31.0	84.5	I = 0.50	
	48	11.0	77.5	R = 2.50	
	72	5.0	71.0	H = 1.58	
	96	4.0	57.0	Irritant	
	7d	2.0	47.0		
	16d	2.5	22.0		
	22d	2.5	16.0		
10	1	11.0	15.0	C = 3.5	Weiner *et al*,
	24	107	108	I = 1.67	1990
	48	107	108	R = 3.0	
	72	71.0	81.0	H = 2.8	
	96	44.5	65.5	Irritant	
	7d	40.5	49.5		
35	1	39.2	41.3	C = 2.33	Weiner *et al*,
	24	62.5	49.0	I = 1.72	1990
	28	69.5	69.7	R = 1.27	
	72	69.5	59.7	H = 2.28	
	96	63.7	48.7	Irritant	
	7d	79.3	76.7		
	14d	74.8	76.0		
	22d	72.7	74.3		
70	Scores could not be determined from the report			Severe irritant	FMC, 1979c

a Scores were determined by the method of Draize with a maximum possible score of 110. Scores were also determined by the EEC Criteria and came out to be the same.

b Irritation scores were based on separate calculation of the mean 24, 48 and 72 hour scores for cornea damage (C); iris damage (I); redness (R) and chemosis (H) for all animals tested, as described in the 6th and 7th EEC Amendment (Directive 67/548/EEC and Labelling Guide, July 1991). Based on the EEC criteria, the term 'irritant' or 'non-irritant' has been assigned.

hyperemia and slight corneal haze, followed by recovery (Koster, 1921; quoted in Grant, 1986).

Evaluation of 10% and 35% solutions in the EYETEX *in vitro* model for ocular irritancy confirmed the irritancy of these solutions under EEC regulations (Regnier, 1990).

8.2.5 Sensitisation

Ten guinea pigs were exposed to 3 or 6% H_2O_2 solutions on intact or abraded skin and by intradermal injections of 0.1ml of test solution in saline. Test solutions were re-applied nine times over a 2 week period prior to a challenge exposure to evaluate sensitisation. The final reactions did not indicate induction of skin sensitisation with either solution (Du Pont, 1953).

8.2.6 Evaluation

H_2O_2 solutions of 35% or less would not be classified as skin irritants in rabbits by the EC criteria. Higher concentrations (≥50%) are corrosive to rabbit skin. H_2O_2 (6%) was not a skin sensitiser in guinea pigs. Solutions of 5% or less can not be classified as irritant to rabbit eyes. Concentrations of 6% to 8% caused reversible eye damage in rabbits. Concentrations of 10% or higher caused irreversible corneal damage. First effects on the rabbit cornea are observed with 1% H_2O_2. Effects on the mucosa of the gingiva and tongue of dogs were found after direct contact with a 1% solution.

8.3 SUBCHRONIC TOXICITY

8.3.1 Oral

Numerous subchronic toxicity studies have been conducted with H_2O_2 administered in drinking water or by gavage to rats and mice.

Drinking water, Male dd strain mice showed a decrease in body weight gain and died within 2 weeks when their drinking water contained >1% H_2O_2. Significant decreases in body weight gain were observed after administration of 0.6%, but not 0.3% for 3 weeks (Aoki and Tani, 1972).

Solutions of 0.5-1.5% H_2O_2 given to male Holtzman rats in place of drinking water for 8 weeks induced extensive carious lesions and pathological changes in the peridontium, the intensity of the effect varying with the H_2O_2 concentration. Significant inhibition of body weight gain was also noted in these rats. Seven out of 24 rats receiving 1.5% H_2O_2 died during the course of the experiment (Shapiro *et al*, 1960).

CRJ-CDE rats were administered 0.05% to 1.2% and JCL-JCR mice 0.05% to 0.8% H_2O_2 for 10 weeks. The only effect observed was a slight decrease in body weight among the mice drinking 0.8% H_2O_2 (Koseishyo, 1977-1981; Ito *et al*, 1981a,b).

Male NMR1 mice and male Wistar rats drinking a 0.5% H_2O_2 solution for 40d and 56d respectively showed a depression in water consumption after one week. A group of animals with similar conditions of water deprivation was included as a control. Rats drinking H_2O_2 continued to show a depression in water consumption and body weight gain until the end of the study. This also happened in the water-deprived control rats. Effects on enzyme activities were also reported (section 7.3.2). The only significant change in mice was an increase in kidney weights compared to controls (Kihlstrom *et al*, 1986).

Male Osborne-Mendel rats drinking water containing 0.45% H_2O_2 for 3 weeks showed a decreased body-weight gain which correlated with a decreased liquid intake (Hankin, 1958).

Concentrations of 0.25 to 10% H_2O_2 were administered to male albino rats in drinking water for 43d. All rats receiving a concentration ≥2.5% died. In a 146 day study, 9/10 rats drinking 0.25% (250mg/kg/d) and 8/10 drinking 0.5% (500mg/kg/d) survived; body weight gain was reduced compared to control animals (Romanovski *et al*, 1960).

Gavage, Six-week old male Wistar-JCL rats were administered doses of 56.2, 168.7 and 506.0mg/kg H_2O_2 (calculated, based on 0.5% solution) by gavage 6d/week for 12 weeks. There was decreased body weight gain in the high dose group. Haemoglobin concentration, erythrocyte count, blood corpuscle volume, serum SGOT, SGPT, and alkaline phosphatase activity were markedly reduced in this group. Slight abnormalities in liver function were also seen at 168.7mg/kg bw. Kidney, liver and heart weights were decreased in high dose animals while adrenal and testes weights were markedly increased. Organ weight changes were not accompanied by histopathological changes. The only histopathological abnormalities were erosion and scars of the gastric mucosa in the 506mg/kg group (Ito *et al*, 1976).

Kawasaki *et al* (1969) administered doses of 6, 10, 20, 30 and 60 mg/kg/d H_2O_2 by gavage to male Wistar rats for 40 or 100d. From 20d onwards, body weight was decreased in the high dose animals. After 40d, spleen weights were slightly elevated in the high dose group but liver and kidney weights were not affected. There were no changes in organ weights after 100d of treatment. Plasma protein, haematocrit and catalase activity were all slightly, but significantly ($P < 0.05$) decreased in the 60mg/kg dose group after 100d of treatment. Blood catalase activity was slightly but significantly lower in the 30mg/kg dose group. No other effects were observed in the 30mg/kg dose group. Since the magnitude of these

changes was small and the number of animals employed was low (about 9/group with half killed after 40d), the validity of these effects is difficult to judge.

Antonova (1974) administered H_2O_2 to rats for 45d at doses of 1/5 and 1/10 the LD_{50}, although the exact dose in mg/kg was not stated. Both doses resulted in a depressed body weight gain, increased blood peroxidase activity, decreased liver catalase activity, an increase in circulating reticulocytes and increased urinary albumin. Inflammatory responses were observed in the stomach wall at both doses but were less severe at the lower dose.

8.3.2 Dermal

No information is available.

8.3.3 Inhalation (Table 26)

Ten rats (strain unspecified) were exposed to an average concentration of 67ppm (95mg/m^3) for 30 exposures over a 7 week period (Comstock *et al*, 1954; Oberst *et al*, 1954). There were signs of nasal irritation and profuse nasal discharge after 2 weeks of exposure. Lung congestion was seen in all animals and tracheal congestion was noted during weeks 5 and 7. No significant microscopic changes were found in the tissues.

Kondrashov (1977) conducted subchronic inhalation/dermal exposure studies in rats (strain not specified) at vapour-concentrations of 0.1mg/m^3 to 10.1mg/m^3 (atmosphere analysis not reported). The numbers of animals in each group and the number which were shaved to give both dermal and inhalation exposure were not reported. It was concluded that a concentration of 10mg/m^3 was the Lowest Observable Effect Level (LOEL) for the respiratory organs, and that 1mg/m^3 was the No Effect Level (NOEL). Changes in serum and lung enzyme activity were seen at 10mg/m^3. The NOEL for skin changes was considered to be 0.1mg/m^3. Kondrashov concluded that skin is less resistant to long-term effects of H_2O_2 vapour than lung tissue. The lack of experimental details makes it difficult to evaluate this study.

Groups of mice were exposed to 57ppm (81mg/m^3) for a total of 8 exposures or 77ppm (109mg/m^3) H_2O_2 for a total of 18 exposures (Comstock *et al*, 1954; Oberst *et al*, 1954). Toxic signs were similar to those for rats, although mice were more sensitive as noted by increased mortality. No descriptions of gross or microscopic pathology were provided.

In a 12 week inhalation study, rabbits were exposed for 6h/d, 5d/week to 22ppm (31mg/m^3) H_2O_2 vapour. No effects other than bleaching of the hair and some nasal irritation were seen. No changes were seen in the eyes following ophthalmoscopic examination, indicating that vapours did not produce delayed corneal damage at this concentration (Comstock *et al*, 1954;

TABLE 26

SUBCHRONIC INHALATION STUDIES

Species, (strain)	Exposure level		Exposure time			Reference
	ppm	mg/m^3	h/d	d/wk	wks	
Rat	67	93	6	2-5	7	Comstock *et al*, 1954; Oberst *et al*, 1954
Rat	0.07	0.1	5	5	16	Kondrashov, 1977
	0.7	1.0	5	5	16	
	7.1	10.1	5	5	16	
Mouse	5.6	8.0	4	5	1	Svirbely *et al*, 1961
Mouse	57	79	6	2-3	3	Comstock *et al*, 1954
	77	107	6	3-5	3	Oberst *et al*, 1954
Rabbit (black)	22	30	6	5	12	
Dog	7	10	6	4-5	26	

Oberst *et al*, 1954).

Similar results were found in a study with two dogs exposed to 7ppm (9.9mg/m^3) for 6 months (Comstock *et al*, 1954; Oberst *et al*, 1954). There were no toxic signs by 14 weeks except for bleaching and loss of hair. After week 23, there was sporadic sneezing and lacrimation. There were no significant weight changes or alterations in clinical chemistry or haematology. Pathological observations included thickening of the skin but no destruction of hair follicles. Other pathological observations were restricted to the lung with atelectatic and emphysematous areas and some hyperplasia of the bronchial musculature.

8.3.4 Evaluation

Repeated exposure to H_2O_2 via drinking water was lethal to mice and rats at concentrations of above 1%. Subchronic oral administration of H_2O_2 (from 0.6% in drinking water in mice; from 0.25% in rats) caused a depression in water consumption and a decrease in bodyweight gain. By gavage, body weight depression in rats was observed at 60mg/kgbw/d from 20d onwards in one study, but not at 169mg/kg in a 12 week study. Organ weight changes, not accompanied by histopathological changes, were observed in rats only at 506mg/kgbw after 12 weeks. The only histopathological changes were erosion and scars of the gastric mucosa (at 506 mg/kg) and changes in the peridontium and dental caries (at 1.5% in drinking water) in rats. Haematological changes and changes in blood and organ enzyme levels have been reported, but the interpretation of these findings is confounded by the decrease in water

intake observed and their inconsistency. Based on the limited data available, the NOEL for subchronic oral administration of H_2O_2 to rats is less than 0.25% in drinking water or 30-56mg/kg by gavage.

Rats exposed to 67ppm H_2O_2 by inhalation for 7 weeks exhibited nasal irritation and lung congestion. Mice exhibited similar signs of toxicity and their mortality was increased after 8 exposures at 57ppm. Inhalation exposure at 22ppm for 12 weeks caused minor nasal irritation and hair bleaching in rabbits. In dogs exposed to 7ppm for 6 months, bleaching and loss of hair, and signs of respiratory and skin irritation were observed. A LOEL for respiratory organs of 7 ppm was mentioned for rats. Effects on the skin of rats were reported down to 1mg/m^3 (0.7ppm). Systemic effects resulting from exposure to H_2O_2 by inhalation have not been reported. A clear NOEL cannot be derived from the available data.

8.4 CHRONIC TOXICITY AND CARCINOGENICITY

8.4.1 Chronic Toxicity (Table 27)

Several longer-term studies were conducted in rats, mice and rabbits with H_2O_2 administered by gavage or in drinking water. The methods used are not well described and, in most cases, there is no indication how often the dosing solutions were prepared or analysed.

Rabbits and male and female rats administered H_2O_2 by gavage for 6 months showed decreases in body weight and blood lymphocyte concentrations at the highest dose level and an increase in the numbers of reticulocytes and haemolysis. There was also a decrease in hepatic catalase activity, an increase in hepatic succinyl-dehydrogenase activity, changes in enzyme activity of the stomach, duodenum, and cerebrum and albuminuria. Structural changes of the gastro-intestinal mucosa and focal adiposis were observed on autopsy. At 5mg/kg/d, no decrease in body weight gain or catalase activity of the liver was observed. The lower doses showed only changes in haematology and enzyme activity. The NOEL for H_2O_2 was considered to be 0.1mg/l (0.005mg/kg/d) by the author (Antonova, 1974).

Aoki and Tani (1972) found focal necrosis of the liver, and thickening of the stomach wall of dd strain male mice administered 0.15% H_2O_2 in drinking water for 16 weeks, but not at 13 weeks. At 22 weeks, there were histological changes in the gastro-intestinal tract, liver and kidney. By 28 weeks, hydropic changes of the liver and epithelial degeneration of the kidney tubule, haemosiderin deposition in the spleen and inflammation with focal necrosis of the stomach were observed, and by 35 weeks, hydropic degeneration of the liver and kidney and necrosis, inflammation and hypertrophy of the intestinal wall were seen. The results show that long-term administration (>13 weeks) of H_2O_2 to mice caused pathological changes in the liver, kidney, gastro-intestinal tract and spleen at a concentration of 0.15% in drinking water, while no effect on body weight gain was observed.

