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Hydrogen Peroxide OEL Criteria Document

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Hydrogen Peroxide OEL Criteria Document, CAS No. 7722-84-1

PREFACE

This report has been prepared by ECETOC for use by the Commission of the EU (DG V) and its Scientific Committee on Occupational Exposure Levels, in accordance with guidance provided (CEC, 1992). It contains a review and assessment of toxicological data to provide a scientific basis for an occupational exposure limit for hydrogen peroxide. A summary evaluation of the health significance and a recommendation for an occupational exposure limit are presented in Section 11.3 and 11.4.

This review has been based on an earlier report on hydrogen peroxide (ECETOC, 1993a), supplemented by data that have subsequently become available.

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1. SUBSTANCE IDENTIFICATION

Common name:	Hydrogen peroxide (H ₂ O ₂)
CAS name:	Hydrogen peroxide
CAS registry No.	7722-84-1
EEC No.	008-003-00-9
EEC classification:	Solution with a concentration $\geq 60\%$: corrosive (C) and oxidising (O) 20% $\leq$ concentration < 60%: corrosive (C) 5% $\leq$ concentration < 20%: irritant (Xi) Solution with a concentration < 5% not classified
EEC labelling:	5% $\leq$ concentration < 20%: R36/38, S28-36/39-45, nota B 20% $\leq$ concentration < 60%: R34, S28-36/39-45, nota B Concentration $\geq 60\%$: R8-34, S28-36/39-45, nota B
RTECS No.	MX 899000
IUPAC name:	Hydrogen peroxide
EINECS name:	Hydrogen peroxide
EINECS No.	231-765-0
Synonyms and tradenames:	Hydrogen dioxide Hydrogen superoxide
Danish:	Hydrogenperoxid
Dutch:	Waterstofperoxyde Zuurstofwater

Finnish:	Vetyperoksidi
French:	Eau oxygénée Peroxyde d'hydrogène
German:	Wasserstoffperoxid
Greek:	Υπεροξειδίου του υδρογόνου
Italian:	Perossido di idrogeno Acqua ossigenata
Norwegian:	Hydrogenperoksid
Portuguese:	Peróxido de hidrógeno Água oxigenada
Spanish:	Peróxido de hidrógeno Água oxigenada
Swedish:	Väteperoxid
Chemical group:	Inorganic compounds
Formula:	H_2O_2
Structure:	$\text{H}-\text{O}-\text{O}-\text{H}$
Molecular mass:	34.0
Purity:	99.75-99.99%
Stabilisers:	Common stabilisers (amounts in Section 1.1) 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 (Section 4.2).

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

1.1 GRADES OF HYDROGEN PEROXIDE

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.

In technical and chemical grades, the total stabilisers and impurities (total non-volatile compounds) range from 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 $\leq 0.006\%$ 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 ($\leq 0.5\%$) added by individual formulators. This is because the product is diluted with water before use in cosmetics, e.g. to a 12% solution in hair-care preparations, 4% in skin care preparations, 2% for nail hardening preparations and 0.1% in oral hygiene products (EEC, 1993). In general, the cosmetic user industry complies with the European Specification for 3% and 30% H_2O_2 (Pharmacopée Européenne, 1985; COLIPA, 1991).

2. PHYSICAL AND CHEMICAL PROPERTIES

H₂O₂ 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 H₂O₂ increases as its concentration in water increases; the total vapour pressure of a solution is due to both H₂O₂ and water and decreases with the concentration of H₂O₂.

Physical and chemical properties of H₂O₂ solutions are given in Table I.

2.1 STABILITY

A pure solution of H₂O₂ 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. Stabilisers are added to the commercial product to prevent its decomposition into oxygen and water.

The decomposition reaction is strongly exothermic ($\Delta H = -98.3 \text{ kJ/mol}$), and large quantities of oxygen gas are evolved (e.g. 1 litre 70% solution of H₂O₂ yields about 250 l O₂ at 0 °C and 1,013 hPa) (Becewa, 1984). The decomposition of H₂O₂ is generally a catalysed reaction. Common catalysts are dissolved transition metals (e.g. copper, iron, manganese), solid metals (e.g. platinum, 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). H₂O₂ 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.

In the gas phase, H₂O₂ at high vapour concentrations ($\geq 26\% \text{ v/v}$) may explode when ignited by an electric spark (Satterfield *et al*, 1951) or when heated $\geq 150 \text{ °C}$ (Schumb *et al*, 1955).

H₂O₂ is miscible with many polar organic solvents, e.g. low molecular weight alcohols, glycols, ketones. Concentrated aqueous H₂O₂ solutions may become explosive with these solvents (Merrifield, 1988).

2.2 CHEMICAL REACTIVITY

H₂O₂ oxidises compounds such as nitrites, cyanides, sulphites and hydrogen sulphide. The oxidising ability of H₂O₂ is greater at low pH. H₂O₂ can also reduce compounds such as hypochlorites, permanganates and cerium salts (Ce⁴⁺).

H₂O₂ oxidises organic compounds such as alcohols, olefins and amines and also forms organic peroxides (Schirmann and Delavarenne, 1979).

Table I: Physical and Chemical Properties of H₂O₂ Solutions

Property	Concentration of H ₂ O ₂ (by weight)					Reference
	10%	35%	50%	70%	90%	
Freezing temperature (°C)	-6	-33	-52	-40	-11	Schumb <i>et al</i> , 1955
Boiling temperature (°C) at 1,013 hPa	102	108	114	125	141	MCA, 1969
Density (kg/m ³) at 20 °C	1,034	1,133	1,195	1,288	1,387	MCA, 1969
Viscosity (10 ³ kg/m•s) at 20 °C	1.01	1.11	1.17	1.24	1.26	MCA, 1969
Vapour pressure (Pa)	-					
total (H ₂ O ₂ +H ₂ O) at 20 °C	-	1,700	1,300	800	-	Atochem, 1989a-e, 1990
total (H ₂ O ₂ +H ₂ O) at 22 °C	-	-	1,486	873	375	Solvay, 1991
partial (H ₂ O ₂) at 22 °C	-	-	46	98	178	Solvay, 1991
total (H ₂ O ₂ +H ₂ O) at 30 °C	-	3,070	2,400	1,470	667	MCA, 1969
partial (H ₂ O ₂) at 30 °C	-	48	99	200	333	MCA, 1969
Vapour saturation in air (mg/m ³) at 25 °C	-	-	787	1,685	3,049	Solvay, 1991
Vapour density (air = 1) at 20 °C	1.02 ^a					Du Pont, 1995
Threshold odour concentration (ppm)	Not applicable					
Surface tension (N/m) at 20 °C	0.0731	0.0746	0.0757	0.0773	0.0792	Schumb <i>et al</i> , 1955
Solubility in water	Miscible at any ratio					
Henry's Law Constant (Pa•m ³ /mol) at 20 °C	-	-	1 x 10 ⁻³	-	-	Hwang and Dasgupta, 1985
Flash point (°C)	Not applicable					
Explosion limits (%) at 131 °C	26 (lower limit) ^b					Satterfield <i>et al</i> , 1951
Auto-flammability, ignition temperature (°C)	Not applicable					

a Calculated

b Hot platinum wire method; upper limit not determined

2.3 CONVERSION FACTORS

Conversion factors for H₂O₂ concentrations in air, calculated at 20 °C and 1,013 hPa:

$$1 \text{ ppm} = 1.414 \text{ mg/m}^3$$

$$1 \text{ mg/m}^3 = 0.707 \text{ ppm}$$

3. OCCURRENCE

3.1 SOURCES

There are industrial, domestic and natural sources of H_2O_2 .

3.1.1 Industrial Sources

Total losses of H_2O_2 during its production are estimated to be 0.3%, the main release being in works waste water where the concentration can be 200 mg/l. Following biological or Fenton based treatment, the maximum concentration in the final effluent is normally in the range of 0-50 mg/l or 0-30 mg/l respectively for a plant using H_2O_2 (Chemoxal, 1992) and 0-10 mg/l for a manufacturing plant (Interox, 1990). The half-life of H_2O_2 in waste water is estimated to be a few minutes to hours (Degussa, 1990).

The emission of H_2O_2 to the atmosphere from manufacturing plants is limited by its Henry's Law constant (Table I) 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.

At the workplace H_2O_2 releases can be expected during drum and tanker filling, general operations (synthesis and distillation), laboratory operations, cleaning, disinfection and maintenance. H_2O_2 may also be released during industrial applications (Section 5.1).

In the paper pulp industry, the amount of H_2O_2 lost to waste water is 2-10 kg/t pulp for mechanical processes, 0-2 kg/t pulp for chemical processes and < 1 kg/t pulp for 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).

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 0.1 mg/l in the former USSR (Antonova, 1974) and Germany (Bundesminister für Jugend, Familie, Frauen und Gesundheit, 1990) and 0.5 mg/l in France (Ministère de la Solidarité, de la Santé et de la Protection Sociale, 1990).

3.1.2 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 0-5 mg/l, assuming that 4 kg clothing, 80 l water and 120 g washing powder containing 15% tetrahydrate perborate (with 10% unreacted H_2O_2) are used in the whole washing cycle. For France, with a perborate consumption of 80 kt/y, the amount of H_2O_2 released would be 1.7 kt/y (Chemoxal, 1992). The decomposition resulting from the mixing of washing effluents with other domestic waste water in sewerage systems greatly reduces the H_2O_2 concentration before it reaches the inflow of municipal sewage treatment works.

3.1.3 Natural Sources

3.1.3.1 Air

H_2O_2 occurs naturally in rural, unpolluted (ambient) air as a result of photolysis of ozone (Wayne, 1988). In polluted air most H_2O_2 formation begins with photolysis of aldehydes (Calvert and Stockwell, 1983). Both reactions give rise to the formation of free radicals such as $\cdot\text{HO}_2$, a precursor of H_2O_2 .

H_2O_2 formation in a conifer forest is considerably higher due to the presence of olefins emitted by the trees (Sakugawa *et al*, 1990). High concentrations of volatile organic compounds (VOC), methane (CH_4) and carbon monoxide (CO) in the atmosphere aid H_2O_2 generation, because of greater photochemical production of free radical species (Wayne, 1988). H_2O_2 is a significant, active component of photochemical smog (Bufalini *et al*, 1972; Kok *et al*, 1978a).

Solar UV irradiation enhances photolysis and H_2O_2 production is therefore higher during the day, especially in the afternoon, than at night (Das *et al*, 1983; Dollard *et al*, 1988; Possanzini *et al* 1988; Sakugawa and Kaplan, 1989) and higher in summer than in winter (Olszyna *et al*, 1988; Boatman *et al*, 1989).

Twice as much H_2O_2 is formed at 100% relative humidity than at 50% humidity (ambient temperature 25 °C) (Calvert and Stockwell, 1983). Levels of H_2O_2 may rise by 70% when the air temperature increases from 10 to 30 °C when other meteorological factors and air pollutant concentrations remain constant (Sakugawa *et al*, 1990).

H₂O₂ formation may be inhibited in various ways, particularly by SO₂ and NO_x which are scavengers for •HO₂. For example, the relatively low NO levels in marine air may explain the enhanced H₂O₂ production when compared to continental air (Stockwell, 1986).

It should be noted that the concentration ratio of VOC/NO_x together with their absolute concentrations, influences the formation of H₂O₂ in either direction.

H₂O₂ can also be degraded by photolysis, but this is a relatively slow process with a tropospheric half-life of 10-20 hours (Kleinman, 1986).

H₂O₂ accumulates in the atmosphere during extended dry periods (Jacob and Klockow, 1987). Airborne H₂O₂ shows a strong tendency to dissolve in the aqueous phase (Henry's Law constant is 10⁻³ Pa·m³/mol at 20 °C for H₂O₂ concentrations of 5.1-5,100 mg/l) (Hwang and Dasgupta, 1985). On the other hand, evaporating clouds can release considerable amounts of gaseous H₂O₂, a phenomenon which occurs even at night. The appearance of clouds lowers the gas-phase H₂O₂ mixing ratio. During wet deposition, H₂O₂ is efficiently removed from the atmosphere.

3.1.3.2 Aquatic Environment

In surface water, H₂O₂ is generally produced 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). A large number of organic (humic) compounds can serve as promoters (Draper and Crosby, 1983). In sea-water, the Weiss mechanism (involving the oxidation of iron and copper ions) may also be responsible for the production of H₂O₂. Metabolites excreted by a number of marine organisms may act as promoter, e.g. riboflavin (Moffet and Zika, 1987). H₂O₂ formation rates in sea-water and freshwater are similar, measured values ranged from 0.34 to 17 µg/l/h (Johnson *et al*, 1987). Experimental rates of H₂O₂ production in sea-water were 1-10 nmol/l/h (34-340 ng/l/h) after illumination. In deep sea-water no H₂O₂ is found under natural conditions (Johnson *et al*, 1989).

The overall half-life of H₂O₂ formed in seawater was measured to be 60 hours (Petasne and Zika, 1987), in freshwater 10-20 hours (Cooper and Lean, 1989; Air Liquide, 1991).

3.1.3.3 Soil

In ground water the H_2O_2 concentration is related to the content of dissolved oxygen through the coupled O_2 : H_2O_2 redox activity, indicating the possibility of a Weiss type oxidation mechanism in the soil (Holm *et al*, 1987).

3.1.3.4 Living Organisms

H_2O_2 is produced naturally by living organisms. For example, H_2O_2 has been detected in human exhaled breath at relatively high concentrations ($1.0 \pm 0.5 \times 10^{-8}$ to $3.0 \pm 1.5 \times 10^{-8}$ molar, 210 to 720 ppb or 300 to 1,020 $\mu\text{g}/\text{m}^3$) (Williams *et al*, 1982). For other mammals, see Section 7.1.2 and 7.1.3.2.

Other organisms capable of producing H_2O_2 are certain blue-green and green algae under light irradiation (Zepp *et al*, 1987; Stevens *et al*, 1973) and phytoplankton (Johnson *et al*, 1989). The bombardier beetle (*Brachinus crepitans* L.) can produce H_2O_2 solutions up to 28.5% (Schildknecht and Holubek, 1961).

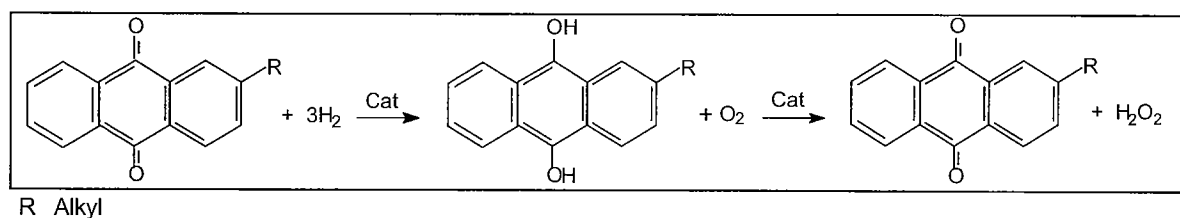
4. PRODUCTION AND USE DATA

4.1 PRODUCTION METHODS

4.1.1 Anthraquinone Autoxidation

The predominant industrial method of H_2O_2 manufacture is by anthraquinone autoxidation involving 2 main steps (Figure I) (SRI, 1988; Goor *et al*, 1989). The first step is the catalytic hydrogenation of 2-alkyl-9,10-anthraquinone or other anthraquinone derivative to its corresponding anthrahydroquinone using a palladium or nickel catalyst, followed by separation of the solid catalyst from the "working solution". The second step involves oxidation of the anthrahydroquinone by bubbling air or oxygen through the solution. When the anthrahydroquinone is oxidised back to anthraquinone 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.

Figure I: Anthraquinone Autoxidation Method (Goor *et al*, 1989)



The crude aqueous solution contains about 20-40% H_2O_2 which is further purified in 2 or 3 extraction steps with an organic solvent 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.

4.1.2 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 former Soviet Union (Goor *et al*, 1989).

4.2 HANDLING, STORAGE AND TRANSPORT

To prevent decomposition of H_2O_2 in aqueous solution, measures are taken to avoid contamination during storage and transport. Tanks of aluminium ($\geq 99.5\%$) or stainless steel (AISI 316L or 304L) are used for bulk storage of H_2O_2 solutions $\leq 70\%$ and also for higher concentrations. 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 $\leq 60\%$ H_2O_2 . Smaller quantities (concentration $\leq 60\%$) 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.

4.3 USES

4.3.1 Quantities Used and Produced

The estimated world-wide consumption of H_2O_2 in 1994 was 1,450 kt, distributed between Western Europe 39.9% (580 kt), North America 33.6%, South America 4.9%, Asia 18.8% and Africa/Middle East 2.8% (CEFIC, 1995).

H_2O_2 is produced at approximately 75 production plants world-wide (excluding China); 30 of these are in Western Europe. Each plant has a production capacity of 2-90 kt/y (CEFIC, 1995). The production in Western Europe has increased over the past decade to approximately 600 kt in 1993 (CEFIC, 1994).

4.3.2 Usage

There are 3 main uses of H_2O_2 ; the percentage of the total world consumption in 1987 is given in parentheses (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 ($\leq 2\%$) is used in applications which are particularly relevant to human exposure (Section 5.2), namely: disinfection 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), sanitation of chemical instruments, disinfection of eye contact lenses, disinfection of wounds, mouthwashing and hair bleaching (cosmetic grade).

5. QUANTITATIVE INFORMATION ON EXPOSURE AND UPTAKE

5.1 EXPOSURE LEVELS AT THE WORKPLACE

The number of workers potentially exposed during H₂O₂ production in the EU is very limited, e.g. 3/shift at one plant and 9/shift at another plant (CEFIC, 1996).

The US-National Institute for Occupational Safety and Health estimated that 52,800 US-workers in 18 different industry sectors were potentially exposed to H₂O₂ (level not specified) in 1982 (OSHA, 1989a). Similar data on EU industries are not available.

Table II summarises exposure data from routine operations during H₂O₂ production collected by CEFIC (1996) and ECETOC (1991). The data concern 13 production plants of 8 major suppliers in the EU.

Table II: Occupational Exposure Levels (CEFIC, 1996)

Job category	Year of measurement	Sample type ^a	Concentration ^b (mg/m ³)	
			TWA	Short-term level
Synthesis	1990-95	Personal	0.49	-
Distillation	1990-95	Personal	0.35-0.47	-
General work area	1994	NS	0.10	-
General work area	1989	Area	-	0.07-0.14
Stabilisation	1991-95	Personal	ND-0.5	-
Stabilisation	1989-95	Area	-	5.66-6.34
Drum filling	1990-95	Personal	0.08-0.56	-
Drum filling	1982-95	Personal	-	0.3-2.83
Drum filling	1990-95	Area	0.2-1.3	0.1-4.7
Tank filling	1990-95	Personal	0.1-2	-
Tank filling	1990-95	Area	-	0.45-1.8
Storage	1990-95	NS	0.05	< 0.2-2
Laboratory	1994-95	Personal	0.5-0.75	-
Laboratory	1989-95	Personal	-	0.28-0.5

a Personal = monitoring in the worker's breathing zone

Area = background monitoring at the work place

NS = not stated

b TWA = time-weighted average exposure concentration (4 to 8-h working period)

Short-term level = exposure concentration during short (15 min to 1 h) periods

ND = not detectable (detection limit not stated)

Although there were differences in the way the measurements were performed and the results reported, the available data indicate that, in general, exposure levels during a 4 to 8-hour workshift were below 1.4 mg/m^3 . During specific operations, like drum and tank filling and stabilisation short-term (15 min to 1 h) levels were measured up to 4.7 mg/m^3 . The highest exposure concentrations (around 6 mg/m^3) occur during stabiliser make-up (CEFIC, 1996).

Incidental exposures may be higher. For example, in an unmanned pump house at a production site H_2O_2 concentrations were 10 and 2 mg/m^3 before and after reparation of a leaking seal box (ECETOC, 1991).

Limited data are available on exposure during use. In the packaging industry, where H_2O_2 solutions are used for disinfection, $< 0.20 \text{ mg H}_2\text{O}_2/\text{m}^3$ (range $0.21\text{-}1.2 \text{ mg/m}^3$) was recorded during start-up of a coffee cream packaging machine (Suenaka *et al*, 1984). H_2O_2 levels near a fruit juice packaging machine were $0.2\text{-}0.66 \text{ mg/m}^3$ (Tetra Pak, 1991). Kaelin *et al* (1988) reported an incident of high H_2O_2 exposure near a milk packaging machine where the levels were $12\text{-}42 \text{ mg H}_2\text{O}_2/\text{m}^3$ before and $1.5\text{-}4.5 \text{ mg/m}^3$ after installation of ventilation.

5.1.1 Biological Monitoring

No data are available (Section 6.3 and 7.1.5). Due to the nature of the compound, its reactivity and its endogenous formation/degradation it is not appropriate to monitor H_2O_2 levels in biological tissues.

5.2 LEVELS IN FOOD AND DRINKING WATER, AND EXPOSURE RELATED TO LIFESTYLE

Studies or other quantitative information on human exposure levels or intake of H_2O_2 via foodstuffs, drinking water or preparations containing H_2O_2 are not available.

H_2O_2 is only permitted for food processing if residual quantities are removed by appropriate physical and chemical means. In the USA, 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).

It should be noted that endogenous H_2O_2 levels in plant tissues are relatively high. For example, the concentration in tomatoes is $3.1\text{-}3.5 \text{ mg/kg}$, in castor beans 4.7 mg/kg and potato tubers 7.6 mg/kg (Warm and Laties, 1982).

People may be exposed to H_2O_2 from its use in antiseptics, cleaning agents and cosmetics, (hair bleach, tooth paste, deodorant and mouthwash), which are generally sold as a 3% solution. H_2O_2 is also used as a sterilising agent for polymeric food packaging and for juice and wine processing, and in the USA as a whitener for teeth, applied in dentists offices.

H_2O_2 (3%) as is widely used to sterilise contact lenses (Holden, 1990). Following sterilisation residual levels of 10 to 50 mg/l or more may be retained on soft lenses (McNally, 1990). The normal concentration range of H_2O_2 in aqueous humour of the human eye is between 10-660 $\mu\text{mol H}_2\text{O}_2/\text{l}$ (0.34-22.44 mg/l), with a mean value of 24 $\mu\text{mol/l}$ (0.82 mg/l) (Spector and Garner, 1981; see also Chalmers, 1989).

5.3 ENVIRONMENTAL LEVELS

5.3.1 Air

Tropospheric H_2O_2 concentrations may vary by 2 orders of magnitude depending on the intensity and duration of light irradiation, water vapour content and presence of promoters (CH_4 , CO, VOC) and inhibitors (NO_x , SO_2) (Section 3.1.3.1). Wet deposition and transport by wind naturally influence local H_2O_2 concentrations.

In general, for the reasons explained in Section 3.1.3.1, H_2O_2 concentrations increase with altitude (mountain regions) (Heikes *et al*, 1987; Van Valin *et al*, 1987; Daum *et al*, 1990; Van Valin *et al*, 1990) and geographical latitude from north to south by approximately 0.05 ppb (0.07 $\mu\text{g}/\text{m}^3$) per degree (Heikes *et al*, 1987; Van Valin *et al*, 1987; Jacob *et al*, 1987; Jacob *et al*, 1990).

Seasonal differences in H_2O_2 concentration have been reported by Olszyna *et al* (1988) and Boatman *et al* (1989), the highest concentrations being measured during the summer. The latter author observed that average H_2O_2 concentration varied by a factor of 16 (0.3-4.8 ppb, 0.4-6.7 $\mu\text{g}/\text{m}^3$) between winter and summer.

In rural air, H_2O_2 concentrations ranged from 0.3-3 ppb (0.4-4 $\mu\text{g}/\text{m}^3$) during day-time but could not be detected at night (< 0.01 ppb, < 0.014 $\mu\text{g}/\text{m}^3$) (Das *et al*, 1983). Other results from a variety of locations and under different conditions ranged from 0.02-2.4 ppb (0.03-3.4 $\mu\text{g}/\text{m}^3$) (Sakugawa and Kaplan, 1987; Slemr *et al*, 1986; Tanner *et al*, 1986; Barth *et al*, 1989).

Levels as high as 0.18 ppm (0.25 mg/m³) have been reported in severe photochemical smog (Bufalini *et al*, 1972). Concentrations during periods of moderate smog ranged from 10-30 ppb (14-42 µg/m³) (Kok *et al*, 1978a).