TABLE 27

CHRONIC TOXICITY STUDIES

Species (strain)	Concentration in solution (%)	n	Dose (mg/kg/d)	Duration (months)	NOEL (%)	LEL (%)	Reference
Gavage							
Rabbit	0.00001, 0.0001 0.001, 0.01, 0.1[a]	45	0.005, 0.05, 0.5, 5, 50	6	0.00001	-	Antonova, 1974
Rat	0.00001, 0.0001 0.001, 0.01, 0.1[a]	85/sex	0.005, 0.05, 0.5, 5, 50	6	0.00001	-	
Drinking Water							
Mouse (dd)	0.15[b]	24	23[c]	8.5[e]	-	0.15	Aoki and Tani, 1972
Mouse (DBA/2N)	0, 0.4[d]	22	0, 62[c]	7[f]	-	0.4	Ito *et al*, 1982
Mouse (BALB/C)	0, 0.4[d]	39	0, 62[c]	7[f]	-	0.4	
Mouse (C57BL/6N)	0, 0.4[d]	34	0, 62[c]	7[f]	-	0.4	
Mouse (C3H)	0.4[b,d]	18	62[c]	6-7	-	0.4	Ito *et al*, 1984
Mouse (B6C3F1)	0.4[b,d]	22	62[c]	6-7	-	0.4	
Mouse (C57BL/6N)	0.4[b,d]	21	62[c]	6-7	-	0.4	
Mouse ($C3H/C^{s}_{b}$)	0.4[b,d]	24	62[c]	6-7	-	0.4	

a Dosing solution prepared daily.
b No indication of frequency of dosing solution preparation.
c Dose calculated on basis of 2.0ml solution and 13g mouse.
d Fresh dosing solution prepared every other day; stability of H_2O_2 in solution was analytically determinated.
e 35 weeks.
f 210d.

Ito *et al* (1982) examined the occurrence of gastro-intestinal lesions in three strains of mice administered 0.4% H_2O_2 in drinking water for periods up to 210d. Between 2 and 7 mice were killed and examined at 60-90 day intervals. The incidence of gastric lesions was higher among C57BL/6N mice than other strains by 90d. Gastric lesions were not observed in the other strains until 150d. The lesions in the duodenum did not differ in size, location or characterisation and the incidence was similar across all strains. The average number per mouse was approximately two-fold greater in C57BL/6N mice than in DBA/2N or BALB/c mice. The authors concluded that C57BL/6N mice are more "sensitive" to the induction of duodenal lesions.

In an additional study (Table 28) conducted by Ito *et al* (1982), the incidence and type of gastro-intestinal lesions in C57BL mice given 0.4% H_2O_2 were examined before and after cessation of H_2O_2 administration. Mice were treated for up to 180d with H_2O_2, and then given distilled water for 10, 20 or 30d. Administration of H_2O_2 for 140d followed by replacement with distilled water resulted in a regression of stomach erosions and decrease in the number of mice having lesions. The average number of stomach nodules per mouse remained similar during the test period, although the total number of nodules declined with increasing duration of distilled water administration. In the duodenum, cessation of H_2O_2 administration followed by distilled water resulted in a decreased incidence of erosions and nodules within 20d, and by 30d of distilled water treatment, all duodenal erosions and nodules had resolved. H_2O_2 administration for 150 or 180d followed by distilled water administration for 30d also resulted in a regression of stomach lesions and duodenal plaques. The total incidence of duodenal nodules of mice treated for 150 or 180d, then given distilled water for 30d, also declined. However, the average number of nodules per mouse either remained the same or decreased.

Glandular stomach lesions (section 8.4.2) were also found in mice after administration of 0.1% and 0.4% H_2O_2 in their drinking water in a carcinogenicity study by Ito *et al* (1981a,b).

8.4.2 Carcinogenicity

Oral carcinogenicity studies have been reported by two groups of Japanese investigators. The study designs are shown in Table 29.

An increase in duodenal tumours was observed in male and female C57BL/6J mice administered H_2O_2 (30% food grade) as 0.1% or 0.4% solutions in drinking water for up to 100 weeks; fresh solutions were provided daily, but the concentrations were not verified analytically. Control mice received distilled water. Body weights of the H_2O_2 treated groups were comparable to those of control mice, except for a slight decrease in body weight of

TABLE 28

EFFECT OF H_2O_2 WITHDRAWAL ON GASTRO-DUODENAL LESIONS[a]

(after Ito *et al*, 1982)

Administration (days) of		Stomach			Duodenum		
H_2O_2	distilled water	Lesions	Erosions	Nodules	Lesions	Plaques	Nodules
150	-	78	33 (1.3)[b]	56 (1.2)	89	67 (2.2)	56 (2.0)
140	10	60	0	60 (1.7)	100	60 (1.7)	60 (2.0)
140	20	40	0	40 (1.0)	40	20 (1.0)	20 (1.0)
140	30	20	0	20 (1.0)	0	0	0
150	-	63	50 (1.8)	25 (1.0)	75	63 (2.0)	38 (1.3)
150	30	14	14 (1.0)	0	57	43 (1.3)	57 (1.3)
180	-	67	56 (1.4)	22 (1.5)	89	78 (1.9)	22 (2.0)
180	30	0	0	0	67	33 (1.0)	33 (1.0)

a Explanation in text, section 8.4.1

b Incidence (%) of erosions; numbers in parentheses are average numbers of erosions per mouse.

TABLE 29

DESIGN OF CARCINOGENICITY STUDIES

Species, strain	Concentration (%)	n	Duration (weeks)	Reference
Drinking Water				
Mouse, C57BL/6J	0, 0.1, 0.4[a]	50/sex	100	Ito *et al*, 1981a, b
Mouse, C57BL/6N	0, 0.4[b]	138	104[b]	Ito *et al*, 1982
Rat, Fischer F344	0, 0.3, 0.6	50/sex	78[c]	Ishikawa and Takagawa, 1984

a Fresh dosing solution was prepared daily.

b Fresh dosing solution was prepared every other day; stability of H_2O_2 in solution was determined analytically.

c Some animals (number unknown) in each group continued to live for an additional 26 weeks and were not administered H_2O_2.

females of the 0.4% group at 15 months of age. Food consumption was not measured. Survival among control mice (54%) was lower than for mice treated with H_2O_2 (63% for high dose and 61% for low dose). Mortality observed during the study was related to bronchopneumonia or infections associated with amyloidosis. A greater incidence of gastric lesions was found in the glandular stomach and the duodenal lesions were restricted to the peri-pyloric and proximal portion of the duodenum (Ito *et al*, 1981a, b).

In the same study, the incidence of gastro-intestinal erosions was increased in treated mice (Table 30). There was no inflammatory response within the oral cavity, forestomach or distal intestinal tract. Erosions within the glandular stomach found at the antrium (proximal side of the pyloric ring on the lesser curvature) occurred at a greater incidence in the treated groups; the incidence was dose dependent. The incidence of duodenal erosions was much lower than that observed in the glandular stomach and the incidence in the treated groups was similar to that of control animals. The erosions were not characterised by their degree of severity, so it is difficult to determine whether they are associated with H_2O_2, spontaneous disease, e.g. gastro-intestinal amyloidosis, or both. Given the incidence of erosions, an effect on mortality and on body weights would have been expected as observed with other gastro-intestinal irritants. Single or multiple domed-shaped, smooth, surface nodules occurred most commonly in the duodenum between the pyloric ring and Vater's papilla. Histologically, the nodules showed hyperplastic, adenomatous or carcinomatous changes.

The incidence of hyperplasia, adenomas and carcinomas found by Ito *et al* (1981a,b) is shown in Table 31. In the stomach, hyperplasia within the glandular part occurred with

TABLE 30

INCIDENCE OF GASTRO-INTESTINAL EROSIONS IN C57BL/6J MICE

(Ito *et al*, 1981a,b)

Concentration in drinking water (%)	Glandular stomach		Duodenum	
	♂	♀	♂	♀
0	2 (4%)*	2 (4%)*	0 (0%)	2 (4%)
0.1	13 (25%)	7 (14%)	0 (0%)	1 (2%)
0.4	19 (38%)	23 (47%)	2 (4%)	2 (4%)

* Number of erosions in males and females, followed by percent incidence (number of animals with erosion/number of animals examined).

similar frequency in all groups; one adenoma was observed in a female mouse in the 0.1% group but no carcinomas. A dose-dependent, increased incidence of duodenal hyperplasia was noted in the treated groups compared to controls. Duodenal adenomas did not have a dose-dependent distribution. The incidence of duodenal carcinomas was higher in the females of the 0.4% group compared to the controls, and one carcinoma was observed in one male mouse in each of 0.1% and 0.4% treated groups. It is not certain whether animals exhibiting a hyperplastic response also exhibited a carcinoma or adenoma since individual animal data were not available.

When the data for male and female mice were combined (Ito *et al*, 1981a), there was a statistically significant increase in the incidence of duodenal carcinomas, but when treated separately and analysed statistically with Fisher's Exact Test, there was no significant difference between dosage groups. Rowlatt *et al* (1969, 1976) have reported that the normal incidence of this tumour type is low, although it appears with greater frequency in females. Ito *et al* reported an invasion of the duodenal carcinomas into the muscular layer and small vessels, but no metastatic tumours were evident. No treatment-related tumours were noted elsewhere. The latency of tumour induction was decreased in the treated mice, the first lesion occurring at about 42 weeks in mice treated with 0.4% H_2O_2. The decreased latency was based on animals which died and not those from interim kills. The authors suggested that the neoplastic nodules developed mainly in the duodenum because H_2O_2 is unstable under alkaline conditions. Interaction of H_2O_2 with bile might be vital for the tumorigenesis.

In another study by Ito *et al* (1982), male and female C57BL/6N mice were administered 0.1% or 0.4% H_2O_2 (30% food grade) in drinking water for up to 740d. A complete description of data relating to the 0.1% concentration was not given. Interim kills were made at 30 or 60 day intervals to evaluate the development of gastro-intestinal lesions (Table 32).

TABLE 31

INCIDENCE OF GASTRO-DUODENAL LESIONS IN C57BL/6J MICE

(Ito *et al*, 1981a,b)

Concentration in drinking water (%)	Glandular stomach				Duodenum					
	Hyperplasia		Adenoma		Hyperplasia		Adenoma		Carcinoma	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
0	2(4%)*	5(10%)*	0(0%)	0(0%)	2(4%)	7(14%)	0(0%)	1(2%)	0(0%)	0(0%)
0.1	6(12%)	7(14%)	0(0%)	1(2%)	16(31%)	24(48%)	2(4%)	4(8%)	1(2%)	0(0%)
0.4	3(6%)	7(14%)	0(0%)	0(0%)	30(60%)	31(63%)	2(4%)	0(0%)	1(2%)	4(8%)

* Number of lesions in males and females, followed by percent incidence (number of animals with lesions/number of animals examined).

Gastric erosions and duodenal "plaques", i.e. round, flat, avillous areas, were observed in animals killed on day 30 and were present in most animals subsequently killed. After prolonged administration, a hyperplasia and some neoplasia appeared. Hyperplastic nodules, adenomas and carcinomas were found in the stomach and duodenum at all times except days 210 and 360. The lesions did not increase in frequency during the study, but atypical hyperplasia appeared and 5% of the animals developed duodenal adenocarcinomas. By day 740, there was a concentration-dependent increase in the incidence of gastro-intestinal lesions, consisting of erosions, adenomas and carcinomas.

Investigations by Ito *et al* (1984) revealed that the incidence of duodenal lesions was inversely related to duodenal, liver and blood catalase activities. In this study, 0.4% H_2O_2 was administered in drinking water for six or seven months to strains of female mice exhibiting high (C3H/HeN), low (C57BL/6N) or "normal" (F1 hybrids, B6C3F1) catalase activity. Duodenal tumours were found in the H_2O_2-treated mice with the greatest incidence occurring in mice having low catalase activity (Table 33, 16). The incidence of duodenal tumours in mice with low catalase activity was comparable to the incidence in mice exhibiting hypocatalasemia ($C3H/C^s_b$).

TABLE 33

INCIDENCE OF DUODENAL TUMOURS IN 4 STRAINS OF FEMALE MICE TREATED WITH 0.4% H_2O_2 IN DRINKING WATER

(Ito *et al*, 1984)

Strain*	n	Number of mice with tumours (% incidence)	Total number of tumours
C3H/HeN	18	2 (11.1%)	2
B6C3F1	22	7 (31.8%)	8
C57BL/6N	21	21 (100%)	82
$C3H/C^s_b$	24	22 (91.7%)	63

* C3H/HeN, high catalase activity; B6C3F1, F, hybrid of C3H and C57BL, with "normal" catalase activity; C57BL/6N, low catalase activity; $C3H/C^s_b$, mice with hypocatalasemia.

H_2O_2 (30%) was administered to Fischer F344 rats in drinking water at concentrations of 0%, 0.3% or 0.6% for 78 weeks followed by a six month recovery phase. The frequency of preparation or analysis of dosing solutions was not stated. Survival was similar to that of the controls (41/50), except for male rats in the 0.3% group (approximately 30% mortality; 36/50 alive at 97 weeks). Tumours of the testes, mammary gland and skin were observed in rats that died during the study; there were no differences in tumour incidence between control and treated rats. After 45 weeks of administration, body weight was decreased by about 6% in

TABLE 32

FREQUENCY OF GASTRO-DUODENAL LESIONS IN C57BL/6N MICE ORALLY GIVEN 0.4% H_2O_2

(Ito *et al*, 1982)

Period (d)	No. of mice tested	Stomach						Duodenum					
		Lesions		Erosions		Nodules		Lesions		Plaques		Nodules	
30	7	29	(2.0)*	29	(2.0)	0		14	(2.0)	14	(2.0)	0	
60	5	40	(2.5)	40	(2.5)	0		80	(3.8)	80	(3.8)	0	
90	6	33	(2.0)	0		33	(2.0)	100	(3.2)	100	(2.3)	67	(1.3)
120	6	67	(2.8)	17	(4.0)	50	(2.3)	83	(4.0)	33	(3.5)	83	(2.6)
150	17	71	(1.6)	41	(1.1)	41	(1.1)	82	(2.5)	65	(2.1)	47	(2.0)
180	9	67	(1.7)	56	(1.4)	22	(1.5)	89	(2.1)	78	(1.9)	22	(2.0)
210	5	60	(1.3)	60	(1.3)	0		100	(3.8)	80	(2.8)	100	(1.6)
300	10	90	(1.3)	20	(1.0)	70	(1.4)	100	(2.6)	70	(1.3)	90	(1.9)
360	7	86	(1.5)	71	(1.6)	0		100	(2.4)	86	(2.7)	14	(1.0)
420	14	93	(2.2)	79	(1.9)	43	(1.3)	100	(4.4)	43	(4.0)	93	(2.9)
490	12	100	(2.3)	83	(2.4)	33	(1.0)	100	(5.0)	58	(4.4)	92	(2.4)
560	7	100	(2.7)	86	(2.7)	43	(1.0)	100	(5.0)	57	(4.0)	86	(2.7)
630	4	100	(1.5)	75	(1.7)	25	(1.0)	100	(5.8)	75	(4.0)	75	(3.7)
700	29	83	(2.0)	76	(1.5)	34	(1.4)	100	(5.8)	66	(3.7)	100	(3.2)

* Incidence (%) of lesions; numbers in parenthesis are average numbers of lesions per mouse.

male and female rats in the 0.3% group and 10% in the 0.6% group. Nasal bleeding was observed in the treated groups; the significance of this is uncertain. At the end of the study (104 weeks), all surviving animals were killed. No significant differences were observed between treated rats and controls relative to the incidence and types of tumours. The authors concluded that, under the conditions of this study, H_2O_2 was not carcinogenic to Fischer F344 rats. Because this study was not published in detail, its quality cannot be assessed. Furthermore, no account was taken of other measurements made during the study and a full characterisation of the pathological changes was not given (Ishikawa and Takayama, 1984).

In other studies, forestomach papillomas were observed in rats exposed to H_2O_2 in drinking water (section 8.4.3).

Sencar mice (n=60) were treated topically twice a week with a 15% solution of H_2O_2 (0.2ml) for up to 50 weeks to examine possible dermal carcinogenicity (Klein-Szanto and Slaga, 1982). Likewise, Kurokawa *et al* (1984) topically treated Sencar mice with a 5% solution of H_2O_2 (0.2ml) for up to 51 weeks. Control mice in each study were treated dermally with acetone (0.2ml). Pathological evaluation of the application site was undertaken at intervals; no increased incidence of dermal tumours was noted in either study. The authors concluded that, under the conditions of these studies, H_2O_2 was not considered to be a complete dermal carcinogen. However, the Sencar mouse study design does not allow full assessment of the potential dermal carcinogenicity.

8.4.3 Initiation-Promotion of Hydrogen Peroxide

Several investigators have examined the potential of H_2O_2 as an initiator or promoter in two-stage carcinogenesis studies. Only those studies that utilised oral or topical administration were reviewed. Details of the study protocols are given in Table 34.

Topical application, Klein-Szanto and Slaga (1982) treated Sencar mice topically with dimethylbenz(a)anthracene (DMBA) followed by once or twice weekly topical applications of H_2O_2 in acetone for 25 weeks. As the concentration of H_2O_2 decreased, the incidence of papillomas increased to a maximum of 10% (H_2O_2 concentrations of 6% and 10%). Repetitive treatment with solutions more concentrated than 15% was considered to be too irritating for the epidermis to permit survival of initiated cells. However, the authors speculated that concentrations greater than 15% did have tumour-promoting potential. Concentrations less than 15% did not cause tumour promotion.

In similar studies, mice were treated dermally for up to 58 weeks with 3% or 5% H_2O_2 following initiation with DMBA (Shamberger, 1972; Bock *et al*, 1975; Kurokawa *et al*, 1984). In these studies there were no significant increases in the incidence of skin tumours, although

TABLE 34

TUMOUR INITIATION-PROMOTION STUDIES[a]

Initiator*	Promoter (and frequency of treatment)	Duration of treatment (weeks) + observation	Species (n)	Reference
DMBA (10nmol)[b]	H_2O_2: 30%, 15%, 10%, 6%; (1 or 2x/week)[c]	25 + 1	Sencar mice (60/group)	Klein-Szanto and Slaga, 1982
DMBA (20nmol)[b]	H_2O_2 5% (2x/week)[c]	51 + 1	Sencar mice (20)	Kurokawa *et al*, 1984
DMBA (125µg)[b]	H_2O_2 3% (5x/week)	56 + 2	ICR Swiss mice (30 f)	Bock *et al*, 1975
DMBA (125µg)	H_2O_2 3% (7x/week)	40	Female ICR Swiss mice	Shamberger, 1972
DMBA (0.25%)[d]	H_2O_2 3%[d]	19 or 22	Syrian hamsters (11)	Weitzman *et al*, 1986
DMBA (0.25%)[d]	H_2O_2 30%[d]	19 or 22	Syrian hamsters (5)	
MNNG (0.1%)[e]	H_2O_2 1%	40	Wistar rats (10)	Takahashi *et al*, 1986
H_2O_2 (15%)[f]	TPA: 2 µg (2x/week)[g]	25	Sencar mice (60)	Klein-Szanto and Slaga, 1982
H_2O_2 (1.5%)[h]	MAM (25 mg/kg)[i]	21[j]	Fischer 344 rats (8/group)	Hirota and Yokoyama, 1981

* DMBA, Dimethylbenzanthracene; MNNC, N-Methyl-N'-nitro-N-nitrosoguanidine.

a Animals treated topically; control animals treated with acetone.

b DMBA administered once, topically, in acetone.

c H_2O_2 administered topically in 0.2ml acetone, 1 week before DMBA: concentrations measured.

d DMBA painted onto one buccal pouch 2x/week; H_2O_2 (3 or 30%) painted on opposite buccal pouch 2x/week (on days other than DMBA painting).

e MNNG administered in drinking water; feed supplemented with 10% NaCl.

f 0.2ml H_2O_2 administered once.

g TPA administered 2x/week.

h H_2O_2 administered in drinking water.

i MAM given 3x/every 2 weeks; H_2O_2 administration continued during MAM treatment interval except for 2d following injection.

j At end of 8 weeks, tap water given to one group and H_2O_2 (1.5%) administration continued in second group for an additional 13 weeks.

epidermal hyperplasia was evident in most of the mice treated by Kurokawa *et al* (1984). The results of these studies, therefore, confirm the conclusion of Klein-Szanto and Slaga (1982) that concentrations of less than 15% have no tumour promoting effect.

DMBA and/or H_2O_2 was painted onto the left buccal pouch of four groups of male Syrian golden hamsters twice weekly for 19 or 22 weeks. Animals in Group A were painted 2x/week with a 0.25% solution of DMBA in heavy mineral oil. Animals in Group B were painted 2x/week with DMBA and 2x/week (on days other than the DMBA painting) with 3% H_2O_2. Group C animals were painted in exactly the same way as Group B animals except that the concentration of H_2O_2 used was 30%. Group D animals were painted 2x/week with 30% H_2O_2 alone. Cheek pouches from animals which had not been painted and from animals which had been painted 2x/week with only the mineral oil vehicle served as controls. Six of 11 hamsters (55%) treated with DMBA and 3% H_2O_2 developed epidermoid carcinomas by 22 weeks, whereas all 5 hamsters treated with DMBA and 30% H_2O_2 developed epidermoid carcinomas by 22 weeks. No carcinomas were observed in hamsters treated with 30% H_2O_2 alone, but 3/7 (43%) of the hamsters treated with DMBA alone developed carcinomas. Only one carcinoma was observed in a hamster treated with DMBA and 30% H_2O_2 at 19 weeks. In all hamsters, chronic inflammation, hyperchromatic cells and dysplasia were also noted at 19 weeks. The authors concluded that long-term, twice weekly application of 3% or 30% H_2O_2 could induce inflammatory changes, but that pathological changes associated with preneoplastic lesions and augmentation of the oral carcinogenesis of DMBA was observed only with 30% H_2O_2 (Weitzman *et al*, 1986).

A group of Sencar mice was tested for tumour initiation by topical administration of H_2O_2 followed by twice weekly applications of 12-0-tetradecanoylphorbol-13-acetate (TPA). The authors found no significant increase in the incidence of dermal tumours and concluded that H_2O_2 was not a tumour initiator (Klein-Szanto and Slaga, 1982).

Oral studies. Takahashi *et al* (1986) examined the potential of H_2O_2 to promote N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) initiated gastric tumours in rats. Two groups of rats (n=30 and 21) received MNNG-treated drinking water and feed supplemented with 10% sodium chloride for eight weeks *ad libitum* after which the animals were maintained on normal feed and tap water. The second group the water was supplemented with 1% H_2O_2. A third group (n=10) was not given MNNG or a sodium chloride supplemented diet, but was administered 1% H_2O_2 in the drinking water. Adenocarcinomas were observed in the pyloric stomach and duodenum of the MNNG-treated rats, and "preneoplastic hyperplasia" was observed in the pylorus (Table 35). In rats treated with MNNG and H_2O_2, there was no enhancement in the number of gastro-intestinal tumours, although all treated animals

exhibited forestomach papillomas; these also occurred in rats treated only with H_2O_2 in the drinking water. No carcinoma development was noted in the stomach or duodenum. Erosions and ulcerations also occurred in the fundic mucosa of the stomach of the H_2O_2 treated rats. The authors concluded that, in contrast to the study of Hirota and Yokoyama (1981, see below), no enhancement of duodenal tumours occurred, although characteristic diffuse lesions, showing fusion of the villi, were observed throughout the duodenum.

Hirota and Yokoyama (1981) examined the tumour promotion potential of 1.5% H_2O_2 in drinking water in the duodenum and jejunum of Fischer F344 rats. After 4 weeks of administration, methylazoxymethanol acetate (MAM) was administered i.p.; H_2O_2 administration was continued except for 2d following injection. At the end of 8 weeks, one group of rats continued on H_2O_2 whereas a second group was given tap water to drink for an additional 5 weeks. A third group of 3 rats received only H_2O_2 throughout the study. A fourth group of 3 rats received only tap water. There was no control MAM group. Animals were killed 21 weeks after the study started. Proximal duodenal (Ito *et al*, 1984) and upper jejunal tumours were observed in groups 1 and 2, with a higher incidence in Group 1 (100% incidence) compared to Group 2 (25%). Tumours were classified as adenocarcinomas, mucosal or invasive. No tumours were observed in tap water control animals or animals treated only with H_2O_2, although duodenal and upper jejunal hyperplasia were noted in the latter group. The authors concluded that H_2O_2 had a tumour promoting effect on MAM-initiated intestinal tumours. Because of the lack of a MAM control group and details of the method, it is not possible to evaluate this study.

Evaluation. These studies confirm that H_2O_2 induces inflammatory changes in tissues following topical application. Furthermore, H_2O_2, at concentrations which induce significant cellular damage, may act as a weak tumour promoter in the skin. At concentrations <15% no tumour promotion is evident. Likewise, when administered by drinking water, inflammatory changes of the gastro-intestinal tract are observed similar to these found in longer-term studies by several investigators, e.g. Ito *et al* (1981a, b). Promotion of initiated gastro-intestinal tumours by H_2O_2 has not been proven. Overall, H_2O_2 concentrations of <1% do not appear to have gastro-intestinal tumour promoting potential.

Anti-tumour effects of Hydrogen Peroxide. Several investigators have examined the potential anti-cancer effects of H_2O_2. Studies in which H_2O_2 was injected are not reviewed.

Regression of implanted Walker 256 adenocarcinomas in 6 rats treated with 0.45% H_2O_2 in the drinking water was observed by Holman (1957). The time to complete tumour regression was approximately 15-60d, depending on tumour size. No other details were provided.

TABLE 35

EFFECT ON GASTRO-DUODENAL CARCINOGENESIS INDUCED BY MNNG

(Takahashi *et al*, 1986)

Treatment group (n)	Glandular stomach				
	Forestomach Papilloma	Fundus Hyperplasia[a]	Pylorus Adenocarcinoma	Preneoplastic hyperplasia	Duodenum Adenocarcinoma
MNNG controls (30)	0 (0%)[b]	0 (0%)	1 (3.3%)	7 (23.3%)	3 (10%)
MNNG-H_2O_2 (21)	21 (100%)	8 (38.1%)	2 (9.5%)	6 (28.6%)	0 (0%)
H_2O_2 (10)	5 (50%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)

a Adenomatous hyperplasia.
b Number of rats with tumours (% incidence).