5.3.1.1 Rain-water and Cloud-water

H₂O₂ levels reported in rain-water were 0-2,414 µg/l in Western Europe (Lagrange and Lagrange, 1990; Klockow and Jacob, 1986; Keuken *et al*, 1987; Römer *et al*, 1985), 1-2,800 µg/l in the USA/Canada (Kok, 1980; Zika *et al*, 1982; Cooper *et al*, 1987; Olszyna *et al*, 1988; Kelly *et al*, 1985; Daum, 1990), 578-6,766 µg/l in Brazil (Jacob *et al*, 1990) and 6.8-1,064 µg/l in Japan (Yoshizumi *et al*, 1984). H₂O₂ concentrations in cloud-water in the USA ranged from 3.4-8,398 µg/l (Kelly *et al*, 1985) and above the North Sea < 41-3,000 µg/l H₂O₂ (Römer, *et al*, 1985).

5.3.2 Aquatic Environment

Spatial and temporal variations of H₂O₂ concentrations in surface waters are principally due to changes in photochemical production (Section 3.1.3.2).

H₂O₂ has been detected in sea-water at concentrations ranging from 0.14-58 µg/l (Van Baalen and Marler, 1966; Zika, 1978, 1980 both as quoted in Cooper *et al*, 1988; Helz and Kieber, 1985; Zika *et al*, 1985a,b; Kieber and Helz, 1986; Johnson *et al*, 1989). In freshwater, the concentrations ranged from 0.34 to 109 µg/l (Sineln'nikov, 1971 as quoted in Cooper *et al*, 1988; Kieber and Helz, 1986; Cooper and Lean, 1989; Cooper *et al*, 1989).

H₂O₂ concentrations decline with increasing depth and follow light attenuation. For example, the concentration level dropped from 5 µg/l to 0.3 µg/l at 100 meters depth at an ocean station (Zika *et al*, 1985a) and from 6 µg/l at the surface to < 0.7 µg/l at 20 meters depth of lake Erie (Cooper *et al*, 1989; Cooper and Lean, 1989).

H₂O₂ concentrations vary throughout the day with mid to late afternoon maxima (diel cycle). For example the concentration in sea-water was 9.9 µg/l at 6 p.m. but 6.8 µg/l at 6 a.m. at a coastal station (Zika *et al*, 1985a) or 27.2 µg/l by late afternoon and 0.34 µg/l during the night in Jacks Lake (Cooper *et al*, 1989; Cooper and Lean, 1989).

5.3.3 Soil

H₂O₂ was found in concentrations of 0.34-2.2 µg/l in ground-water taken from wells 11, 15, 21 and 32 meters deep in a shallow sand and gravel aquifer (Holm *et al*, 1987).

5.4 SUMMARY AND EVALUATION

In general, exposure levels during production in a 4 to 8-hour workshift are below 1.4 mg/m³. During specific operations, like drum and tank filling and stabilisation short-term (15 min to 1 h) levels were up to 4.7 mg/m³. The highest exposure concentrations (around 6 mg/m³) occurred during stabiliser make-up.

In the packaging industry, where H₂O₂ solutions are used for disinfection, the limited data available show levels of < 0.2-4.5 mg H₂O₂/m³.

Exposure of the general population from ambient air is usually < 4 µg/m³. However, exhaled air contains 300-1,000 µg/m³. Therefore ambient air is not a significant source of exposure, except in fog and smog. Total human exposure cannot be assessed due to a lack of data on foodstuffs, drinking water, non-prescriptive pharmaceuticals and cosmetics.

6. MEASUREMENT TECHNIQUES AND ANALYTICAL METHODS

6.1 WORKPLACE

Methods for the determination of H₂O₂ in air at the workplace are summarised in Table III.

Table III: Determination of H₂O₂ in Air at the Workplace

Sample preparation	Analysis	Limit of detection		Reference
		(ppb)	(µg/m ³)	
Absorption in water containing TiCl ₄ /HCl	Colorimetry at 415 nm	10	14	Pilz and Johann, 1974
Absorption in acidic potassium titanium oxalate	Colorimetry at 400 nm	140-2,800	198-3,960	Interox, 1991

6.2 ENVIRONMENTAL MONITORING

6.2.1 Air

Sakugawa *et al* (1990) reviewed a large number of studies of the H₂O₂ content of air and rain/cloud-water and stated that the different analytical procedures used to measure atmospheric concentrations yielded different results. Further improvements in H₂O₂ sampling and analysis would be needed. Sampling presents a special problem at low concentrations in air. Lee *et al* (1991), for example, found that for low atmospheric levels (2 ppb, 3 µg/m³) as much as 90% of the gaseous H₂O₂ could be lost by surface reactions at the inlet of the sampling line.

Methods for the determination of H₂O₂ in ambient air are summarised in Table IV. In ambient air, a sensitive, direct fluorescence method is available (Lazrus *et al*, 1985, 1986). Other methods involve trapping H₂O₂ in wash traps or on filters, followed by analytical determination. The lowest detection limit is approximately 3 ppt (v/v) (4 ng/m³) (Ferm, 1988).

Table IV: Determination of H₂O₂ in Ambient Air

Sample preparation	Analysis	Limit of detection		Reference
		(ppb)	(µg/m ³)	
Collect in impingers containing distilled water; react with luminol using alkaline Cu ²⁺ catalyst	Chemiluminescence	0.5	0.7	Kok <i>et al</i> , 1978b Das <i>et al</i> , 1983
Collect in gas washing traps filled with water; react with scopoletin, add horseradish peroxidase and phenol	Fluorometry at 365/490 nm	At least 70 ng/l in trap water	-	Zika and Saltzman, 1982
Collect in aqueous stripping solution; react with horseradish peroxidase and <i>p</i> -hydroxyphenylacetic acid; decompose H ₂ O ₂ by catalase	Automated fluorometry at 326/400 nm	0.050 (gas phase) ^a 0.07 µg/l (liquid phase) ^a	-	Lazrus <i>et al</i> , 1985, 1986
Trap in Ti ⁴⁺ + H ₂ SO ₄ on glass filter, leach with water	Colorimetry at 475 nm	0.003	0.004	Ferm, 1988

^a Detection limits in a commercial analyser (Aero-Laser, 1992)

6.2.2 Water

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

Table V: Determination of H_2O_2 in Water

Sample preparation	Analysis	Limit of detection ($\mu\text{g/l}$)	Reference
Surface Water			
Add leucocrystal violet, horseradish peroxidase in acetate buffer	Colorimetry at 596 nm	50	Draper and Crosby, 1983
Boil, cool, filter and irradiate	Thin-layer chromatography with peroxidase-catalysed leucocrystal violet spray	50	Draper and Crosby, 1983
Rain and Cloud Water			
React with alkaline luminol	Chemiluminescence	1	Kok, 1980
Acidify with H_2SO_4 , cool to 5°C , degas, add KMnO_4	Conversion of H_2O_2 -derived O_2 to CO_2 , manometry of CO_2 collected in cool trap	$1\text{-}10^a$	Holt and Kumar, 1986
Sea Water			
Add phenylarsine oxide, KI and Mo catalyst in acetate buffer	Iodometry of excess phenylarsine oxide	0.68	Kieber and Helz, 1986
Ground Water			
Add scopoletin, measure fluorescence; add horseradish peroxidase, measure fluorescence	Fluorometry at 365/495 nm	0.12-1.52	Holm <i>et al</i> , 1987
Cooling Water			
Mix with phenolphthalein leuco base	Photometry	Not stated	Zabelin and Karbovnichii, 1983

^a Reported as "in the ppb range"

6.3 BIOLOGICAL TISSUES

Standard methods for assaying H_2O_2 are not available. A summary of methods used 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 VI.

Table VI: Analysis of Biological Media and Food

Sample preparation	Analytical measurement	Limit of detection	Reference
Plant Tissue			
Transfer frozen sliced tissue into 5% thyrocalcitonin; homogenise; centrifuge; pass over anion exchange resin; add to ammoniacal luminol; add potassium ferricyanide	Chemiluminescence	At least 1 ng (corresponding to 0.1-1 g fresh tissue)	Warm and Laties, 1982
Animal Cells and Tissue			
Mix tissue homogenates or subcellular fractions with catalase	Spectrophotometry (640-660 nm)	Unknown	Sies, 1981
Blood Serum			
Add sodium azide, ascorbate oxidase, catalase and 1,4-piperazine-diethane-sulphonic acid or phosphate buffer	H_2O_2 -selective electrode with oxidase meter	Unknown	Nakane and Kosaka, 1980
Plasma			
Blood centrifuged, deproteinated	HPLC and spectrometry	Unknown	Nahum <i>et al</i> , 1989
Milk			
Treat with trichloro-acetic acid; filter	Colorimetry ($TiCl_4/HCl$) 415 nm	2 mg/l 0.4 mg/l	Matz and Dietze, 1971 Gupta <i>et al</i> , 1977
Food			
Extract with 0.5% $KBrO_3$ at pH 7.0	O_2 release by catalase, oxygen electrode	0.01 mg/l (liquid) 0.1 mg/kg (solid)	Toyoda <i>et al</i> , 1982

7. TOXICOLOGY

7.1 TOXICOKINETICS

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

7.1.1 Uptake

7.1.1.1 Absorption from Exogenous Exposure

There is limited information on the uptake of H_2O_2 . After local application of H_2O_2 solutions (1-30%) to human skin, tongues of cats and dogs, and foot pads and heart of rats, 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 ($^{18}\text{O}_2$) being transported to the lungs. After 18 minutes 7% and after 34 minutes 30% of $^{18}\text{O}_2$ 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 (0.12-0.48%) 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).

7.1.1.2 Mechanism of Absorption

The permeability constant of erythrocyte membrane for H_2O_2 is approximately 0.04 cm/min (Nicholls, 1972), and for peroxisomal membrane 0.2 cm/min (De Duve, 1965 as quoted in Chance *et al*, 1979). 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 blanching of the exposed tissue area (Hauschild *et al*, 1958). Large volumes of gaseous oxygen (1 ml of 30% H_2O_2 yields approximately 100 ml 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 intravenous (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 (Hrubetz *et al*, 1951).

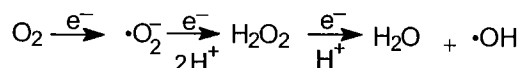
Administration by other routes also shows decreased absorption rates with higher concentrations of H_2O_2 (Hrubetz *et al*, 1951). Dieter (1988) explained this by the local, massive liberation of oxygen bubbles which obstructed blood flow, thus preventing H_2O_2 entering the general circulation and exerting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

Thus, there is little data on 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.

7.1.2 Endogenous Formation

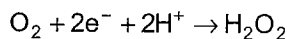
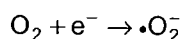
H_2O_2 is normally found in each aerobic cell as an endogenous metabolite. It is generated during cell respiration by various metabolic process (e.g. oxidase-catalysed reactions), by oxidative stress (i.e. superoxide anion degradation by superoxide dismutase) and by patho-physiological reactions such 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 ($^1\text{O}_2$), superoxide radical anion ($\cdot\text{O}_2^-$), H_2O_2 and hydroxyl radical ($\cdot\text{OH}$):



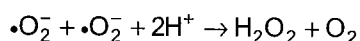
These reactive oxygen species are formed during enzymatic reactions, but also through spontaneous redox reactions such as the reduction of hydroquinone, xanthin, haemoglobin or catecholamines (Vuillaume, 1987). Characteristic for the enzyme reactions is the role of transition metals, especially copper, 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; cytochrome 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):



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 24 nmol $\cdot\text{O}_2^-$ /min/mg protein and the intramitochondrial steady-state concentration 10-11 mol $\cdot\text{O}_2^-$ /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):



In an aqueous solution, spontaneous dismutation is a second-order reaction and the half-life of $\cdot\text{O}_2^-$ is an inverse function of its initial concentration, whereas the SOD-catalysed reaction is first order and the $\cdot\text{O}_2^-$ half-life is independent of its initial concentration. Assuming typical concentrations of 10^{-5} mol SOD/mg

tissue and 10^{-11} mol $\bullet\text{O}_2^-$ /mg tissue (steady state), Fridovich (1983) calculated the rate of catalysed elimination of $\bullet\text{O}_2^-$, and subsequent formation of H_2O_2 , to be 10^{10} x higher than spontaneous dismutation.

SOD is specific for $\bullet\text{O}_2^-$ substrate and is present in several subcellular compartments such as cytosols, mitochondria, microsomes and nuclei, where $\bullet\text{O}_2^-$ formation may occur. Higher concentrations of SOD in cells can be induced by increased $\bullet\text{O}_2^-$ levels in rat lung and rat liver (Section 7.1.4.1, *Induction of Enzyme Activity*), in endothelial cells and also in bacteria (Fridovich, 1983).

In perfused rat liver, H_2O_2 formation is dependent on the substrate used and a great variation in the results is observed. Uric acid led to a production rate of 100-300 nmol H_2O_2 /min/g liver (wet weight) (Boveris *et al*, 1972). Sies (1981) measured a formation of 80 nmol 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 0.5 μmol H_2O_2 /min/mg protein (Boveris and Chance, 1973; Nohl and Hegner, 1978). The maximum rate of induced H_2O_2 formation was 11-15 μmol 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 90 nmol H_2O_2 /min/g liver (wet weight) under physiological conditions.

Using the value reported by Boveris *et al* (1972), the rate of H_2O_2 production in human liver was estimated to be 270 mg/h (7,941 μmol /h) under normal conditions or 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).

7.1.3 Distribution

7.1.3.1 Exogenous H_2O_2

As already described in Section 7.1.1.2, the local spontaneous or enzymatic-catalysed breakdown of small quantities of H_2O_2 prevent it to enter the general circulation and thus its systemic distribution. In the case of large quantities, the local and massive liberation of oxygen bubbles obstruct the blood flow and prevent systemic distribution (Dieter, 1988).

7.1.3.2 Endogenous H_2O_2 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} $\mu\text{mol/l}$ (0.034 $\mu\text{g/l}$); after maximum stimulation of H_2O_2 production its concentration increases to 0.1 $\mu\text{mol/l}$ (3.4 $\mu\text{g/l}$) (Sies, 1974). This limited variation is attributed to the catalase activity in rats (97,000 $\mu\text{mol } H_2O_2/\text{min/g liver}$) (Tamura *et al*, 1990).

In rats, H_2O_2 levels of 43-87 mmol/kg kidney (dry weight; 1,460-2,960 mg/kg) and 3.6-8.3 mmol/kg liver (dry weight; 122-282 mg/kg) have been reported. The corresponding values in mice were 74-147 mmol/kg kidney (dry weight; 2,520-5,000 mg/kg) or 15-31 mmol/kg liver (dry weight; 510-1,050 $\mu\text{g/kg}$) (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 65 mmol/l (2,210 mg/l) before to 125 mmol/l (4,250 mg/l) immediately after a heart bypass operation. The spectrophotometric analytical method was later criticised by Nahum *et al* (1989).

Using a radioisotopic technique, an average plasma level of 34 $\mu\text{mol } H_2O_2/\text{l}$ (1.16 mg/l) with a range of 13-57 $\mu\text{mol/l}$ (0.44-1.94 mg/l) was found in human volunteers. The average blood level appeared to be 288 $\mu\text{mol/l}$ (9.79 mg/l) with a range of 114-577 $\mu\text{mol/l}$ (3.88-19.6 mg/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 12.2 $\mu\text{mol/l}$ (0.415 mg/l) in the extracellular pool of phorbol myristate ester stimulated neutrophils (Test and Weiss, 1984).

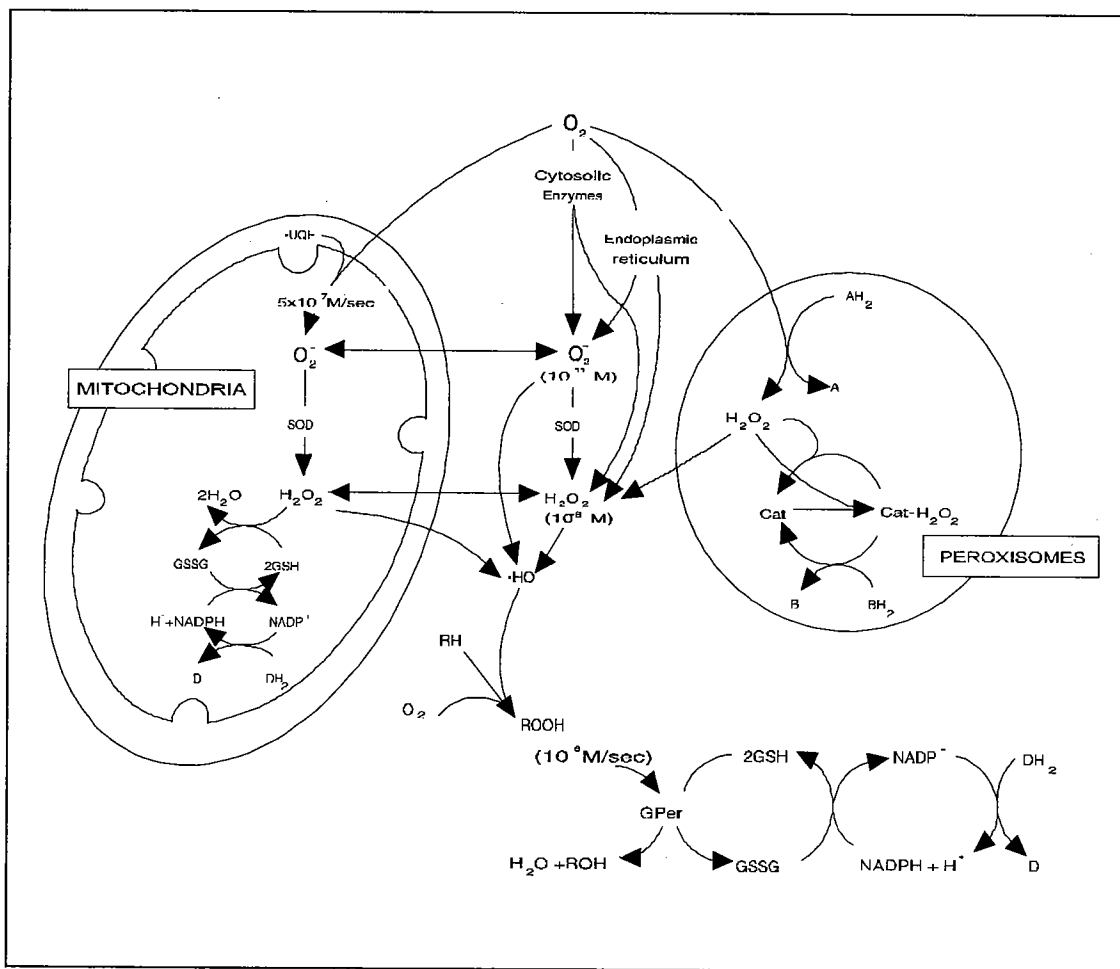
In the lens of rabbit eyes, the concentration of H_2O_2 was approximately 59 $\mu\text{mol/l}$ (2.01 mg/l) (Bhuyan and Bhuyan, 1977) and in bovine eyes approximately 20 $\mu\text{mol/l}$ (0.68 mg/l) (Pirie *et al*, 1970). The normal concentration range of H_2O_2 in aqueous humour of the human eye was between 10-660 $\mu\text{mol } H_2O_2/\text{l}$ (0.34-22.44 mg/l), with a mean value of 24 $\mu\text{mol/l}$ (0.82 mg/l) (Spector and Garner, 1981; see also Chalmers, 1989).

7.1.4 Biotransformation

7.1.4.1 Enzymatic Metabolism

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

Figure II: Metabolism of Hydrogen Peroxide (Chance *et al*, 1979)

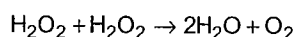


Concentrations and formation rates of oxygen metabolites are estimated.

•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; $\text{O}_2^{\cdot-}$, superoxide anion; •OH, hydroxyl radical; •ROOH, an alkyl hydroperoxide; GPer, glutathione peroxidase; Cat, catalase; B and BH_2 , hydrogen donors of a specificity appropriate to catalase, such as ethanol.

Catalases

The overall decomposition reaction of H_2O_2 in the presence of a catalase is as follows:



Catalases are present at a wide range of concentrations in nearly all mammalian cells; the enzymes are particularly efficient in dealing with large amounts of H_2O_2 (Chance *et al*, 1979). Catalases are located in subcellular compartments, mainly in peroxisomes (De Duve and Baudhuin, 1966). Soluble catalases were found in erythrocytes (Saito *et al*, 1984).

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

Ito *et al* (1984) measured catalase activity in duodenal mucosa, blood and liver homogenates of 4 strains of mice. Activity in the liver was 8-10 x that in blood and 14-83 x that in duodenal mucosa. Differences in catalase activity as high as 20-fold were seen in the same organs of the different strains (Table VII). In gastrointestinal 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 VIII). This difference may explain the different responses of these 2 species to exogenous H_2O_2 in drinking water (Section 7.2.5.2). Data on catalase levels in human beings are limited. Catalase levels in human jejunum biopsies were several times higher than those in mice (Table VIII). However, the data on different species are not completely comparable due to differences in sampling, tissue preparation and sensitivity of the analytical methods used.

Table VII: 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	B6C3F ₁ ^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, F₁ hybrid of C3H and C57BL, with "normal" catalase activity;

c C57BL/6N, low catalase activity;

d C3H/C, mice with hypocatalasemia

Table VIII: Gastrointestinal Levels in Various Species

Species, strain	Tissue	Catalase level (U/mg protein)	Reference
Human	Jejunum	0.065 ± 0.0047 (n=3)	Dawson <i>et al</i> , 1981
	Gastric antrum	0.048 ± 0.0007 (n=5)	
Mouse, C3H/HeN	Duodenum	0.0317 ± 0.0084 (n=11)	Ito <i>et al</i> , 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 ^a	Manohar and Balasubramanian, 1986
	Jejunum	1.60 ± 0.1 ^a	

a Number of samples used to calculate the mean not stated

In human serum, catalase activity was 3,600 x 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 rate ranges from 0.01-0.05 mol H₂O₂/l/min (Yamagata and Seino, 1953).

Erythrocytes (human and animal) can degrade gram quantities of H₂O₂ in several minutes (Yamagata *et al*, 1952; Winterbourn and Stern, 1987). During and after i.v. infusion of a dog with 1.0 M H₂O₂ in a 0.9% saline solution at a rate of 3 x 10⁻³ mol H₂O₂/min for 60 min, no H₂O₂ could be detected in the plasma (Nahum *et al*, 1989).

A rare genetic defect in red blood 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 red blood cells of acatalasemic patients shown greater sensitivity to oxidative stress and *in vitro* are particularly sensitive to lipid peroxidation by H₂O₂ (Aebi and Suter, 1972). 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 occurrence of hypocatalasemia lies between 0.2% and 0.4% of the population. In Japan the prevalence of acatalasemia was estimated to be 0.0087% (Ogata, 1991).

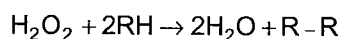
In addition, approximately half the Japanese acatalasemic patients develop progressive gangrene of the mouth, called Takahara's disease. This condition is characterised by small, painful ulcers in the gingival crevices and tonsillar lacunae, attributed to excess levels of H₂O₂ generated by various micro-organisms 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 blood cell catalase activity levels. For both types of individuals, the defect is compensated for by an increase of hepatic glutathione peroxidase and catalase levels and by increased blood glutathione peroxidase activities (Chance *et al*, 1979; Vuillaume, 1987 and Ogata, 1991). Apart from the extremely rare occurrence of occasional oral lesions, acatalasemic patients lead normal lives, suggesting an alternative degradation mechanism for H₂O₂ (Ogata, 1991).

Increased sensitivity to H₂O₂ has also been noted *in vitro* in isolated red blood cells of individuals with deficiency in other enzymes involved in the degradation of H₂O₂ (Chiu *et al*, 1982). The sensitivity of these individuals to endogenous and exogenous H₂O₂ will depend on the degree of compensation by the alternative pathways for destruction of H₂O₂.

Peroxidases

Peroxidases decompose H₂O₂ through the reaction:



Peroxidases require as an electron donor a co-substrate such as alcohol, nitrite or formate for the reduction of H₂O₂ (Little, 1972; Vuillaume, 1987). When the H₂O₂ 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 medula, liver, kidney, 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 H₂O₂; as a consequence, it can react with both H₂O₂ and organic hydroperoxides (R-OOH) (Guenzler *et al*, 1974). Glutathione peroxidase is more efficient at low concentrations of H₂O₂ compared to catalase (Halliwell, 1974). Glutathione reduces H₂O₂ to water with formation of oxidised glutathione (GSSG), which is regenerated by GSSG-reductase by consuming NADPH.

There are 2 kinds of glutathione peroxidase. The one that is Se-dependent requires selenium for the decomposition of H_2O_2 . The other is Se-independent and cannot use H_2O_2 as a substrate, but uses organic hydroperoxides such as phosphatidylcholine-hydroperoxide or physiological peroxides (prostaglandin G2) (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 2 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 10^7 mol/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) deficient cells, the NADPH levels in erythrocytes are not sufficient for the reduction of oxidised glutathione. 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 II).