The Holman study created some controversy, since several studies disputed his findings. Green and Westrop (1958) implanted Walker 256 tumours and Rd/3 sarcomas into rats (n=6 or 10), and subsequently treated the rats with 0.45% H_2O_2 in their drinking water. All rats died by 21d and no tumour regression was observed. Similarly, Ghadially and Wiseman (1958) implanted Walker tumours or dibenzantracene derived RD3 sarcomas into rats (n=29 or 35). Five days after implantation, the rats were administered 0.45% H_2O_2 in drinking water for up to 136d; controls received tap water. No differences in survival time or tumour weight were observed between the treated rats and controls. These authors concluded that H_2O_2 had no effects on host survival or tumour regression. The tumour regression observed by Holman (1957) may be accounted for by the natural regression observed in transplanted tumours and not by the action of H_2O_2.

8.4.4 Evaluation

Although details needed to fully evaluate the chronic toxicity or carcinogenic potential of H_2O_2 are lacking, several studies reviewed show that long-term oral administration of 0.1-0.15% H_2O_2 causes an inflammatory response in gastro-duodenal tissue of mice. The response is limited to the glandular stomach and, to a lesser extent, to the peri-pyloric and proximal portion of the duodenum. No inflammatory response was observed in the oral cavity, forestomach or distal intestinal tract. The incidence was higher in strains of mice with a low catalase activity. Studies by Ito *et al* (1982) revealed that cessation of H_2O_2 administration causes a regression of lesions induced by prolonged (up to 180d) administration of H_2O_2 in drinking water.

The investigations by Ito *et al* (1981a,b) suggest that this inflammatory response may progress to carcinogenic changes in mice. In rats, H_2O_2 induced only papillomas, no malignant tumours of the forestomach were seen, even at nearly lethal concentrations (1-1.5% H_2O_2 in drinking water). Initiation-promotion studies suggest that H_2O_2 is not an initiator in skin, but may be a weak promoter of intestinal tumours in the rat at high (>15%) concentrations on the skin, or nearly lethal concentrations (1.5%) in drinking water.

Although the *in vitro* genotoxicity data (section 8.5) would indicate that a genotoxic mechanism for tumour induction is feasible for H_2O_2, the *in vivo* data currently point strongly to a non-genotoxic mechanism. The induction of carcinogenicity by a non-genotoxic mechanism has been proposed (Troll and Wiesner, 1985). The fact that tumours were induced only at the sites where H_2O_2 came directly into contact with the tissues and that the tumours were associated with persistent local inflammation supports a non-genotoxic mechanism for the gastro-intestinal tract tumours. Non-genotoxic mechanisms have been discussed in detail by many workers (ECETOC and IPCS, 1991).

The US Food and Drug Admnistration has concluded that there is insufficient evidence of carcinogenicity and IARC that there is 'limited' evidence of carcinogenicity in experimental animals (US FDA, 1988; IARC, 1985).

8.5 GENOTOXICITY

The mutagenicity of H_2O_2 has been extensively studied over the past 30 years. This review has been limited to the more recent and significant studies.

8.5.1 Gene Mutations

Gene mutations in bacteria and yeast

A large number of Ames tests have been undertaken with a wide variety of strains of *Salmonella typhimurium* (Table 36).

H_2O_2 induced gene mutations in some strains of *Salmonella typhimurium* without metabolic activation. The highest mutagenic response was observed with strains sensitive to oxidative mutagens: TA102, TA104, SB1106p, SB1106, SB1111 and TA2638 (Levin *et al*, 1982; De Flora *et al*, 1984; Carlsson *et al*, 1988; Glatt, 1989; Abu-Shakra and Zeiger, 1990; Wilcox *et al*, 1990). L-arabinose forward mutations were also induced in strains BA13 and BA9 (Ruiz-Rubio *et al*, 1985). Inconsistent (negative or weakly positive) results, possibly due to differences in study protocols or concentrations tested, were obtained with strains TA97, TA98, TA100, TA1537 and TA1538 (Stich *et al*, 1978; De Flora *et al*, 1984; Ishidate *et al*, 1984; Glatt, 1989; Kensese and Smith, 1989; Abu-Shakra and Zeiger, 1990). Strains TA92, TA94, TA1532, TA1534, TA1535, D3052 and G46 produced negative results in the few tests where they were used (Mitchell, 1974; Ishidate *et al*, 1984; Glatt, 1989).

The addition of S9-mix, catalase, superoxide dismutase (SOD) (De Flora *et al*, 1984; Kensese and Smith, 1989) or catalase induction (Winquist *et al*, 1984) reduced or abolished the mutagenic response to H_2O_2 in *Salmonella typhimurium* strains. Abu-Shakra and Zeiger (1990) showed that variations in catalase content of the tested strains did not correlate with the responses obtained in the Ames test (catalase content in decreasing order: TA102 >>TA103 >TA104 >TA2638 >SB1106 >TA100 >SB1106p >TA1535).

Positive or negative results were obtained with H_2O_2 (Table 37) for auxotrophic reversion with *Escherichia coli* in the absence of metabolic activation (Mitchell, 1974; Bosworth *et al*, 1987; Wilcox *et al*, 1990). H_2O_2 was positive in a *Bacillus subtilis* multigene sporulation test (Sacks and MacGregor, 1982), and negative or positive in a *Saccharomyces cerevisiae* gene conversion or forward mutation test (Mitchell, 1974; Thacker and Parker, 1976).

TABLE 36

GENE MUTATIONS IN *SALMONELLA TYPHIMURIUM* (AMES TEST)

Strain	Protocol	Metabolic activation	Concentration (μmol)	Results	Reference
TA102, TA104, SB1106p, TA97, SB1111, SB1106	Standard agar	No	0-4/plate	Highly +ve on SB1106p (x4) and TA97 (x2.8) Weakly +ve on TA102, TA104, SB1106 and SB1111	Abu-Shakra and Zeiger, 1990
TA102, TA104, SB1106p, TA97, SB1111, SB1106	Pre-incubation	No	0-1.2/plate	Highly +ve on SB1106p (x4), and TA104 (x4) Weakly +ve on TA97, TA102, SB1111 and SB1106	Abu-Shakra and Zeiger, 1990
TA102	Standard agar	No	0-300μg/plate	Weakly +ve (x2.3)	Wilcox *et al*, 1990
TA97, TA98, TA100, TA102, TA1537, TA1538	Standard agar	No	0-6,000/plate	Weakly +ve on TA97, TA98, TA1537 and TA102 -ve on TA100 and TA1538	Kensese and Smith, 1989
TA97, TA98, TA100, TA102, TA1537, TA1538	Pre-incubation	No	0-340/plate	Weakly +ve (x2-6) in all six test strains	Kensese and Smith, 1989
TA97, TA102, TA1537, TA1538	Pre-incubation	Catalase or SOD	0-340/plate	-ve in all four test strains	Kensese and Smith, 1989
TA97, TA98, TA100, TA102, TA1537, TA1538	Liquid incubation	+/-S9 or +catalase or +SOD	0-6/plate	Weakly +ve on TA1537 without S9 and on TA97 and SOD -ve with catalase os S9	Kensese and Smith, 1989
TA92, TA97, TA100, TA102, TA104, TA1535, TA1537	Pre-incubation	No	0-2.4 /plate	-ve on TA92, TA97, TA1535 and TA1537, weakly +ve on TA100 (x2.5), pronounced mutagenic effects on TA102 (x2.8) and TA104 (x4.4)	Glatt, 1989

TABLE 36 (cntd.)

GENE MUTATIONS IN *SALMONELLA TYPHIMURIUM* (AMES TEST)

Strain	Protocol	Metabolic activation	Concentration (µmol)	Results	Reference
TA102	Pre-incubation	No	400	+ve	Carlsson *et al*, 1988
TA102	Pre-incubation	Sulphide	0-50	Highly +ve (catalase in activated by sulphide)	Carlsson *et al*, 1988
BA9, BA13 (L arabinose forward mutation)	Liquid test	No	0-17.6/plate	-ve on the two strains	Ruiz-Rubio *et al*, 1985
TA97, TA102	Standard agar	No	Unknown	-ve on TA97, weakly positive on TA102	De Flora *et al*, 1984
TA102	Standard agar	S9	Unknown	Decrease of mutagenic potential on TA102	De Flora *et al*, 1984
TA92, TA94, TA98, TA100, TA1535, TA1537	Pre-incubation	No	0-200µg/plate	-ve on TA92, TA94, TA98, TA1535 and TA1537 Weakly +ve on TA100 (x1.9)	Ishidate *et al*, 1984
TA100	Standard agar	Catalase induction	0-7.5/plate	H_2O_2 pre-treatment induced catalase, and protection against toxicity and mutations	Winquist *et al*, 1984
TA102, TA2638	Standard agar	No	100µg/plate	+ve on the two strains	Levin *et al*, 1982
TA98, TA100	Liquid incubation	No	0-10,000/plate	-ve on the two strains	Stich *et al*, 1978
TA1532, TA1534, D3052, G46	Spot test	No	5% solution on filter paper disk	-ve	Mitchell, 1974

TABLE 37

GENE MUTATIONS IN OTHER BACTERIA OR YEAST

Bacteria or Yeast	Metabolic activation	Concentration	Results	Reference
Escherichia coli (trp-)	No	0-300µg/plate	+ve on strain WP2uvrA(pKM101) (x3.6) and WP2(pKM101) (x2.7)	Wilcox *et al*, 1990
Escherichia coli (amp-)	No	0-80µg/ml	+ve on strain DB2 (x5)	Bosworth *et al*, 1987
Bacillus subtilis (Exc-)	No	0-0.003%	+ve in the multigene sporulation test	Sacks and McGregor, 1982
Escherichia coli (trp-)	No	5% solution on filter paper disk	-ve on strains WP2uvrA+ and WP2uvrA-	Mitchell, 1974
Escherichia coli (caca-)	No	5% solution on filter paper disk	Questionable on strain WP2uvrA	Mitchell, 1974
Saccharomyces cerevisiae	No	5% solution on filter paper disk	-ve for forward mutations and gene conversions	Mitchell, 1974
Saccharomyces cerevisiae	No	100µg	+ve for forward mutations	Thacker and Parker, 1976

The overall response to H_2O_2 in bacterial tests is summarised Table 38

These results emphasise that the mutagenicity of H_2O_2 in bacteria is a function of the genotype of the bacterial strain. The strains bearing the mutations hisG428, hisC3108/hisO1242 or araD531/hisG46 are particularly sensitive to oxidative stress. The amount of endogenous catalase is not correlated with the mutagenic response but the destruction of H_2O_2 by exogenous metabolic agents (S9 or catalase) abolishes this mutagenic effect.

Gene mutations in bacteria after *in vivo* treatment (host mediated assay with mice)

Following 2 oral administrations within 2h of 0.5ml 0.3% H_2O_2 to OF1 mice, a statistically significant increase in mutation frequency of i.p. injected *Salmonella typhimurium* TA1530 (hisG46, gal-, uvrB-) was obtained up to 94 fold that of the control group (Keck *et al*, 1980).

Gene mutations in cultured mammalian cells (Table 39)

H_2O_2 is able to induce gene mutations at the HGPRT locus (Nassi-Calo *et al*, 1989), the TK locus (Wangenheim and Bolcsfoldi, 1988) and in 6-thioguanine-resistant clones (Ziegler-Skylakakis and Andrea, 1987), but mutagenicity was not observed in earlier experiments (Bradley *et al*, 1979; Bradley and Erickson, 1981; Tsuda, 1981; Speit, 1986). Presumably the maximum concentrations used in some of the earlier tests were too low because either the cytotoxicity had been overestimated (Ziegler-Skylakakis and Andrea, 1987) or the culture medium contained sodium pyruvate which scavenges H_2O_2 (Andrea *et al*, 1985). The failure of Bradley and Erickson (1981) to observe mutations may be due to the temperature of incubation being 0°C instead of 37°C.

Hsie *et al* (1986) found dose-dependent mutagenicity in a special strain of CHO-BH4 cells which have a bacterial xanthine phosphoribosyl transferase gene incorporated into a chromosomal region where relatively large deletions could be detected.

Moraes *et al* (1990) described mutations arising at the DNA sequence level in plasmid as a result of treatment of mammalian host cells with H_2O_2. When compared with spontaneous mutations, the most predominant feature of the spectrum of induced mutations was the high number of small deletions, many of which arise in runs of identical base pairs.

Gene mutations in D*rosophila melanogaster*

H_2O_2 (3% solution) did not induce sex-linked recessive lethal mutations in *Drosophila melanogaster* following treatment of the larvae (Di Paolo, 1952).

TABLE 38

THE OVERALL RESPONSE TO H_2O_2 IN BACTERIAL TESTS

Strain	Mutation	Type*	Genotype			Result	
			Repair	LPS	Plasmid	-S9	+S9
Salmonella typhimurium							
TA102	hisG428	B	+	rfa	pKM101 /pAQ1	+ve	+/-ve
TA104	hisG428	B	uvrB	rfa	pKM101	+ve	
TA2638	hisG428	B	+	rfa	pKM101	+ve	
SB1106p	hisC3108 /hisO1242	No data	+	+	pKM101	+ve	
SB1106	hisC3108 /hisO1242	No data	+	+	-	+ve	
SB1111	hisC3108	No data	+	+	-	+ve	
BA13	araD531 /hisG46	F	uvrB	+	pKM101	+ve	
BA9	araD531 /hisG46	F	uvrB	rfa	pKM101	+ve	
TA97	hisD6610 /hisO1242	F	uvrB	rfa	pKM101	+/-ve	-ve
TA98	hisD3052	F	uvrB	rfa	pKM101	+/-ve	-ve
TA100	hisG46	B/F	uvrB	rfa	pKM101	+/-ve	
TA1537	hisC3076	F	uvrB	rfa	-	+/-ve	-ve
TA1538	hisD3052	F	uvrB	rfa	-	+/-ve	-ve
TA92	hisG46	B	+	+	pKM101	-ve	
TA94	hisD3052	F	+	+	pKM101	-ve	
TA1532	hisC3076	F	uvrB	gal	-	-ve	
TA1534	No data	F	uvrB	No data	No data	-ve	
TA1535	hisG46	B	uvrB	rfa	-	-ve	
D3052	hisD3052	F	+	+	-	-ve	
G46	hisG46	B	+	+	-	-ve	
Escherichia coli							
WP2	trpE	B	+	+	pKM101	+ve	
WP2uvrA	trpE	B	uvrA	+	pKM101	+ve	
DB2	ampD494	F	uvrB	+	pGW170	+ve	
WP2	trp-	B	+	+	0	-ve	
WP2	trp-	B	uvrA	-	-	-ve	
WP2	caca	A	uvrA	+	- -	Equivocal	

* Type B: Base-pair substitution mutation; F: Frameshift mutation; A: All mutations.

TABLE 39

GENE MUTATIONS IN CULTURED MAMMALIAN CELLS

Test System	Metabolic activation	Concentration (μmol)	Cytotoxicity (LC_{50}) (μM)	Results (lowest effective concentration)	Reference
V79 cells	No	10	Unknown	Induction of mutations at the HGPRT locus (10μM)	Nassi-Calo *et al*, 1989
L5178Y Mouse lymphoma cells	No	18.6-496	37.2-79.5	Concentration-related increase of mutations at the thymidine kinase locus (18.6μM)	Wangenheim and Bolcsfoldi, 1988
V79 cells	No	500-4,000	>4,000	Concentration dependent increase in the of 6-thioguanine-frequency resistant clones (500μM)	Ziegler-Skylakakis and Andrae, 1987
V79 cells	No	10-80	20-40	No induction of mutations at the HGPRT locus (low concentrations tested due to on over-estimation of cytotoxicity)	Speit, 1986
V79 cells	No	100-300	<100	No increase of the frequency of 8-azaguanine or ouabain-resistant mutation	Tsuda, 1981
V79 cells	No	100-585	about 100	No induction of mutations at the HGPRT locus	Bradley and Erickson, 1981
V79 cells	No	353	20% survival at 353	No induction of mutations at the HGPRT locus	Bradley *et al*, 1979

8.5.2 Primary DNA-damage

Primary DNA-damage in bacteria (Table 40)

H_2O_2 induced DNA-repair in *Escherichia coli* (Thielmann and Gersbach, 1978; De Flora *et al*, 1984), SOS responses in *Salmonella typhimurium* (Nakamura *et al*, 1987) and *E. coli* (Von der Hude *et al*, 1988; Zhou *et al*, 1991). In the presence of metabolic activation, catalase or SOD, no effect was observed in the DNA-repair test or the SOS chromotest using *E. coli* strains (De Flora *et al*, 1984; Zhou *et al*, 1991).

DNA-strand breaks in cultured mammalian cells (Table 41)

H_2O_2 induced DNA single-strand breaks in bovine lens epithelial cells (Kleiman *et al*, 1990), rat intestinal epithelial cells (Grisham and Kvietys, 1989), L1210 leukemia cells (Hincks and Gibson, 1988/89), rat hepatocytes (Olson, 1988), L5178 Y mouse lymphoma cells (Garberg *et al*, 1988), P388D1 cells (Schraufstatter *et al*, 1986), rat and human lymphocytes (Schraufstatter *et al*, 1986; Grisham and Kvietys, 1989), human bronchial epithelial cells (Saladino *et al*, 1985), JB6 Cl 21 cells (Gensler and Bowden, 1983) or V79 cells (Bradley *et al*, 1979; Bradley and Erickson, 1981; Prise *et al*, 1989).

DNA double-strand break induction was observed in V79 cells (Prise *et al*, 1989) and in human leucocytes (Cristovão *et al*, 1991) but not in cultured rat hepatocytes (Olson, 1988). Decreased DNA and RNA synthesis in human bronchial cells was observed (Saladino *et al*, 1985) after treatment with H_2O_2, but there was no or a relatively insignificant induction of DNA-protein (Bradley *et al*, 1979; Bradley and Erickson, 1981; Olson, 1988; Kleiman *et al*, 1990) or DNA-DNA crosslinks (Bradley *et al*, 1979; Bradley and Erickson, 1981).

Unscheduled DNA Synthesis (UDS) in cultured mammalian cells (Table 42)

Unscheduled DNA Synthesis was observed in rat hepatocytes (Cattley *et al*, 1988; Beales and Suter, 1989) and human cells (Stich *et al*, 1978; Coppinger *et al*, 1983) after *in vitro* treatment with H_2O_2.

Sister Chromatid Exchanges (SCE) in cultured mammalian cells (Table 43)

H_2O_2 induced Sister Chromatid Exchanges (SCE) in V79 cells (Bradley *et al*, 1979; Speit *et al*, 1982; Mehnert *et al*, 1984a,b; Speit, 1986; Tachon and Giacomoni, 1989), CHO cells (Mehnert *et al*, 1984a,b; Tucker *et al*, 1989; Wilmer and Natarajan, 1981), human D98/AH2 cells (Estervig and Wang, 1984) and human lymphocytes (Mehnert *et al*, 1984a,b). In the presence of S9, catalase or peroxidase, SCE-induction was reduced or inhibited (MacRae and Stich, 1979; Speit *et al*, 1982; Mehnert *et al*, 1984a,b).

TABLE 40

PRIMARY DNA-DAMAGE IN BACTERIA

Test system	Metabolic activation	Concentration (μM)	Results (lowest positive response)	Reference
SOS chromotest				
E. coli PQ37	No	0-500	+ve (20μM)	Zhou *et al*, 1991
	Catalase	20	-ve	
	SOD	20	-ve	
SOS chromotest				
E. coli PQ37	No	0-1,000	+ve (0.3mM)	Von der Hude *et al*, 1988
Umu test				
Salmonella typhimurium TA1535/pSK1002	No	Unknown	+ve (45mg/ml)	Nakamura *et al*, 1987
DNA-repair test				
E. coli WP2, WP67 and CM871	No	Unknown	Minimal inhibitory concentration was 0.02μg	De Flora *et al*, 1984
	S9	Unknown	-ve	
DNA excision repair				
E. coli	No	0-400,000	+ve	Thielmann and Gersbach, 1978

TABLE 41

PRIMARY DNA-DAMAGE IN CULTURED MAMMALIAN CELLS - INDUCTION OF DNA-STRAND BREAKS

Test system	Metabolic activation	Treatment time/ temperature	Concent-ration (μM)	Results (lowest effective concentration)	Reference
Human leucocytes	No	10min/ Unknown	500-1,000	Dose-dependent induction of DNA double strand breaks	Cristovao *et al*, 1991
Bovine lens epithelial cells	No	5min/37°C	10-200	Linear increase of DNA single-strand breaks (50μM)	Kleiman *et al*, 1990
Rat intestinal epithelial cells	No	15min/37°C	100	Induction of DNA-strand breaks	Grisham and Kvietys, 1989
Lymphocytes	No	15min/37°C	100	Induction of DNA-strand breaks	Grisham and Kvietys, 1989
V79 cells	No	10-20min/0°C	10-1,000	Dose-dependent induction of DNA single-strand breaks (10μM)	Prise *et al*, 1989
V79 cells	No	20min/0°C	10-10,000	Induction of DNA double-strand breaks at concentration higher than 100μM	Prise *et al*, 1989
L1210 leukemia cells	No	1h/0°C	50-200	Dose-dependent formation of DNA strand breaks (50μM)	Hincks and Gibson, 1988/89
Rat hepatocytes	No	1h/37°C	10-1,000	Concentration-dependent increases in single-strand DNA breaks (10-50μM) but lack of induction of double-strand DNA breaks	Olson, 1988
Rat hepatocytes	No	1h/37°C	200/500	Lack of H_2O_2-induced DNA-protein cross linking	Olson, 1988

TABLE 41 (cntd.)

RIMARY DNA-DAMAGE IN CULTURED MAMMALIAN CELLS - INDUCTION OF DNA-STRAND BREAKS

Test system	Metabolic activation	Treatment time /temperature	Concent-ration (μM)	Results (lowest effective concentration)	Reference
L5178Y Mouse Lymphoma cells	No	3h/37°C	2,000-2,510	Concentration-related increase in single-strand DNA breaks (2000μM)	Garberg *et al*, 1988
P388D1 cells and human lymphocytes	No	5min/37°C	25-1,000	Concentration-related increase in single-strand DNA breaks (25μM)	Schraufstat-ter *et al*, 1986
Normal Human Bronchial Epithelial Cells	No	1h/37°C	100 or 120	Decrease DNA and RNA synthesis Induced DNA single-strand breaks	Saladino *et al*, 1985
JB6Cl21 cells	No	1h/37°C	1-100	Concentration-dependent increase in single-strand breaks (10μM)	Gensler and Bowden, 1983
V79 cells	No	2h/1°C	7.4-73.5	Concentration-dependent increase in single-strand breaks(14.7μM)	Bradley and Erickson, 1981
	No	1h/1°C	353	No induction of DNA-protein or DNA-DNA crosslinks	Bradley and Erickson, 1981
V79-4 cells	No	Unknown	353	Induction of DNA single-strand breaks and/or alkali-labile lesions No induction of DNA-protein or DNA-DNA crosslinks	Bradley *et al*, 1979

TABLE 42

PRIMARY DNA-DAMAGE IN CULTURED MAMMALIAN CELLS

- INDUCTION OF UNSCHEDULED DNA SYNTHESIS (UDS)

Test system	Protocol	Exposure time (h)	Metabolic activation	Concent-ration (mM)	Results (lowest effective concentration)	Reference
Rat hepatocytes	Liquid scintillation	20	No	0.1-100	Concentration related increase (1mM)	Beales and Suter, 1989
Rat hepatocytes	Auto-radiography	3x1	No	1.9-6.4	Induction of UDS (3.2mM)	Cattley *et al*, 1988
WI-38 CCL75 cells (human diploid fetal lung cells)	Auto-radiography	3-4	No	0.6-2400 µg/ml	Concentration-related increase (9µg/ml)	Coppinger *et al*, 1983
	Differential density labeling	3-4	No	0.15-600 µg/ml	Concentration-related increase (9.4µg/ml)	Coppinger *et al*, 1983
Human fibroblastes	Auto-radiography	3	No	0.1-10	Concentration-related increase (0.6mM)	Stich *et al*, 1978

TABLE 43 - PRIMARY DNA-DAMAGE IN MAMMALIAN CELLS - INDUCTION OF SISTER CHROMATID EXCHANGES (SCE)

Test system	Metabolic activation	Exposure time (h)	Concent-ration (μM)	Cyto-toxicity (IC_{50}) (μM)	Results (lowest effective concentration)	Reference
V79 cells	No	1	5-20	>20 in MEM 5-10 in PBS	No SCE induction in MEM culture medium Concentration related induction of SCE (10μM) in PBS medium	Tachon and Giacomoni, 1989
CHO cells	No	15-22	40-240	160	Induction of highly significant levels of SCE and endoreduplicated cells (40μM and 160μM, respectively)	Tucker *et al*, 1989
V79 cells	No	3	10-80	20-40	Induction of SCE (20μM)	Speit, 1986
Whole human blood or human purified lymphocytes	No	24	20-2,000	Unknown	SCE induction in purified lymphocytes (20μM) but not in whole blood culture	Mehnert *et al*, 1984a
	No	2	80-200	Unknown	SCE induction in purified lymphocytes (80μM) but not in whole blood culture	Mehnert *et al*, 1984a
Human purified lymphocytes	Catalase or peroxydase or S9-mix	2	80-200	Unknown	Reduction of H_2O_2-induced SCEs	Mehnert *et al*, 1984a
D98/AH2 human cells	No	24	15-60	>60	3-fold SCE induction at 60μM	Estervig and Wang, 1984
CHO cells	No	2	0.1-100mM	10mM	Slight increase of the SCE frequency (0.5mM)	Wilmer and Natarajan, 1981

TABLE 43 (Cntd)

Test system	Metabolic activation	Exposure time (h)	Concent-ration (μM)	Cyto-toxicity (IC_{50}) (μM)	Results (lowest effective concentration)	Reference
CHO cells	No	24	0.3-7.8	No toxicity	Two-fold increase of SCE (3.9μm)	MacRae and Stich, 1979
	Catalase	Un-known	5-100	No toxicity	Complete reduction of the H_2O_2-induced SCE	MacRae and Stich, 1979
V79 and CHO cells	No	1 or 24	10-40	Un-known	Concentration-related induction of SCE (10-20μM)	Mehnert *et al*, 1984b
	S9-mix	1	10-40	Un-known	Inhibition of SCE-induction	Mehnert *et al*, 1984b
V79 cells	No	1 or 9	1-800	Un-known	Induction of SCE	Speit *et al*, 1982
	Catalase	9	100-800	Un-known	Complete reduction of the H_2O_2-induced SCE	Speit *et al*, 1982
CHO cells	No	2	100-100,000	>10,000 μM	Slight increase of the SCE frenquency (500μM)	Wilmer and Natarajan, 1981
V79 cells	No	Un-known	353	20% survival	Two-fold increase of the SCE frequency	Bradley *et al*, 1979
CHO cells	No	24	0.31-7.8	No toxicity	Two-fold increase of the SCE frequency (3.9μM)	McRae and Stich, 1979
	Catalase	Un-known	5-100	No toxicity	Complete reductions of the hydrogen peroxide induced SCE's	McRae and Stich, 1979

8.5.3 Clastogenicity

Chromosomal aberrations in cultured mammalian cells (Table 44)

Chromosome aberrations (mainly chromatid breaks or micronuclei) were induced in V79 cells (Tsuda, 1981; Tachon and Giacomoni, 1989), Don-6 Chinese hamster cells (Sasaki *et al*, 1980), human embryonic fibroblasts (Oya *et al*, 1986), human leucocytes (Cristovão *et al*, 1991), CHO cells (Stich *et al*, 1978; Tsuda, 1981; Stich and Dunn, 1986), CHL cells (Ishidate *et al*, 1984), Syrian hamster and Balb/c mouse cells (Tsuda, 1981), but not in D98/AH2 human cells (Estervig and Wang, 1984) after *in vitro* treatment with H_2O_2.