Distribution of Enzymes that Produce and Degrade H_2O_2

The distribution of glutathione peroxidase, catalase and SOD has been studied in various regions of the gastrointestinal tract of fasted Wistar rats, in the villus and crypt cells of the small intestine (Table IX), 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 gastrointestinal 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 to adulthood (Manohar and Balasubramanian, 1986).

Table IX: Regional Distribution of Glutathione Peroxidase, Catalase and Superoxide Dismutase in Rat Gastrointestinal 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 ^a	ND	1.75 ± 0.6	13.22 ± 1.4	1.94 ± 0.3

a Not detectable

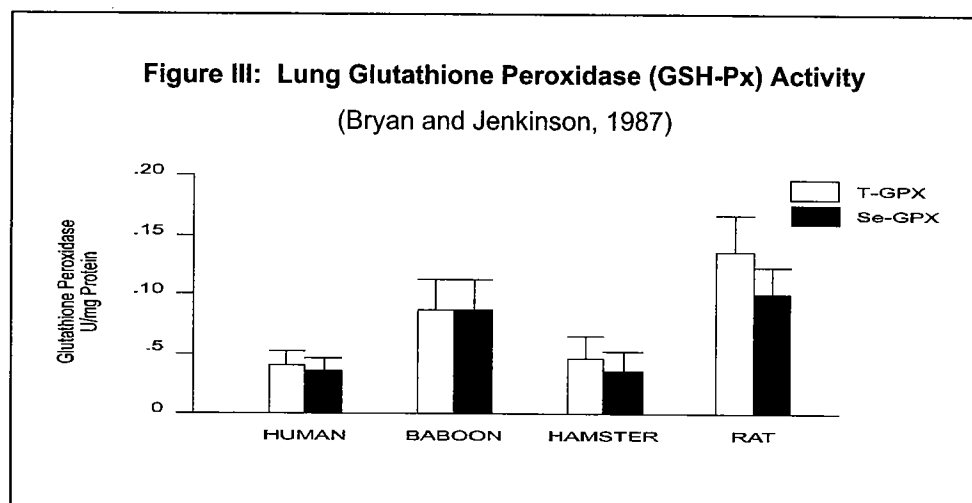
To characterise lung antioxidant enzyme activities in different species, Bryan and Jenkinson (1987) measured the glutathione peroxidase, SOD and catalase activity in the Sprague-Dawley rat, Syrian Golden hamster, baboon (*Papio cytocephalus*) and human lung. SOD activity was similar for all 4 species (Table X). GSH-Px activity was higher in rat than baboon or hamster lung (Figure III). Lung catalase activity was variable with the highest activity present in the baboon, which was 10 x higher than in the rat (Figure IV). 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.

Table X: Lung Superoxide Dismutase Activity in Various Species
(Bryan and Jenkinson, 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

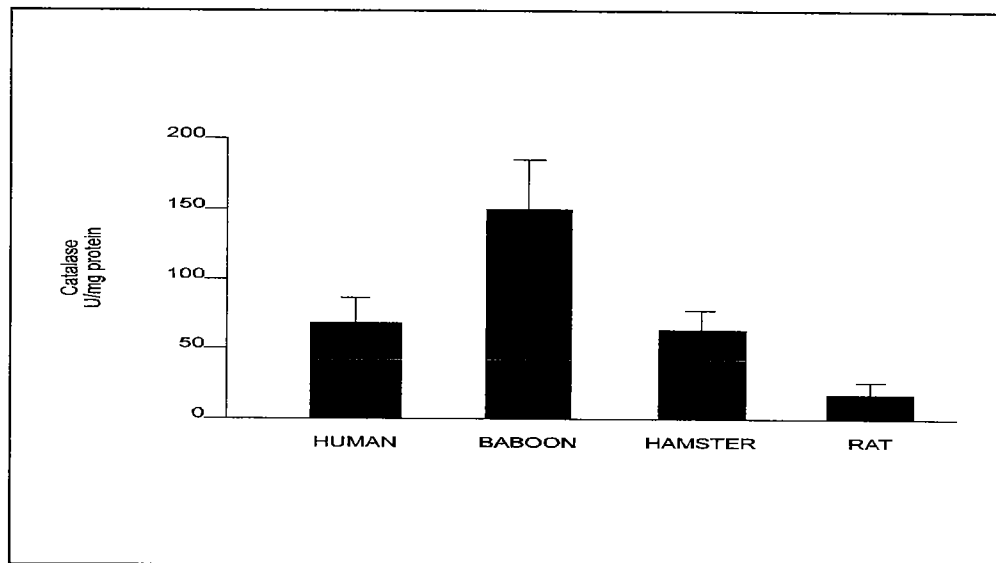
a CuZn, cyanide-sensitive; Mn, cyanide-resistant.

b Significantly different from other species, P < 0.05



Units are μmol of reduced nicotinamide adenine dinucleotide phosphate oxidised /min/mg protein. Values are means $\pm$ 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 activity and was measured using hydrogen peroxide as substrate.

Figure IV: Catalase (Cat) Activity In Lung
(Bryan and Jenkinson, 1987)



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

Induction of Enzyme Activity

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).

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 XI). 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).

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).

It should be noted that the findings on 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.

7.1.4.2 Non-enzymatic Metabolism Degradation

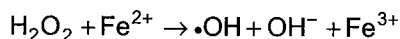
The oxidative reactivity of H_2O_2 with biological molecules such as carbohydrates, proteins, fatty acids or nucleic acids is not pronounced in the absence of transition metals, except for a few nucleophilic reactions.

Table XI: Enzymatic Activity in Organs and Tissues of Rats (Matkovichs and Novak, 1977)

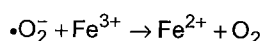
Organ or tissue	Enzymatic activity (U/g wet weight)					
	Superoxide dismutase		Peroxidase		Catalase	
	Controls	H ₂ O ₂ -treated	Controls	H ₂ O ₂ -treated	Controls	H ₂ O ₂ -treated
Organs						
Liver	4,000 ± 600	10,408 ± 750	0	0	4.80 ± 0.43	25.25 ± 2.455
Kidney	1,120 ± 151	1,692 ± 240	361 ± 33	1,200 ± 105	0.36 ± 0.03	0 ± 0.295
Spleen	560 ± 50	470 ± 240	963 ± 91	4,250 ± 418	2.40 ± 0.21	3,300 ± 0.062
Testes	960 ± 63	1,100 ± 53	407 ± 41	-	0.41 ± 0.03	0.730 ± 0.011
Whole brain	240 ± 24	397 ± 38	120 ± 10	140 ± 13	0.04 ± 0.004	0.150 ± 0.001
Lung	210 ± 20	895 ± 85	872 ± 86	2,020 ± 201	0.26 ± 0.02	0.012 ± 0.050
Pancreas	310 ± 31	352 ± 33	136 ± 13	480 ± 46	0.195 ± 0.010	0.520 ± 0.023
						0.255
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 ^a	696 ± 68	787 ± 80	11,666 ± 1,000	17,900 ± 1,340	4.12 ± 0.04	6.98 ± 0.07

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

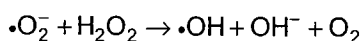
In the presence of transition metals, particularly ferrous ions (Fe^{2+}), H_2O_2 can be reduced to hydroxyl radicals. This corresponds to the metal catalysed Fenton reaction:



Another possibility is reduction of the iron ion by the superoxide anion ($\bullet\text{O}_2^-$):



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



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 (2.3 nm), 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 (Birnbom, 1982; Troll and Wiesner, 1985).

7.1.5 Excretion

Due to its rapid endogenous transformation into water and oxygen there is no specific excretion of H_2O_2 or (determinable) degradation product.

7.1.6 Biological Monitoring

No standard methods are available (Section 6.3). Due to the nature of the compound, its reactivity and endogenous formation/degradation it is not appropriate to monitor H_2O_2 levels in biological tissues.

7.1.7 Summary and Evaluation

H₂O₂ undergoes immediate 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. The formation of gaseous oxygen causes capillary microembolism and prevents irrigation of tissues by blood resulting in a characteristic blanching of exposed tissue.

H₂O₂ is a normal product of aerobic cell metabolism resulting from a number of enzymatic reactions such as the enzymatic catalysed dismutation of peroxide anion. Under physiological conditions H₂O₂ production in rat liver is 90 nmol/min/g liver.

H₂O₂ is metabolised by catalase and glutathione peroxidase. Catalases are located mainly in peroxisomes and the highest activities are found in the duodenum, liver, kidney, mucous membrane and other highly vascularised tissues. H₂O₂ is metabolised to water and oxygen, the decomposition rate in human plasma being approximately 0.01-0.05 mol H₂O₂/l/min. Catalase decomposes high H₂O₂ concentrations whereas glutathione peroxidase is more efficient at lower H₂O₂ concentrations. Glutathione is oxidised to glutathione disulfide and H₂O₂ 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 H₂O₂ is seen among individuals with genetic deficiencies in catalase activity (acatalasemia) or of the glutathione pathway (G6PD deficiency).

In some studies, H₂O₂ was shown to induce increased enzymatic activities in rodent tissue, while in other studies, no changes or decreased activities were reported.

In the presence of transition metals, H₂O₂ 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 H₂O₂ tissue levels is 1-100 nmol/l (0.034-3.4 µg/l) depending upon the organ, cell type, oxygen pressure and cell metabolic activity.

H₂O₂ is decomposed by enzymatic action and does not accumulate in cell systems and therefore not in organisms.

7.2 TOXICODYNAMICS

7.2.1 Acute Toxicity

7.2.1.1 Inhalation (Table XII)

Table XII: Acute Inhalation Toxicity

Species	Strain	Exposure level ^a		Time	Observations and comment	References
		(mg/m ³)	(ppm)	(h)		
Rat	Wistar	4,000 ^b	2,876	8	No mortality; exposure to "saturated" vapours of 90% H ₂ O ₂	Comstock <i>et al</i> , 1954; Oberst <i>et al</i> , 1954
Rat	Wistar	338-427 ^c	243-307	8	No mortality; scratching/licking during exposure; pulmonary edema and emphysema at necropsy	Comstock <i>et al</i> , 1954; Oberst <i>et al</i> , 1954
Rat	Unknown	2,000	1,458	4	"LC ₅₀ " ^d	Kondrashov, 1977
Rat	Wistar	23	16.1	4	No mortality	Svirbely <i>et al</i> , 1961
Rat	Sprague-Dawley	170	121	4	No mortality or necropsy findings; nasal discharge	FMC, 1989c
Mouse	Swiss	110	78.1	4	No mortality (0/10)	Svirbely <i>et al</i> , 1961
		159	113	4	Delayed mortality (4/10)	
		274	194	4	Delayed mortality (6/10)	
Mouse	Swiss-Webster	930	670	2	Mortality (3/4)	Janssen, 1995a
		3,130	2,254	1	Mortality (4/4)	
		2,170	1,562	1	No mortality (0/4); ptosis, noisy breathing	
		3,220	2,318	30 min	No mortality (0/4); ptosis, noisy breathing	
		3,590	2,585	15 min	No mortality (0/4); noisy breathing	
		4,960	3,571	7.5 min	No mortality (0/4); ptosis, skin irritation	
Mouse	Unknown	16,809	12,007	15 min	Mortality (9/10)	Punte <i>et al</i> , 1953
		11,877	8,484	15 min	Mortality (5/10)	
		13,287	9,491	10 min	Mortality (5/10)	
		9,462	6,759	5 min	No mortality (0/10)	
		5,234	3,739	5 min	Nasal and eye irritation, gasping and loss of muscular coordination were seen in all exposed groups; severity was dose-related	

a Form of H₂O₂ (vapour or aerosol) not always clear, see text

b Calculated

c Measured

d Combined inhalation exposure and skin application

In several studies on the acute inhalation toxicity of H₂O₂ to rats (Table XII), it is not always clear to what form of H₂O₂ (vapour or aerosol) animals were exposed.

Thus in studies of Comstock *et al* (1954) and Oberst *et al* (1954), groups of Wistar rats were exposed for 8 hours to "saturated" vapours of 90% H₂O₂ at a calculated concentration of 4,000 mg/m³ (2,880 ppm). There were no signs of toxicity or deaths. Animals killed between days 4 and 14 showed severe pulmonary congestion and emphysema, but no signs of necrosis of the pulmonary mucosa.