In vitro exposure of murine splenocytes to H_2O_2 did not increase the frequency of micronucleated cells (Dreosti *et al*, 1990). This negative result may be due to the use of the culture medium as the solvent for H_2O_2 and inadequate H_2O_2 concentrations (Oya *et al*, 1986). Combined treatment with ferrous ions and H_2O_2, led to a synergistic enhancement of the frequency of micronuclei (Dreosti *et al*, 1990).

Chromosomal aberrations and micronuclei induction in mammals

Forty-eight hours after implantation of ascite-tumours (S2 sarcoma, Ehrlich ascites carcinoma and sarcoma 180), mice were injected i.p. with 0.2ml H_2O_2 solution at concentrations ranging from 0.01 to 0.5M; 48h later, the treated and untreated tumours were examined. A dose-related increase in the number of chromosomal aberrations of the tumour cells was observed in the H_2O_2 treated mice (Schoeneich, 1967).

H_2O_2 did not induce chromosomal aberrations in bone-marrow cells of rats (Kawachi *et al*, 1980) and was negative in a micronucleus test in mice (Keck *et al*, 1980). Details of these two studies are lacking.

8.5.4 Genotoxicity in Cultured Mammalian Cells - Other End-points

H_2O_2 caused morphological transformation of C3H/10T1/2 cells (Nassi-calo *et al*, 1989).

8.5.5 Mechanistic Considerations

According to several authors, DNA appears to be the main cellular target of H_2O_2, but its cytotoxicity limits the expression of its genotoxic potential *in vitro* (Sanford *et al*, 1986; Meneghini, 1988; Cantoni *et al*, 1989; Ruch *et al*, 1989).

The formation of adenine N-1-oxide (Mouret *et al*, 1990), 8-hydroxyguanosine (Kasai *et al*, 1986), thymidine glycol (Frenkel and Chrzan, 1987), H_2O_2-induced alkali-labile sites (including

TABLE 44

CHROMOSOMAL ABERRATIONS IN CULTURED MAMMALIAN CELLS

Test system	End-point	Exposure time (h)	Metabolic activation	Concent-ration (μM)	Results (lowest effective concentration)	Reference
Human leucocytes	Chromosomal aberrations	24	No	15 and 20mM	Six-fold increase at 20mM	Cristavao *et al*, 1991
Murine splenocytes	Cytokinesis-block micronucleus assay	Unknown	No	10 and 20	No increase of the frequency of micronucleated splenocytes	Dreosti *et al*, 1990
		Unknown	Ferrous ions	20	Synergistic enhancement in micronucleus frequency	Dreosti *et al*, 1990
V79 cells	Micronuclei	1	No	10-20	Concentration-related increase of micronuclei (10μM) in PBS* medium, no effect in MEM* medium	Tachon and Giacomoni, 1989
Human embryonic fibroblasts	Chromosomal aberrations Chromatid aberrations	10 min	No	10-1,000	Concentration-related increase of chromosomal and chromatid aberrations (20μM)	Oya *et al*, 1986
CHO cells	Chromosomal aberrations and micronuclei	3	No	<10-25	Concentration-related increase of chromosome aberrations (12.5μM), chromatid translocations (12.5μM) and micronuclei (12.5μM)	Stich and Dunn, 1986
CHL cells	Chromosomal aberrations	24/48	No	0-7.3	Positive at 24h (3.7μM) and 48h (1.8μM)	Ishidate *et al*, 1984

* PBS, Phosphate-buffered saline; MEM, Eagles Modified Minimal Essential Medium.

TABLE 44 (cntd)

CHROMOSOMAL ABERRATIONS IN CULTURED MAMMALIAN CELLS

Test system	End-point	Exposure time (h)	Metabolic activation	Concentration (μM)	Results (lowest effective concentration)	Reference
D98/AH_2 human cells	Chromosomal aberrations	24	No	15-60	Negative	Estervig and Wang, 1984
CHO-K1 cells	Chromosomal aberrations	3	No	100-1,000	Concentration-related increase of chromatid breaks (200μM)	Tsuda, 1981
V79 cells	Chromosomal aberrations	3	No	100-1,000	Concentration-related increase of chromatid breaks (200μM)	Tsuda, 1981
Syrian hamster cells	Chromosomal aberrations	3	No	100-1,000	Concentration-related increase of chromatid breaks (200μM)	Tsuda, 1981
Balb/c mouse cells	Chromosomal aberrations	3	No	10-100	Concentration-related increase of chromatid breaks (50μM)	Tsuda, 1981
CHO cells	Chromosomal aberrations	2	No	100-100,000	Only positive at 10,000μM	Wilmer and Natarajan, 1981
CHO cells	Chromosomal aberrations	3	No	100-1,000	Concentration related increase of chromosome aberrations (100μM), toxic at concentrations higher than 300μM	Stich *et al*, 1978
Don-6 cells (Chinese hamster cells)	Chromosomal aberrations	3	No	500-2,000	Dose related increase of chromosome aberrations (1,000μM)	Sasaki and Sugimura, 1980

DNA strand-breaks), SCEs, gene mutations, chromosomal aberrations, DNA-repair and cell transformations are all mediated by hydroxyl radicals (·OH) aimed at the DNA by DNA-bound transition metals (Emerit *et al*, 1982; Oya *et al*, 1986; Joenje, 1989; Tachon, 1990). These effects can be inhibited by the addition of a strong Fe-chelating agent or a hydroxyl radical (·OH) scavenger (Mello Filho and Meneghini, 1984; Oya *et al*, 1986; Cantoni *et al*, 1989; Nassi-Calo *et al*, 1989) and enzymes that catalytically scavenge intermediates of oxygen reduction ($\cdot O_2^-$ and H_2O_2) (section 7).

No specific defences are known against ·OH radicals and singlet oxygen which react quickly with almost any biologically active molecule.

The *in vivo* data show discrepancies between positive results observed in host-mediated assays (gene mutations and chromosomal aberrations) and negative results in chromosomal aberration tests in rats and micronucleus tests in mice. Several reports confirm that sensitivity to exogenous H_2O_2 is negatively correlated with cellular catalase activity in mammalian cells (Winquist *et al*, 1984; Vuillaume, 1987; Sawada *et al*, 1988). The low catalase activity of tumour cells used in the host mediated assay (Schoeneich, 1967) and the direct contact with H_2O_2 by i.p. administration may explain the positive results in the host-mediated assays. On the other hand, the absence of chromosomal abnormalities in the bone-marrow of the orally treated animals may be explained by decomposition of H_2O_2 in the bowel before absorption and by the high catalase activity of red blood cells, which can decompose H_2O_2 after its absorption.

8.5.6 Evaluation

The *in vitro* and *in vivo* genotoxic potential of H_2O_2 is summarised in Table 45.

In conclusion, since only hydroxyl radicals and singlet oxygen are capable of damaging DNA directly, the genotoxic potential of H_2O_2 depends on the accessibility of the extremely reactive hydroxyl radical to its target DNA. *In vitro*, the bacteria or other cells come into direct contact with H_2O_2 and genotoxic effects can be induced; in general, the addition of an exogenous metabolic agent or catalase reduces or abolishes the mutagenic response. *in vivo*, many factors contribute to the reduction of the bioavailability of H_2O_2 for systemic genotoxic action. However, the occurrence of genotoxic effects on cells which are in direct contact with H_2O_2 (at the site of application) cannot be excluded.

TABLE 45

EVALUATION OF GENOXICITY

End-Point	Test System	Results
IN VITRO		
Gene mutation	*Salmonella typhimurium*	+ve without activation decrease of the genotoxic potential in presence of exogenous S9 or catalase
	Escherichia coli	+ve without activation
	Saccharomyces cerevisiae	+/-ve without activation
	Bacillus subtilis	+ve without activation
	Mammalian cells	+ve without activation
Primary DNA-damage		
DNA-repair	*Salmonella typhimurium*	+ve without activation
	Escherichia coli	+ve without activation -ve with activation
DNA-strand break SCE	Mammalian cells	+ve without activation
	Mammalian cells	+ve without activation
	Mammalian cells	+ve without activation decrease of the SCE induction in presence of exogenous S9 or catalase
Chromosomal aberration	Mammalian cells	+ve without activation
Morphological transformation	Mammalian cells	+ve without activation
IN VIVO		
Gene mutation	Drosophila melanogaster	-ve
	Salmonella typhimurium (host mediated assay in mice)	+ve
Chromosomal aberration		
Micronucleus Metaphase analysis	Mice	-ve
	Rat	-ve
	Tumour cells (host mediated assay in mice)	+ve

8.6 REPRODUCTIVE TOXICITY/TERATOGENICITY

8.6.1 Male Fertility

The spermicidal activity of H_2O_2 was investigated both *in vivo* and *in vitro*. Washed and unwashed sperm from bull, rabbit, ram, fowl, dog and mouse were exposed to solutions containing .3, 3, 30, 300 and 3,000ppm H_2O_2. Sperm were evaluated for mobility *in vitro* following washing and when added directly to diluent. There were large species differences in the tolerance to H_2O_2. Rabbit spermatozoa were the most resistant and bull and fowl were the least resistant to the immobilising effect of H_2O_2. Although 3ppm had small, but significant effects on bull and fowl spermatozoa, concentrations 10 to 100 times greater were required to immobilise rabbit spermatozoa. In general, washing increased the toxicity of H_2O_2, but this effect varied with the species tested. Bull and fowl spermatozoa were least affected by washing and rabbit spermatozoa were most affected (Wales *et al*, 1959). Endogenous catalase is removed when spermatozoa are washed; thus, washing the spermatozoa is expected to increase the toxicity of H_2O_2.

Male albino mice were given 0.33%, 1.0% or 3.0% H_2O_2 solutions in place of drinking water. There were no controls. The mice at the highest dose (3.0%) would not drink the solution and were taken off the study. Mice were mated after 7 and 21d on H_2O_2. All females became pregnant within a few days and delivered litters of normal size. The concentration, morphology and mobility of the spermatozoa of the male mice and rabbits receiving H_2O_2 in their drinking water over 3 and 6 weeks remained normal. *In vivo*, H_2O_2 has no significant spermicidal action in mice at concentrations up to 1% in solution (Wales *et al*, 1959).

8.6.2 *In Vitro* Embryotoxicity

The embryotoxicity of 30% H_2O_2 was investigated using 3-day old (72-76h) Leghorn chicken eggs (Korhonen *et al*, 1984). H_2O_2 solutions (0.05, 0.09, 0.19 and 0.37mg/egg) were dropped on the inner shell membrane in the egg's air chamber, focussing on the embryo visible under the membrane. The ED_{50} for all effects observed was 0.9mg/egg. The LD_{50} was 1.2mg/egg. The NOEL was 0.05mg/egg based on embryo-lethality and toxicity. The significance of this type of study to mammalian species is uncertain.

8.6.3 Reproductive Organs

Male and female rats were administered H_2O_2 by gavage at doses of 1/10-1/5 LD_{50}. Several effects were reported: modification of the oestrus cycle in females and a decrease in sperm mobility in males. Male and female rats received daily doses of 0.005-50mg/kg H_2O_2 by gavage for 6 months. At the high dose, females showed modifications of the oestrus cycle and males reduced mobility of spermatozoa. Treated animals were mated. Among high dose

females, only 3/9 produced litters, compared to 7/9 in the control group. In addition, the body weight of the offspring of the high dose females was reduced relative to those of control females (Antonova, 1974).

8.6.4 Teratology

An attempt was made to assess the teratogenic potential of H_2O_2 in the rat. H_2O_2 was mixed with powdered feed to give 0.02, 0.1, 2 and 10% in the diet which was fed to female rats for 1 week during their 'critical period' of gestation. Unfortunately, this study has multiple deficiencies including a poor design which led to severe reduction of food intake, lack of diet analysis and too few animals for a meaningful statistical analysis. The authors acknowledged that H_2O_2 decomposes readily and showed that the level in the 2% diet declined to background level within 3d. Thus no conclusion regarding the teratogenic potential of H_2O_2 can be made from this study. (Moriyama *et al*, 1982).

A brief communication published over 30 years ago described a study in which 3 rat dams were given 0.45% H_2O_2 to drink. After 5 months, the H_2O_2 was replaced with water and they were mated; all 3 produced normal litters (Hankin, 1958). However, no details regarding study design or conduct were given, thus precluding any evaluation of the adequacy of this study.

8.6.5 Evaluation

Data on the teratogenic potential and reproductive toxicity are too limited to allow an evaluation.

SECTION 9. EFFECTS ON MAN

9.1 ACCIDENTAL EXPOSURE INCIDENCE (TABLE 46)

Reports of accidental exposure resulting in treatment at poison control centres have been tabulated by the American Association of Poison Control Centers since 1985 (Litovitz *et al*, 1990). Similar data on the European situation are not available. In 1989, there were 7,354 cases of accidental exposure due to consumer use of H_2O_2 as a household topical disinfectant (4,293 cases in 1987). There were 8,557 additional cases due to contact with cosmetic/personal care products containing peroxide (7,587 cases in 1987). The combined number in 1989 (15,911) was greater than the total number of cases reported in 1987 (11,871) (Litovitz *et al*, 1988).

TABLE 46

ACCIDENTAL EXPOSURES IN THE USA IN 1989

(Litovitz *et al*, 1990)

Total number of exposures	Age Group (y)			Reason		Treated in Health Care Facility
	<6	6-17	>17	Accident	Intention	
Topical use						
7,354[a]	4,613	590	2,100	7,199	138	487
Cosmetic and personal care use						
8,557[b]	5,073	669	2,717	8,291	229	727

a Outcomes of exposure included 2,071 cases with no toxicity, 1,395 cases of minor toxicity and 37 cases of moderate toxicity. There were no deaths.

b Outcomes of exposure included 2,619 cases with no toxicity, 1,917 cases of minor toxicity, 75 cases of moderate toxicity and 3 cases of major toxicity. There were no deaths.

9.2 ACUTE ORAL TOXICITY

A 1-year-old child died of respiratory failure within one hour after ingesting an unknown quantity of a 30% H_2O_2 solution (Giusti, 1973). Post-mortem examination revealed extensive hypostasis and petechiae of the thymus, epicardium and duodenum. The stomach was distended with gas and the viscera were congested. There was some necrosis of the gastro-intestinal mucosa.

A 2-year-old boy ingested an unknown amount of a 3% H_2O_2 solution. He was foaming around the mouth and had vomited three times. A gas embolism was visualised in the portal venous system. It was concluded that liberation of oxygen from H_2O_2 in the stomach ascended through the gastric veins to the portal venous system. The child recovered (Rackoff and Merton, 1990).

A 26-month-old girl ingested a mouthful of a 35% H_2O_2 solution. The child vomited spontaneously. Clinical signs included lethargy and bright red-tinged, frothy emesis. She experienced one fainting episode with short cessation of respirations which returned spontaneously. Endoscopy 16h after exposure showed erosion of the cardiac stomach and erythema of the lower oesophageal sphincter and a gastric burn. Follow-up endoscopy 12d later showed normal esophageal and duodenal mucosa with minimal hyperemia of the cardiac stomach and no ulceration or eschar of the gastric mucosa. The child recovered completely within 2 weeks (Humberston *et al*, 1990).

A 3-year old girl died following ingestion of an unknown quantity of 40% H_2O_2 solution. Pathological findings at autopsy included oedema of the lungs and small erosions of the stomach mucosa, probably due to the lavage tube used to wash out the stomach. The report indicates that the probable cause of death was asphyxia due to obstruction of the distal respiratory tract by the foam liberated by the peroxide (oxygen gas) (Zecevic and Gsparec, 1979).

A 6-year old girl died and her four-year old friend was in critical condition with oropharyngeal burns after accidentally ingesting a solution of 35% H_2O_2 (Thompson, 1989).

Five non-fatal poisonings in adults were reported following consumption of about 25-100ml of H_2O_2 (concentration unknown). The victims experienced sharp pains in the abdomen, foaming at the mouth, vomiting, transitory loss of consciousness, sensory and motor impairment and elevated temperature. Microhaemorrhages of the skin and conjunctiva and moderate leukocytosis were also reported. One person exhibited marked visual (including temporary blindness) and neurological symptoms after swallowing 100ml of the H_2O_2 solution. Symptoms were considered to be due to microemboli of generated oxygen. All victims recovered completely in two to three weeks (Budagovskij *et al*, 1971).

A near-fatality occurred when a 33 year-old woman unintentionally ingested the contents of a 1 pint bottle (<500ml) of 35% H_2O_2. The patient was cyanotic and experienced seizures and foaming at the mouth. The abdomen was slightly distended. Neurological signs included lack of spontaneous eye movement, no verbal response and withdrawal from noxious stimuli. Laparotomy revealed air bubbles in the stomach but no perforations. Recovery was complete following treatment (Giberson *et al*, 1989).

9.2.1 Evaluation

Young children are particularly vulnerable to accidental ingestion of H_2O_2. Deaths have been reported from ingestion of unknown quantities of H_2O_2 solutions of 30-40% concentration.

9.3 INTENTIONAL EXPOSURE: MISUSE

There are a number of unofficial reports that some individuals self-administer H_2O_2 under the impression that it can provide health benefits. Such intentional exposure can lead to life-threatening toxicity, including death. A recent publication highlights the dangers of misuse of H_2O_2. A 39-year old man was admitted to a hospital emergency room in haemolytic crisis 24h after alleged intravenous injection of H_2O_2 (amount and concentration not specified), provided by a local physician, for treatment of cancer. Clinically, the patient was experiencing an acute haemolytic crisis (haematocrit 13.5%). He died following cardiopulmonary arrest on his twelfth day in the hospital (Jordan *et al*, 1991).

9.4 ACUTE EFFECTS FROM IRRIGATING AND CLEANSING SOLUTIONS

9.4.1 Oral Rinses

In a study designed to evaluate the antiseptic activity of a mouth rinse containing 0.5% or 0.75% H_2O_2 in 33% or 50% glycerine, respectively, no irritation of the oral mucosa was noted at the lower concentration in six subjects over a five-day use period with two one-minute rinses daily. Some subjects noted irritation of the mouth and gums at the higher concentration of H_2O_2, but these effects may have been due to the humectant effect of the 50% glycerine (Slanetz and Brown, 1949).

Rees and Orth (1986) report that the use of 3% H_2O_2 three to five times/day as a mouthrinse resulted in mucosal irritations in two individuals with prior tissue injury. The pre-existing lesions worsened after exposure to 3% solution. Herrin *et al* (1987) has shown that use of 3% H_2O_2 with sodium bicarbonate did not cause any lesions in healthy individuals. Gingival lesions were seen in patients who used home care solutions employing 5M sodium chloride in addition to 3% H_2O_2 and sodium bicarbonate.

A group of 88 dental students self-administered 6-12.5% H_2O_2 solutions. They used it as a mouthwash and dipped their toothbrushes into the solution before brushing their teeth. Application of the H_2O_2 was 2-3 times/day for 1-2.5 months. Some gingival changes were noted: 6.4% of the subjects showed "redder" gums, 3.4% showed "paler" gums and 6.6% developed hyperkeratinised filiform papillae of the tongue (Miller *et al*, 1938).

The US FDA concluded that concentrations of up to 3% H_2O_2 are safe for over-the-counter use on the mucous membranes of the mouth and throat (US FDA, 1983).

9.4.2 Wound Irrigating and Disinfecting Solutions

A 3% solution of H_2O_2 is widely used as a topical antiseptic agent for suppurative wounds and inflammations of the skin and mucous membranes; as an irritating solution during root-canal therapy and as a mouth rinse for acute necrotising gingivitis (US FDA, 1983).

Cases of rupture of the colon, inflammation of the anus or rectum and ulcerative colitis have been reported following H_2O_2 use of a 1% or 2% solution as an enema (Pumphrey, 1951; Ludington *et al*, 1958; Sheehan and Brynjolfsson, 1960). This is no longer used.

Shock and coma developed suddenly in a 54-year old patient in which 3% H_2O_2 was being used for wound irrigation of the inguinal area during surgery. The patient recovered within one hour. The cause of the near-fatality was considered to be microembolisms formed by H_2O_2 degradation in the closed spaces of the wound irrigation (Bassan *et al*, 1982).

Oxygen embolism has been reported in several infants following intestinal irrigation with H_2O_2 to remove meconium (Danis *et al*, 1967; Shaw *et al*, 1967). In one case, a 36-h old infant died following use of 1% H_2O_2 to remove inspissated meconium from the bowel due to meconium ileus (Shaw *et al*, 1967).

There are several reports of chemically-induced colitis in patients undergoing endoscope examination with instruments which had been cleaned with 3% H_2O_2 (Jonas *et al*, 1988; Bilotta and Waye, 1989). Discrete white plaques adherent to the colonic mucosa and mild to severe erythema of the surrounding mucosa were observed in one report of twenty patients (Jonas *et al*, 1988). In the other reports, areas of white mucosa extending over areas of the colon were seen following endoscopic procedures (Bilotta and Waye, 1989). Pathological examination of biopsy specimens revealed nonspecific inflammation. Colitis has also been reported in patients following the use of 3% H_2O_2 diluted with water as an enema (Meyer *et al*, 1981).

Use of H_2O_2 to sanitise haemodialysis equipment has resulted in haemolysis in three pediatric patients, as shown by a significant decline in blood haemoglobin levels (Gordon *et al*, 1990). Apparently, the H_2O_2 sanitising solution was not adequately rinsed out prior to use.

A United States Food and Drug Administration panel indicated that 3% H_2O_2 is safe for over-the-counter use as a topical antiseptic and cleansing solution and sanitising lotions (US FDA, 1983).

9.5 OCULAR EXPOSURE

Several early reports indicate that H_2O_2 was used directly in human eyes to treat corneal ulcers, particularly in herpetic dendritic keratitis (Grant, 1986). In one case, a 20% solution was applied after a local anaesthetic every two hours, as a localised cautery to the ulcer. No apparent corneal damage resulted (Vala, 1965). A 10% solution of H_2O_2 was dropped on one eye of a patient after cocaine with no adverse consequences (Lewin and Guillery, 1913). It is not known how much reliance can be placed on these reports.

Historically, 1-3% solutions have been used as topical ocular antibacterial agents three to five times per day without significant injury (Grant, 1986). Dropping 3% H_2O_2 solution into human eyes causes severe pain, which soon subsides. Even a 0.5% solution caused pain and conjunctival hyperemia (Grant, 1986).

An accident report describes ocular inflammation, hyperaemia, tears and eyelid spasm in a woman who inadvertently placed in her eye a contact lens which had been stored in 3% H_2O_2. Vision was reduced insignificantly and recovery was complete in a few days following treatment with anti-inflammatory drops (Knopf, 1984).

Contact lens solutions of H_2O_2 generally contain buffers and preservatives which may reduce the ocular toxicity. The threshold irritant concentration was determined by introducing hydrogel contact lens treated with various concentrations of H_2O_2 into human eyes. Concentrations of 0, 25, 50, 100, 200, 400 and 800ppm were tested; none affected the physiological integrity of the eye. An initial conjunctival hyperemia occurred with the 800 and 400ppm levels by not with 200ppm and below. Levels of H_2O_2 in excess of 100ppm were associated with an initial subjective stinging response. Subjective effects, such as stinging were first noticed between 50 and 100ppm; the NOEL was 50ppm. There were no disruptive changes to the corneal or conjunctival epithelium at any concentration (Paugh *et al*, 1988).

The mean ocular threshold for discomfort in human subjects was 247ppm when solutions were instilled directly into the eyes (McNally, 1990).

H_2O_2 has gained wide-spread use as a disinfectant of contact lenses, resulting in maximum residual concentrations of 50-60ppm after neutralisation; this is close to the natural background level of H_2O_2 in the eye lens (section 7).

9.6 DERMAL EXPOSURE

A characteristic temporary whitening of the skin occurs after dermal application of 20% to 30% H_2O_2. The whitening is due to oxygen bubbles acting as microemboli in the capillaries and blocking circulation (Hauschild *et al*, 1958). H_2O_2 is used topically to bleach hair at concentrations of 3% to 6%. No clear data are available on the skin irritation effects of various H_2O_2 solutions in human beings (see also next section).

9.7 VAPOUR EXPOSURE

There are few reports of adverse effects resulting from inhalation exposure to H_2O_2, although bleaching of the hair is a common occurrence. Bleaching has been known to occur with levels of 0.5-1ppm vapour (FMC, 1990e), but it is uncertain whether bleaching was due to vapour exposure or to transfer of H_2O_2 from the hands to hair.

Eye and throat irritation and gradual bleaching of hair have been reported among factory workers exposed to H_2O_2 aerosol concentrations of 12 to 41mg/m^3 (8.6 to 29.5ppm) (Kaelin *et al*, 1988). The workers were operators of a semi-automatic milk packing machine which used a H_2O_2 bath to sanitise cardboard packaging for milk. One worker developed interstitial lung disease and impaired gas exchange, but since he was a heavy smoker, the cause of the pulmonary disease could not be ascertained.

In a USSR study, the irritant threshold to the lungs was determined in human volunteers exposed from 5 minutes to 1-4h. The threshold level was considered to be 10mg/m^3 for the full period (up to 4h) with a NOEL of 5mg/m^3 (Kondrashov, 1977). Increasing the time over which skin was exposed to H_2O_2 aerosols from 5min to 4h decreased the irritation threshold from 180 to 20mg/m^3 (Krondrashov, 1977).