In other studies, 3 groups of 10 male Wistar rats were exposed for 4 or 8 hours to H₂O₂ vapour concentrations of 338-427 mg/m³ (243-307 ppm) as determined analytically. "Scratching" and "licking" occurred but there were no deaths. These studies indicate that rats are affected by acute exposures to vapours from 90% H₂O₂, while death does not occur (Comstock *et al*, 1954; Oberst *et al*, 1954).

Whole-body exposure of white rats for 4 hours to various concentrations of H₂O₂ (vaporised from a solution) resulted in a LC₅₀ of 2,000 mg/m³. The LOEC for the respiratory mucosa (increase in NAD-diaphorase) was 60 mg/m³ and exposure at 110 mg/m³ produced hyperaemia and transient thickening of the skin (Kondrashov, 1977).

A 4-hour inhalation toxicity study was conducted in which the atmosphere was generated by bubbling air flow through a reservoir containing 1,000 ml of 50% H₂O₂. Five male and 5 female Sprague-Dawley rats were exposed to a maximum attainable vapour concentration of 170 mg/m³ (122 ppm). The test atmosphere was analysed by colorimetry 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 4-hour LC₅₀ was greater than 170 mg/m³ (122 ppm), the highest concentration tested (FMC, 1989c).

Groups of 10 mice (strain not given) were exposed to aerosols of 90% H₂O₂ at concentrations varying between 3,600 and 19,000 mg/m³ for 5, 10 or 15 minute intervals (Punte *et al*, 1953). These concentrations were near or well above the saturation level of 3,049 mg/m³ for gaseous H₂O₂ (Table I). The average mass median droplet size as determined by cascade impaction was approximately 3.5 µm. Exposure to concentrations of up to 5,000 mg/m³ (3,535 ppm) 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 20 mice exposed to 5,200 mg/m³ (3,676 ppm) showed necrosis of the bronchial epithelium. Animals exposed to concentrations of 9,400 mg/m³ (6,645 ppm) or more for 5-15 minutes showed similar but more severe signs and 10-50% of animals died within 1 hour 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 9,400 mg/m³ (6,645 ppm) or more showed slowly developing corneal damage which appeared 5 weeks after exposure (Punte *et al*, 1953).

Nineteen groups of 4 male Swiss-Webster mice were exposed nose-only to a test atmosphere containing an aerosol of H₂O₂ generated from a 70% solution. The animals were exposed for periods between 7.5 and 120 minutes. The mean actual concentrations of H₂O₂ during exposure ranged from 880 to 4,960 mg/m³. All animals were observed for clinical symptoms and for body weight changes up to 14 days after exposure. Treatment related mortalities were not observed in animals exposed to concentrations as high as 3,220 mg/m³ for up to 30 minutes, but prolonged exposure (1 h) to 3,130 mg/m³ was lethal, as was exposure to concentrations as low as 880 mg/m³ for 2 hours. The onset of the clinical symptoms such as respiratory irritation was almost immediate. The length of the recovery period increased with increasing exposure duration and exposure concentration but did not exceed 1 week. The findings at macroscopy of the decedents were regarded attributable to bleaching or the corrosive nature of the test material. The absence of macroscopical findings in the survivors, except the bald area between the eyes, suggests that no permanent damage was induced by the treatment. No clear changes in lung weights were observed (Janssen, 1995a). The results of the study indicate that a single inhalatory exposure in mice caused effects limited to the exposed area which can be attributed to the corrosive nature of the substance.

7.2.1.2 Oral

Acute oral LD₅₀ values have been determined for H₂O₂ solutions ranging in concentration from 9.6-90% (Table XIII).

Table XIII: Acute Oral Toxicity

Concentration of solution (%)	Species, strain	LD ₅₀ (mg/kgbw)	Observation period (d)	Reference
9.6	Rat, Wistar-JCL	1,517 (m) 1,617 (f)	7 7	Ito <i>et al</i> , 1976
10	Rat, Sprague-Dawley	> 5,00	14	FMC, 1989a
35	Rat, Sprague-Dawley	1,193 (m) 1,270 (f)	14 14	FMC, 1983a
50	Rat, Sprague-Dawley	> 225 < 1,200	14	FMC, 1986
60	Rat, Wistar	872 (m) 801 (f)	14	Mitsubishi, 1981
70	Rat, CrI:CD®BR	805	14	Du Pont, 1996
70	Rat, Unknown	75 (m)	Unknown	FMC, 1979a
90	Mouse, Unknown	2,000	Unknown	Liraskii <i>et al</i> , 1983

Predominant clinical signs in rats administered 35% H₂O₂ 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).

7.2.1.3 Dermal

The acute, dermal toxicity of H₂O₂ solutions (35-90%) has been determined in various species (Table XIV).

Table XIV: Acute Dermal Toxicity

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

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.

7.2.1.4 Evaluation

Acute exposure to saturated H₂O₂ vapour produced only slight clinical signs of toxicity.

Brief (15 min) exposure of mice to H₂O₂ aerosols at concentrations of 9,400 mg/m³ 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 4-hour LOEC for respiratory mucosa was 60 mg/m³ and exposure at 110 mg/m³ induced hyperaemia and transient thickening of the skin.

In mice exposure to an aerosol concentration as high as 3,220 mg/m³ for 30 minutes did not cause mortalities. Prolonged (1 h) exposure to the same concentration was lethal, as was exposure for 2 hours to 880 mg/m³.

The rat oral LD₅₀ varies from > 5,000 mg H₂O₂/kgbw for a 10% solution to 1,200 mg/kgbw for a 35% solution. The highest toxicity was shown by a 60-70% solution with an LD₅₀ of 801 mg/kgbw. An earlier study which showed an LD₅₀ of 75 mg/kgbw for a 70% solution was not confirmed by a more recent study conducted according to OECD guidelines. A value of 2,000 mg/kgbw has been reported for a 90% solution for the mouse but details of the study are not available.

The acute dermal toxicity of H₂O₂ is low at all concentrations tested.

7.2.2 Irritation, Sensitisation and Immunotoxicity

7.2.2.1 Skin Irritation (Table XV)

Table XV: Skin Irritation - EU Classification

Concentration of solution (%)	Exposure (h)	Interval (h)	Score ^a	Classification ^b	Reference
3	24	24 72	0 ^c 0	Non-irritant	Du Pont, 1953, 1972a
6	24	24 72	0 ^c 0 ^c	Non-irritant	Du Pont, 1973a
8	24	24 72	0 ^c 0 ^c	Non-irritant	Du Pont, 1974a
10	4	4.5 24 48 72	0.3 0.2 0 0	Non-irritant	FMC, 1989b; Aguinaldo <i>et al</i> , 1992
35	4	4 24 48 72 96 5-14 d	2.8 2.6 0.58 0.58 0.42 0	Non-irritant	FMC, 1983c; Aguinaldo <i>et al</i> , 1992
50	3 min	24 48 72	e	Irritant	Sarver, 1994
50	1	24 48 72	e	Corrosive	Sarver, 1994
50	4	24 48 72	d	Corrosive	Aguinaldo, 1992
70	3 min	24 48 72	e	Corrosive	Sarver, 1994
70	0.5	24 48 72	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 1 animal was used in the 50% and 70% studies, no scores could be calculated. Evaluation was based on histopathological evaluation of skin sections.

e Since only 1 animal was used in the 50% and 70% in this reference, no scores were calculated. Evaluation was based on visual examination of treated skin sites.

H₂O₂ 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 24 hours under occlusive dressing (Du Pont, 1953, 1972a, 1973a). Irritation was slight following 4 hours

exposure to 10% H₂O₂ and mild with 35% H₂O₂. Desquamation occurred in 2 of 6 animals at day 14 at the latter concentration (Aguinaldo *et al*, 1992). Application of a 35% solution to intact skin for 24 hour under an occlusive dressing induced mild erythema and moderate to slight oedema at 24 hours and severe to moderate erythema and slight to very slight oedema at 48 hours (Du Pont, 1974b).

Solutions of 50% and 70% H₂O₂ were severe skin irritants; studies were carried out on 1 anaesthetised rabbit in each case. Histopathological examination 48 hours after 4 hours 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 minutes contact (FMC, 1987b; Aguinaldo *et al*, 1992).

In preliminary studies, 30% and 15% H₂O₂ solutions (0.2 ml) were applied to the shaved dorsal skin of Sencar mice. Both solutions had caused epidermal necrosis 24 hours after application. Marked epidermal hyperplasia and leukocytic infiltration were observed within 6 days of application but by day 10 the epidermis was essentially normal (Klein-Szanto and Slaga, 1982).

The intact skin of a single rabbit was exposed to 0.5 ml of 50% H₂O₂ under a semi-occlusive wrap for 3 minutes, 1 hours or 4 hours. Following 3 minutes exposure, mild erythema and mild or no oedema were observed at 24, 48 or 72 hours post-dosing. No irritation was observed at 7 or 14 days. Following 1 hour exposure, slight erythema and severe oedema were observed at 24, 48 and 72 hours post-dosing. Blanching was also observed at 1 hour post-dosing. Superficial necrosis was noted at 72 hours, followed by necrosis and sloughing at 7 days and scar tissue at 14 days. Following 4 hours exposure, mild erythema, moderate oedema and blanching were observed. Blanching persisted through 48 hours. Mild, moderate or slight erythema and mild to no oedema were observed through day 7 post-dosing. Superficial necrosis was observed at 72 hours and persisted through day 7. Scar tissue developed by day 14. A 50% solution of H₂O₂ was considered to be corrosive after 1 hour exposure and irritating after 3 minutes exposure (Sarver, 1994).

The intact skin of a single rabbit was exposed to 0.5 ml of 70% H₂O₂ under semi-occlusive wrap for 3 minutes or 1 hour. Following 3 minutes exposure, mild erythema, severe oedema and blanching were observed. Moderate or mild erythema and moderate or mild oedema were observed at 24, 48 and 72 hours post-dosing. Sloughing and fissuring were observed after 24 hours. Superficial necrosis, observed at 24, 48 and 72 hours, developed into necrosis by day 7. Scar tissue was noted on day 14. H₂O₂, 70% solution, was considered to be corrosive following 3 minutes exposure (Sarver, 1994).

7.2.2.2 Mucous Membrane Irritation

H₂O₂ (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).

7.2.2.3 Respiratory Tract Irritation

H₂O₂ generated from a 70% solution was evaluated for respiratory irritancy in mice using the method of Alarie *et al* (1981). Five groups of 4 male mice were exposed nose-only for a single 30-minute period to a test atmosphere containing an aerosol of H₂O₂. Before, during and after exposure the respiratory movements of the animals were recorded and the respiratory rates and the tidal and minute volumes calculated. All animals were observed for clinical symptoms and for body weight changes up to 1 day after exposure. Hereafter, the animals were killed and necropsied. The mean actual concentrations of H₂O₂ during exposure were 300, 616, 1,135 and 1,856 mg/m³. Treatment related decreased respiratory rates, tidal and minute volumes were observed in all treatment groups. Statistical evaluation of the data revealed significant ($P < 0.05$) dose response relationships for the respiratory rates and minute volumes during as well as after exposure. The exposure concentrations (95% confidence limits between brackets) at which a 50% reduction of the respiratory rates and minute volume is observed (RD₅₀) were 665 (280-1,139) and 696 (360-1,137) mg/m³ respectively. From the findings in this study it is concluded that H₂O₂ is a respiratory irritant in mice at relatively high aerosol concentrations compared to present occupational exposure levels. In the absence of changes in lung weights and lung macroscopy it is concluded that levels of H₂O₂ sufficient to cause macroscopic findings do not reach the lower respiratory tract (Janssen, 1995b).

7.2.2.4 Eye Irritation

The ocular irritancy of H₂O₂ solutions (5-70%) has been evaluated in rabbits; 0.1 ml was instilled into the conjunctival sac and in certain cases, the eyes were washed with 100 ml tap water after 20-30 seconds (Table XVI).