9.8 INDIVIDUAL SUSCEPTIBILITY

Some individuals are more susceptible to H_2O_2 exposure because of hereditary disorders in H_2O_2 metabolising enzymes (section 7).

9.9 SKIN SENSITISATION

No data are available.

9.10 CHRONIC TOXICITY AND CARCINOGENICITY

No data are available.

9.11 REPRODUCTIVE TOXICITY

Human sperm was exposed to solutions containing 0.3, 3, 30, 300 and 3,000ppm H_2O_2 *in vitro*. In unwashed spermatozoa, the ED_{50} (the concentration that results in a 50% reduction of the mobility index of spermatozoa) of H_2O_2 was between 30 and 300ppm (Wales *et al*, 1959). Removal of endogenous catalase by washing increased the toxicity of H_2O_2 to spermatozoa.

9.12 NEUROTOXICITY

No information is available.

9.13 EVALUATION

Accidental exposure to H_2O_2 has occurred from its use in household topical disinfectants and cosmetic/personal care products. Young children are particularly vulnerable to accidental oral exposure. Deaths have been reported from ingestion of unknown quantities of 30-40% solutions. The effects seen were related to a corrosive action on the gastro-intestinal tract and to generation of large volumes of oxygen. Complete recovery has occurred in near fatal cases within 2-3 weeks with medical care.

H_2O_2 solutions of 1-3% have been used as anti-bacterial agent in the eye and mouth washes without causing significant injury. In the eye, H_2O_2 solutions of >200ppm caused hyperemia; pain and stinging occurred with concentrations of ≥100ppm.

In volunteers exposed to aerosol for 4h, the irritation threshold for the respiratory tract was 10mg/m^3 H_2O_2 and for the skin 20mg/m^3. At these concentrations, eye and throat irritation and gradual bleaching of hair has been reported. At concentrations at or below the present occupational exposure limit of 1.4mg/m^3 only hair bleaching was observed; this may, however, also have been caused by H_2O_2 transferred from the hands to the hair.

SECTION 10. FIRST AID AND SAFE HANDLING ADVICE

This section has been based on Material Safety Datasheets from L'Air Liquide (1990b-j), Amylum (1990), Atochem (1989a-e, 1990), Degussa (1986, 1988, 1989), Du Pont (1990a,b), Eka Nobel (1986a-c, 1988a,b, 1989), FMC (1990a-d), Mallinckrodt (1989), Montefluos (1990a-c) and Solvay (1984a,b), and on information from CEDRE (1990) US DOT (1987) and Weiberg and Leuchtenberger (1978).

10.1 FIRST AID AND MEDICAL TREATMENT

This advice applies to all commercially available grades of H_2O_2 solutions (3-70%), unless stated otherwise.

10.1.1 First Aid

Eye contact. Immediately flush eyes with plenty of water for at least 15 minutes (keep eyelids apart). Seek medical attention; with ≤5% solutions, medical attention is required only if irritation or burning sensation persists.

Skin contact. Remove contaminated clothing and shoes. Soak contaminated clothes in water to prevent risk of fire. Wash contaminated skin with water for at least 15 minutes. Keep patient warm and quiet. Seek medical attention. With a ≤5% solution, medical attention is required only if irritation or ulcers develop.

Inhalation. Move subject to fresh air and keep warm and quiet. If not breathing, give artificial respiration. Obtain medical attention immediately.

Ingestion. Do not induce vomiting. Flush mouth with water. Give several glasses of water to drink if subject is conscious. Keep subject quiet and warm. Seek medical attention.

10.1.2 Notes to Physicians

H_2O_2 solutions and their vapours and airborne mists are of low toxicity, but, depending on the concentration and duration of exposure, may cause mild (≤5% solutions) to severe damage to the skin and mucous membranes. Symptoms will subside after exposure ceases except for highly concentrated solutions (≥70%) when symptoms are likely to persist. It is often difficult to estimate the amount of H_2O_2 ingested or the concentration of H_2O_2 solutions.

Eye contact. Stinging and burning sensation of the eyelids and conjunctivitis occur with 5% solutions and with vapours. Direct contact with 8% solutions may cause burning of the eyelids, keratitis; necrosis of mucous membranes, ulceration of the cornea. Appearance of

damage, even corneal ulceration, may be delayed for hours or days after an initial (false) healing period.

Following first aid, instil 1-2 drops of an anaesthetic or apply local corticosteroids. Keep subject under surveillance for 1-2 weeks.

Skin contact. Contact on a burn or open skin may cause a stinging or burning pain. A short period (2-3h) of whitening may occur at the point of contact, followed by redness, blisters, deep cutaneous necrosis. If large areas of skin are affected, the subject may suffer from shock. Treat as for thermal burns. No adverse effects are expected from dilute (≤5%) solutions on intact skin.

Inhalation. Effects depend on vapour or aerosol concentration and may include irritation of the nose, eyes and throat. Coughing, dyspnoea, shortness of breath may occur and, in the worst cases, there is a risk of lung oedema.

Ingestion. Ingestion may produce a burning sensation in the throat, oesophagus and stomach, abdominal pain, vomiting and diarrhoea. High concentrations may produce buccal foaming, micro-haemorrhage of the gastric mucosa with risk of micro-embolism. Blood-stained vomit, glottis oedema and shock may occur in severe cases.

Do not induce vomiting because of the danger of aspiration of foam. Prevent and treat shock, treat digestive tract burns. Large amounts of oxygen may rapidly be released from H_2O_2. Severe distension may be treated by flexible nasogastric or orogastric tube. Evacuation of the stomach by emesis induction or gastric lavage should be avoided.

10.2 SAFE HANDLING

General precautions.

Protect against heat.

Work in strictly clean areas and use only the minimal quantity necessary in the workplace.

All installations, containers and equipment must be made of completely pure materials compatible with H_2O_2 (section 3.2) and provided with the necessary devices to avoid pressure build-up, such as pressure relief valves and/or bursting discs, these should release to a safe area.

The design and operation of the system should ensure that no contamination can enter it. If a breather vent or similar device is used it should be provided with a filter to avoid any airborne contaminants entering the H_2O_2 system.

Depending upon the quantity of product being stored/handled, additional venting may be required by means of a ventable lid.

All materials must be cleaned before coming into contact with H_2O_2 according to the suppliers recommendations. Vessels and other ancillary items used for H_2O_2 handling should be exclusively reserved for this purpose. Never return unused solution to containers of H_2O_2.

When emptying plastic containers the preferred methods are syphoning or pumping, pressure should not be used. For stronger containers, pressure may be used, but only good quality air (or nitrogen), free from oil and other airborne contaminants, should be used.

Never confine H_2O_2 between two valves.

Personnel protection. Personnel must be educated in the hazards of H_2O_2 and that it slowly decomposes. Contamination causes the decomposition to take place at a much enhanced rate, and can be initiated even with trace amounts (a few ppm) of transition metal ions, and pH changes. Other factors such as direct sunlight and high temperatures can also lead to decomposition.

Decomposition results in a rapid increase in the rate of gas (plus steam) production which can lead to a pressure build up and bursting of the container if adequate relief is not provided.

H_2O_2 at strength >8% is an oxidising substance, having the potential to ignite combustible materials. Evaporation of water, e.g. by heat, may increase the concentration of a H_2O_2 solution, the higher the strength of the solution the greater is the potential for ignition.

When handling solutions of <8%, normal work clothing can be worn with impervious gloves, apron and shoes as well as eye protection. For stronger solutions, wear a non-flammable worksuit made of natural rubber, neoprene, nitrile rubber or PVC, including gloves, boots, trousers, jacket, and hood.

Leather shoes should not be worn as these can ignite within a few minutes of contact with H_2O_2. Cotton clothing can also ignite quickly. Protective skin creams offer no protection from H_2O_2.

Eye protection using chemical safety goggles must be worn when handling H_2O_2. In addition, a face-shield is desirable to protect against accidental splashing.

Safety showers and eye fountains must be provided at the work place, in addition sufficient clean water should be provided to deal with spillages.

Respiratory protection should be provided by natural or general ventilation of the workplace, with local exhaust points, where necessary, to control mists or vapours. Where there is a potential for airborne exposure in excess of applicable limits (section 5.2), a face-mask with an approved cartridge must be provided, used as an escape mask. In cases of severe exposure, e.g. in dealing with an emergency, self-contained breathing apparatus should be used by qualified personnel.

Storage. Store H_2O_2 solutions outside in a cool, clean place or in clean, well ventilated rooms, built with non-combustible materials. Do not stack containers next to the air vents. The floors should be impermeable so that when accidental leakage occurs, the product will flow away to a safe area or be retained.

Do not store on wooden pallets. Store away from reactive substances (e.g. finely divided metals and their salts, strong acids and alkalis, organic substances, oils and greases) and combustible materials (e.g. straw, paper, wood, textile), and heat sources.

Containers should be regularly checked for signs of abnormality, e.g. bulging or increased temperature. Regularly verify the availability of ample supply of water for emergencies. For bulk storage, the supplier should be consulted regarding the design and installation.

10.3 MANAGEMENT OF SPILLAGE AND WASTE

Spillage. In the event of a container spillage, evacuate and isolate the hazard area and keep people away, stay upwind and keep out of low-lying areas. Contain the spill, if possible, and dilute with large amounts of water before disposal. If containment is not possible wash down with copious amounts of water. If water pollution occurs, notify the appropriate authorities.

If containers are involved in a fire situation, drench with water, keep adjacent containers cool by spraying with water. These operations should only be undertaken by personnel equipped with fire fighting protection. For bulk storage spillages, a pre-arranged plan must be worked out in conjuction with the supplier and the competent authority, if applicable.

Waste disposal. In most countries, there will be statutory regulations governing the disposal of H_2O_2. Solutions containing <1% can normally be flushed down drains (no explosion hazard); in certain cases it may need to be more dilute.

For further and more detailed safety instructions, contact your H_2O_2 supplier.

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Joint Assessment of Commodity Chemicals No. 22, Hydrogen Peroxide

Errata (4 October 1994)

Page 7, Table 1: 45% should read 35%, density should read 1,133.
Density: decimal points should be read as comma's.

Page 16, Section 4.1.1 Suface water. The second and third reaction should be equated electrically: $Org^* + O_2 \rightarrow Org^{\cdot +} + \cdot O_2^-$

$$2 \cdot O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$$

In seawater, similarly, the reactions should read:

$$Fe^{2+} + O_2 \rightarrow Fe^{3+} + \cdot O_2^-$$

$$Fe^{2+} + \cdot O_2^- + 2H^+ \rightarrow Fe^{3+} + \cdot O_2^-$$

Page 36, Table 11: Concentrations measured by Kaelin *et al* without ventilation other than the windows were 41 mg/m^3 on the floor and 12 mg/m^3 close to the machine; with new ventilation the respective values were 4.5 mg/m^3 and 1.5 mg/m^3.

Page 40, Table 13: µg/l should read mg/l

Page 33, Section 5.2.1: The second sentence should be replaced by:

"It is generally only permitted ... chemical means. The residual level in food immediately after aseptic packaging should not exceed 0.5 ppm (US FDA, 1990). Under the GRAS regulation, the maximum treatment levels for H_2O_2 in food applications can be up to 1.25% depending on the use (US FDA, 1992)."

References:

US FDA (US Food and Drug Administration) (1992). Maximum usage levels permitted [in food]. CFR 21, 184.1366c.

US FDA (US Food and Drug Administration) (1990). Substances utilized to control the growth of microorganisms. CFR 21, 178.1005d [unchanged in 1993 edition].

Page 54/55, Table 20 Please insert the attached table.

Page 69, Gavage: calculated, based on 0.5% should read 5% solution

Page 140: ATOCHEM should read ELF ATOCHEM and A. MAYR should read W. MAYR.

Encl. Table 20

Table 20: Enzymatic Activity in Organs and Tissues of Rats (Matkovics and Novak, 1977)

Organ or tissue	Enzymatic activity (U/g wet weight)					
	Superoxide dismutase		Peroxidase Controls	H_2O_2-treated	Catalase Controls	H_2O_2-treated
	Controls	H_2O_2-treated				
Organs						
Liver	4,000 ± 600	10,408 ±750	0	0	4.80 ± 0.43	25.250 ± 2.455
Kidney	1,120 ± 151	1,692 ±240	361 ± 33	1,200 ± 105	0.36 ± 0.03	3.300 ± 0.295
Spleen	560 ± 50	470 ±240	963 ± 91	4,250 ± 418	2.40 ± 0.21	0.730 ± 0.062
Testes	960 ± 63	1,100 ± 53	407 ± 41	-	0.41 ± 0.03	0.150 ± 0.011
Whole brain	240 ± 24	397 ± 38	120 ± 10	140 ± 13	0.04 ± 0.004	0.012 ± 0.001
Lung	210 ± 20	895 ± 85	872 ± 86	2,020 ± 201	0.26 ± 0.02	0.520 ± 0.050
Pancreas	310 ± 31	352 ± 33	136 ± 13	480 ± 46	0.195 ± 0.010	0.255 ± 0.023
Muscles						
Heart	480 ± 47	950 ± 93	2,690 ± 300	1,615 ± 155	0.245 ± 0.023	0.725 ± 0.065
Skeletal	300 ± 27	530 ± 50	105 ± 10	750 ± 69	0.11 ± 0.009	0.092 ± 0.009
Haemolysate*	696 ± 68	787 ± 80	11,666 ± 1,000	17,900 ± 1,340	4.12 ± 0.04	6.98 ± 0.07

* U/ml wet weight; n = 10; catalase activity-values in U/g wet weight