Table XVI: Eye Irritation - EU Classification

Concentration of solution (%)	Interval (h)	Index ^a		Score ^b	Reference
		unwashed	washed		
5	1	8.0	6.0	C=0	Weiner <i>et al</i> , 1990
	24	3.0	3.0	I=0	
	48	2.0	1.0	R=0.83	
	72	0	0	H=0 Non-irritant	
6	1	19	27	Unwashed:	Du Pont, 1990
	24	5.2	15	C=0/I=0	
	48	3.3	19.7	R=1.33/H=0.2	
	72	0.7	5	Washed:	
	7 d	0	< 4 ^c	C=0.4/I=0.22	
	14 d	-	< 7 ^c	R=1.33/H=0.78	
	21 d	-	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	
	7 d	2.0	47.0		
	16 d	2.5	22.0		
10	22 d	2.5	16.0		Weiner <i>et al</i> , 1990
	1	11.0	15.0	C=3.5	
	24	107	108	I=1.67	
	48	107	108	R=3.0	
	72	71.0	81.0	H=2.8	
	96	44.5	65.5	Irritant	
35	7 d	40.5	49.5		Weiner <i>et al</i> , 1990
	1	39.2	41.3	C=2.33	
	24	62.5	49.0	I=1.72	
	48	69.5	69.7	R=1.27	
	72	69.5	59.7	H=2.28	
	96	63.7	48.7	Irritant	
	7 d	79.3	76.7		
70	14 d	74.8	76.0		FMC, 1979c
	22 d	72.7	74.3		
70	Scores could not be determined from the report			Severe irritant	

a Scores were determined by the method of Draize with a maximum possible score of 110. Scores were also determined by the EU 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 EU Amendment (Directive 67/548/EEC and Labelling Guide, July 1991. Based on the EU criteria, the term 'irritant' or 'non-irritant' has been assigned.

c Score based on 1 animal only.

A 5% H₂O₂ solution caused minimal irritation to unwashed eyes (Weiner *et al*, 1990).

H₂O₂, 6% solution, produced slight corneal opacity, moderate iritis, moderate or severe conjunctival redness, slight or mild chemosis and discharge in unwashed eyes. The eyes of 2 rabbits were normal by 72 hours and the treated eye of the remaining rabbit was normal by 7 days. In washed eyes, H₂O₂, 6%, produced slight or mild corneal opacity, moderate iritis, mild or severe conjunctival redness, slight or moderate chemosis and moderate discharge. Moderate to severe corneal damage was evident in 1 rabbit, but there was no damage in the remaining 2 rabbits. Corneal vascularisation was still evident in the 1 rabbit 21 days after treatment. H₂O₂, 6% solution, is considered to be non-irritant (Du Pont, 1990).

An 8% H₂O₂ 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% H₂O₂ solution was extremely irritating to both washed and unwashed eyes; severe corneal opacity, iritis and conjunctivitis were observed (Weiner *et al*, 1990).

A 35% H₂O₂ 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 studies, 3% H₂O₂ had no effect when instilled into the rabbit eye; a 6% solution caused severe, reversible ocular damage; an 8% solution caused mild, reversible injury to the cornea, iris and conjunctiva with washed eyes returning to normal within 2 days. H₂O₂ administered as a 10% or 12% solution 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 H₂O₂ 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% H₂O₂ 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% H₂O₂ 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 conjunctiva of rabbit eyes. A 1% solution applied repeatedly caused conjunctival hyperaemia and slight corneal haze, followed by recovery (Koster, 1921 as quoted by Grant, 1986).

Evaluation of 10% and 35% solutions in the EYETEX *in vitro* model for ocular irritancy was consistent with the irritancy of these solutions under EEC regulations (Regnier, 1992).

7.2.2.5 Immunotoxicity (Skin Sensitisation)

There are no data on immunotoxicity.

Ten guinea pigs were exposed to 3 or 6% H₂O₂ solutions on intact or abraded skin and by intradermal injections of 0.1 ml of test solution in saline. Test solutions were re-applied 9 x 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).

No other data are available on sensitisation.

7.2.2.6 Evaluation

H₂O₂ solutions of 35% or less would not be classified as skin irritants in rabbits by the EU criteria. Higher concentrations ($\geq 50\%$) are corrosive to rabbit skin. H₂O₂ (50%) is corrosive to rabbit skin after 1 hour of exposure, whereas H₂O₂ (70%) is corrosive to rabbit skin after 3 minutes of exposure. H₂O₂ (6%) was not a skin sensitizer in guinea pigs. Solutions of 5% or less can not be classified as irritant to rabbit eyes. Concentrations of greater than 6% caused irreversible eye damage in rabbits. Ocular damage due to 8% was not reversible by 21 days. Concentrations of 10% or higher caused irreversible corneal damage. First effects on the rabbit cornea are observed with 1% H₂O₂. Effects on the mucosa of the gingiva and tongue of dogs were found after direct contact with a 1% solution. Inhalation of H₂O₂ aerosol (70%) caused respiratory irritation in mice with an RD₅₀ of 665 mg/m³.

7.2.3 Subchronic Toxicity

7.2.3.1 Inhalation (Table XVII)

Table XVII: Subchronic Inhalation Studies

Species (strain)	Exposure level		Exposure time			Reference
	(ppm)	(mg/m ³)	(h/d)	(d/wk)	(wk)	
Rat	67	93	6	2-5	7	Comstock <i>et al</i> , 1954; Oberst <i>et al</i> , 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 <i>et al</i> , 1961
Mouse	57	79	6	2-3	3	Comstock <i>et al</i> , 1954 Oberst <i>et al</i> , 1954
	77	107	6	3-5	3	
Rabbit (black)	22	30	6	5	12	
Dog	7	10	6	4-5	26	

Ten rats (strain unspecified) were exposed to an average concentration of 67 ppm H₂O₂ (95 mg/m³) 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 week 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 H₂O₂ vapour concentrations of 0.1 mg/m³ to 10.1 mg/m³ (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 10 mg/m³ was the Lowest Observable Effect Level (LOEL) for the respiratory organs, and that 1 mg/m³ was the No Effect Level (NOEL). Changes in serum and lung enzyme activity were seen at 10 mg/m³. The NOEL for skin changes was considered to be 0.1 mg/m³. Kondrashov concluded that skin is less resistant to long-term effects of H₂O₂ vapour than lung tissue. The lack of experimental details makes it difficult to evaluate this study.

Groups of mice (strain not stated) were exposed to 57 ppm (81 mg/m³) for a total of 8 exposures or 77 ppm (109 mg/m³) H₂O₂ 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 (6 h/d, 5 d/wk) to 22 ppm (31 mg/m³) H₂O₂ 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; Oberst *et al*, 1954).

Similar results were obtained in a study with 2 dogs exposed to 7 ppm (9.9 mg/m³) 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.

7.2.3.2 Oral

Numerous subchronic toxicity studies have been conducted with H₂O₂ 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% H₂O₂. 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% H₂O₂ 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 H₂O₂ concentration. Significant inhibition of body weight gain was also noted in these rats. Seven out of 24 rats receiving 1.5% H₂O₂ died during the course of the experiment (Shapiro *et al*, 1960).

To assess palatability, toxicity, 4 groups of 10 male and 10 female C57BL/6NCrIBR mice were given drinking water containing 0, 200, 1,000, 3,000 or 6,000 mg H₂O₂/l (w/v) *ad libitum* in distilled water for 14 days. There were no toxic signs observed in mice exposed to 200, 1,000 or 3,000 mg H₂O₂/l. A significant dose-related decrease of water consumption due to low palatability was seen in mice exposed to ≥ 1,000 mg H₂O₂/l. Although body weight gain was depressed in the 3,000 and 6,000 mg/l

groups during the first 3 days of the study, this effect was considered to be secondary to dehydration related to the poor palatability of the treated water. A decrease in body weight and food consumption was observed in mice exposed to 6,000 mg/l in drinking water throughout the study. Gross pathological examination revealed no specific findings attributable to exposure to H₂O₂. Histopathological evaluation of the gastrointestinal tract revealed degenerative (minimal to mild erosions) and regenerative (minimal to mild hyperplasia) changes in the mucosa of the stomach and/or duodenum in the 3,000 and 6,000 mg/l males and females; the overall NOAEL for pathology was 1,000 mg/l for males and females, corresponding to 164 mg/kgbw (males) and 198 mg/kgbw (females) (Ross, 1995; Sarver, 1995).

CRJ-CDE rats were administered 0.05% to 1.2% and JCL-JCR mice 0.05% to 0.8% H₂O₂ for 10 weeks. The only effect observed was a slight decrease in body weight among the mice drinking 0.8% H₂O₂ (Koseishyo, 1977-1981; Ito *et al*, 1981a,b).

Male NMR1 mice and male Wistar rats drinking a 0.5% H₂O₂ solution for 40 days and 56 days respectively showed a depression in water consumption after 1 week. A group of animals with similar conditions of water deprivation was included as a control. Rats drinking H₂O₂ 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 7.1.4.1, *Induction of Enzyme Activity*). 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% H₂O₂ for 3 weeks showed a decreased body-weight gain which correlated with a decreased liquid intake (Hankin, 1958).

Concentrations of 0.25 to 10% H₂O₂ were administered to male albino rats in drinking water for 43 days. All rats receiving a concentration $\geq$ 2.5% died. In a 146 day study, 9/10 rats drinking 0.25% (250 mg/kgbw/d) and 8/10 drinking 0.5% (500 mg/kgbw/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 by gavage (6 d/wk) doses of 56.2, 168.7 and 506.0 mg H₂O₂/kgbw (calculated, based on 5% solution) 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 168.7 mg/kgbw. 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 506 mg/kgbw group (Ito *et al*, 1976).

Kawasaki *et al* (1969) administered doses of 6, 10, 20, 30 and 60 mg H₂O₂/kgbw/d by gavage to male Wistar rats for 40 or 100 days. From day 20 onwards, body weight was decreased in the high dose animals. After 40 days, 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 100 days of treatment. Plasma protein, haematocrit and catalase activity were all slightly, but significantly ($P < 0.05$) decreased in the 60 mg/kgbw dose group after 100 days of treatment. Blood catalase activity was slightly but significantly lower in the 30 mg/kgbw dose group. No other effects were observed in the 30 mg/kgbw dose group. Since the magnitude of these changes was small and the number of animals employed was low (approximately 9/group; half of the animals was killed after 40 days), the validity of these effects is difficult to judge.

Antonova (1974) administered H₂O₂ to rats at doses of 1/5 and 1/10 the LD₅₀ for 45 days, although the exact dose in mg/kgbw 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.

7.2.3.3 Dermal

See Section 7.2.5.3.

7.2.3.4 Evaluation

Repeated exposure to H₂O₂ via drinking water was lethal to mice and rats at concentrations of above 1%. By gavage, body weight depression in rats was observed at 60 mg/kgbw/d from 20 days onwards in one study, but not at 169 mg/kgbw in a 12 week study. Organ weight changes, not accompanied by histopathological changes, were observed in rats only at 506 mg/kgbw after 12 weeks. The only histopathological changes were erosion and scars of the gastric mucosa (at 506 mg/kgbw) 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 H₂O₂ to rats is less than 0.25% in drinking water

or 30-56 mg/kgbw by gavage. In mice, the NOEL was approximately 0.1% in a subchronic drinking water study.

Rats exposed to 67 ppm H_2O_2 (95 mg/m³) 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 57 ppm (81 mg/m³). Inhalation exposure at 22 ppm (31 mg/m³) for 12 weeks caused minor nasal irritation and hair bleaching in rabbits. In dogs exposed to 7 ppm 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 (10 mg/m³) was mentioned for rats. Effects on the skin of rats were reported down to 1 mg/m³ (0.7 ppm). 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.

7.2.4 Genotoxicity of Hydrogen Peroxide

The mutagenicity of H_2O_2 has been extensively studied over the past 30 years. This review has been limited to recent studies, references are shown in Table XVIII to XXVI.

7.2.4.1 *In Vitro* Studies

A large number of *in vitro* studies has been conducted; the studies included many different strains of bacteria (Table XVIII and XIX). The responses in these assays are summarised in Table XX. H_2O_2 was mutagenic in most bacterial strains sensitive to oxidative agents, although the differences in results are probably due to differences in protocols, test concentrations, etc. Addition of S9, catalase, or superoxide dismutase (SOD) reduced or abolished the mutagenic response in *Salmonella typhimurium*, although the level of endogenous catalase was not correlated with the mutagenic response.

H_2O_2 is able to induce gene mutations at the HGPRT locus, the TK locus and in a special strain of CHO-BH₄ cells (Table XXI). Mutations at the DNA sequence level in plasmid as a result of treatment of mammalian host cells with H_2O_2 also have been reported. 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.

In *Escherichia coli* strains, H_2O_2 also induced DNA-repair in the absence of a metabolic system (Table XXII). The responses were not evident in the presence of metabolic activation (catalase, SOD).

Table XVIII: 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 (x 4) and TA97 (x 2.8) 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 (x 4), and TA104 (x 4) Weakly +ve on TA97, TA102, SB1111 and SB1106	Abu-Shakra and Zeiger, 1990
TA102	Standard agar	No	0-300 μg/plate	Weakly +ve (x 2.3)	Wilcox <i>et al</i> , 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 (x 2-6) 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 or 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 (x 2.5), pronounced mutagenic effects on TA102 (x 2.8) and TA104 (x 4.4)	Glatt, 1989

Table XVIII (Continued): Gene Mutations in *Salmonella Typhimurium* (Ames Test)

Strain	Protocol	Metabolic activation	Concentration (μ mol)	Results	Reference
TA102	Pre-incubation	No	400	+ve	Carlsson <i>et al</i> , 1988
TA102	Pre-incubation	Sulphide	0-50	Highly +ve (catalase in activated by sulphide)	Carlsson <i>et al</i> , 1988
BA9, BA13 (L arabinose forward mutation)	Liquid test	No	0-17.6/plate	-ve on the two strains	Ruiz-Rubio <i>et al</i> , 1985
TA97, TA102	Standard agar	No	Unknown	-ve on TA97, weakly positive on TA102	De Flora <i>et al</i> , 1984
TA102	Standard agar	S9	Unknown	Decrease of mutagenic potential on TA102	De Flora <i>et al</i> , 1984
TA92, TA94, TA98, TA100, TA1535, TA1537	Pre-incubation	No	0-200 μ g/plate	-ve on TA92, TA94, TA98, TA1535 and TA1537 Weakly +ve on TA100 (x 1.9)	Ishidate <i>et al</i> , 1984
TA100	Standard agar	Catalase induction	0-7.5/plate	H ₂ O ₂ pre-treatment induced catalase, and protection against toxicity and mutations	Winquist <i>et al</i> , 1984
TA102, TA2638	Standard agar	No	100 μ g/plate	+ve on the two strains	Levin <i>et al</i> , 1982
TA98, TA100	Liquid incubation	No	0-10,000/plate	-ve on the two strains	Stich <i>et al</i> , 1978
TA1532, TA1534, D3052, G46	Spot test	No	5% solution on filter paper disk	-ve	Mitchell, 1974

Table XIX: Gene Mutations in Other Bacteria or Yeast

Bacteria or Yeast	Metabolic activation	Concentration	Results	Reference
<i>Escherichia coli</i> (trp-)	No	0-300 µg/plate	+ve on strain WP2uvrA(pKM101) (x 3.6) and WP2(pKM101) (x 2.7)	Wilcox <i>et al</i> , 1990
<i>Escherichia coli</i> (amp-)	No	0-80 µg/ml	+ve on strain DB2 (x 5)	Bosworth <i>et al</i> , 1987
<i>Bacillus subtilis</i> (Exc-)	No	0-0.003%	+ve in the multigene sporulation test	Sacks and McGregor, 1982
<i>Escherichia coli</i> (trp-)	No	5% solution on filter paper disk	-ve on strains WP2uvrA+ and WP2uvrA-	Mitchell, 1974
<i>Escherichia coli</i> (caca-)	No	5% solution on filter paper disk	Questionable on strain WP2uvrA	Mitchell, 1974
<i>Saccharomyces cerevisiae</i>	No	5% solution on filter paper disk	-ve for forward mutations and gene conversions	Mitchell, 1974
<i>Saccharomyces cerevisiae</i>	No	100 µg	+ve for forward mutations	Thacker and Parker, 1976

Table XX: The Overall Response to H₂O₂ in Bacterial Tests

Strain	Mutation	Type ^a	Genotype			Result	
			Repair	LPS	Plasmid	–S9	+S9
<i>Salmonella typhimurium</i>							
TA102	hisG428	B	+	rfa	pKM101	+ve	+/–ve
TA104	hisG428	B	uvrB	rfa	pKM101	+ve	
TA2638	hisG428	B	+	rfa	pKM101	+ve	
SB1106p	hisC3108	No data	+	+	pKM101	+ve	
	/hisO1242						
SB1106	hisC3108	No data	+	+	-	+ve	
	/hisO1242						
SB1111	hisC3108	No data	+	+	-	+ve	
BA13	araD531	F	uvrB	+	pKM101	+ve	
	/hisG46						
BA9	araD531	F	uvrB	rfa	pKM101	+ve	
	/hisG46						
TA97	hisD6610	F	uvrB	rfa	pKM101	+/–ve	–ve
	/hisO1242						
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	
<i>Escherichia coli</i>							
WP2	trpE	B	+	+	pKM101	+ve	
WP2uvrA	trpE	B	uvrA	+	pKM101	+ve	
DB2	ampD494	F	uvrB	+	pGW1700	+ve	
WP2	trp-	B	+	+	-	–ve	
WP2	trp-	B	uvrA	-	-	–ve	
WP2	caca	A	uvrA	+	-	Equivocal	

a B: Base-pair substitution mutation; F: Frameshift mutation, A: All mutations.

Table XXI: Gene Mutations in Cultured Mammalian Cells

	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 (10 μM)	Nassi-Calo <i>et al.</i> , 1989
L5178Y Mouse lymphoma cells	No	18.6-496	37.2-79.5	Concentration-related increase of mutations at the thymidine kinase locus (18.6 μM)	Wangenheim and Bolcsfoldi, 1988
V79 cells	No	500-4,000	> 4,000	Concentration dependent increase in the of 6-thioguanine-frequency resistant clones (500 μM)	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 an over-estimation of cytotoxicity)	Speit, 1986
V79 cells	No	100-300	< 100	No increase of the frequency of 8-azaguanine or quabain-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 <i>et al.</i> , 1979

Table XXII: Primary DNA-Damage in Bacteria

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

H₂O₂ induced DNA single-strand breaks in a number of cultured mammalian cell lines such as rat intestinal epithelial cells, human bronchial epithelial cells, V79 cells (Table XXIII). Similarly, DNA double-strand breaks were observed in human leukocytes and V79 cells but not in cultured rat hepatocytes. Decreased DNA and RNA synthesis in human bronchial cells was observed after treatment with H₂O₂, but there was no or relatively insignificant induction of DNA-protein or DNA-DNA crosslinks.

Unscheduled DNA synthesis was observed in rat hepatocytes after *in vitro* treatment with H₂O₂ (Table XXIV).

H₂O₂-induced sister chromatid exchanges (SCE) in V79 cells, CHO cells, human D98/AH2 cells and human lymphocytes have also been observed (Table XXV). In the presence of S9, catalase or peroxidase, SCE induction was reduced or inhibited.

Chromosome aberrations (mainly chromatid breaks or micronuclei) were induced in a number of cell types, e.g., V79 or CHO cells but not in D98/AH2 human cells, after *in vitro* treatment with H₂O₂ (Table XXVI). *In vitro* exposure of murine splenocytes to H₂O₂ did not increase the frequency of micronucleated cells. This negative result may be due to the use of the culture medium as the solvent for H₂O₂ and inadequate H₂O₂ concentrations. Combined treatment with ferrous ions and H₂O₂ led to a synergistic enhancement of the frequency of micronuclei.

7.2.4.2 *In Vivo* Studies

Following 2 oral administrations of 0.5 ml 0.3% H₂O₂ to 0F₁ mice within 2 hours, up to a 94 fold increase in mutation frequency of intraperitoneal (i.p.) injected *Salmonella typhimurium* TA1530 (his G46, gal⁻, uvrB⁻) was obtained (Keck *et al*, 1980).

Forty-eight hours after implantation of ascite tumours (S2 sarcoma, Ehrlich ascites carcinoma and sarcoma 180), mice were injected i.p. with 0.2 ml H₂O₂ solution at concentrations ranging from 0.01 to 0.5 M; 48 hours 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 H₂O₂-treated AB/Jena Gat mice (Schöneich, 1967).

H₂O₂ did not induce chromosomal aberrations in bone-marrow cells of male Wistar albino rats (Kawasaki *et al*, 1969) and was negative in a micronucleus test in Swiss albino mice (Keck *et al*, 1980). Details of these 2 studies; e.g. route of administration, H₂O₂ concentration, etc. are lacking.

Table XXIII: Primary DNA-Damage in Cultured Mammalian Cells - Induction of DNA-Strand Breaks

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

Table XXIII (Continued): Primary DNA-Damage in Cultured Mammalian Cells - Induction of DNA-Strand Breaks

Test system	Metabolic activation	Treatment time /temperature	Concentration (µM)	Results (lowest effective concentration)	Reference
L5178Y Mouse lymphoma cells	No	3 h/37 °C	2,000-2,510	Concentration-related increase in single-strand DNA breaks (2,000 µM)	Garberg <i>et al</i> , 1988
P388D1 cells and human lymphocytes	No	5 min/37 °C	25-1,000	Concentration-related increase in single-strand DNA breaks (25 µM)	Schraufstatter <i>et al</i> , 1986
Normal human bronchial epithelial cells	No	1 h/37 °C	100 or 120	Decrease DNA and RNA synthesis Induced DNA single-strand breaks	Saladino <i>et al</i> , 1985
JB6Cl21 cells	No	1 h/37 °C	1-100	Concentration-dependent increase in single-strand breaks (10 µM)	Gensler and Bowden, 1983
V79 cells	No	2 h/1 °C	7.4-73.5	Concentration-dependent increase in single-strand breaks (14.7 µM)	Bradley and Erickson, 1981
	No	1 h/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 <i>et al</i> , 1979

Table XXIV: Primary DNA-Damage in Cultured Mammalian Cells - Induction of Unscheduled DNA Synthesis (UDS)

Test system	Protocol	Exposure time (h)	Metabolic activation	Concentration (mM)	Results (lowest effective concentration)	Reference
Rat hepatocytes	Liquid scintillation	20	No	0.1-100	Concentration related increase (1 mM)	Beales and Suter, 1989
Rat hepatocytes	Auto-radiography	3 x 1	No	1.9-6.4	Induction of UDS (3.2 mM)	Cattley <i>et al</i> , 1988
WI-38 CCL75 cells (human diploid fetal lung cells)	Auto-radiography	3-4	No	0.6-2,400 µg/ml	Concentration-related increase (9 µg/ml)	Coppinger <i>et al</i> , 1983
	Differential density labelling	3-4	No	0.15-600 µg/ml	Concentration-related increase (9.4 µg/ml)	Coppinger <i>et al</i> , 1983
Human fibroblasts	Auto-radiography	3	No	0.1-10	Concentration-related increase (0.6 mM)	Stich <i>et al</i> , 1978

Effort on CEFIC funded LRI 2002

Date	Location	Hours	Task
3.1.02	Ecetoc	4	WG II and QSAR Workshop
8.1.02	Ecetoc	1	Eco 1a
9.1.02	Frankfurt	1	Eco 1a
10.1.02	Ecetoc	2	QSAR Workshop
14.1.02	Ecetoc	1	QSAR teleconference
15.1.02	Ecetoc	2	QSAR Participants
23.1.02	Ecetoc	8	QSAR workshop
25.1.02	London	4	LRI Monitoring Team
28.1.02	Ecetoc	2	QSAR teleconference
1.2.02	Ecetoc	8	QSAR Workshop Letters
6.2.02	Ecetoc	6	QSAR travel
7,8.2.02	Toulon	16	Eco 3b
18.2.02	London	8	Eco 2a
19.2.02	Ecetoc	8	QSAR programme
20.2.02.	Ecetoc	8	QSAR workshop
21.2.02	Ecetoc	4	QSAR programme
22.2.02	London	8	EEM 1
25.2.02	Ecetoc	2	QSAR meeting details
26.2.02	Ecetoc	4	Selection team meeting arrangements
27.2.02	Ecetoc	8	QSAR meeting handouts
4-7.3.02	Portugal	32	QSAR workshop
14.3.02	Brussels	4	Selection team
2.4.02	Ecetoc	4	QSAR report
8.4.02	Ecetoc	4	SETAC posters
15.4.02	Ecetoc	4	SETAC poster
22.4.02	Ecetoc	2	SETAC booth
2.5.02	Ecetoc	4	SETAC posters
3.5.02	CEFIC	3	SETAC
17.5.02	Cranfield	8	EEM 3
30.5.02	Ecetoc	8	Animals in the Wild
12.6.02	Brest	4	Eco 3b
13.6.02	Brest	8	Eco 3b
14.6.02	Brest	8	Eco 3b
20.6.02	Ecetoc	2	Selection team arrangements
26.6.02	Ecetoc	4	QSAR workshop report
4.7.02	Cefic	1	QSAR workshop report
4.7.02	Ecetoc	1	Selection team arrangements
9.7.02	Paris	8 (214)	Eco 1a RLT meeting
8.8.02	Ecetoc	2	Eco 4a
10.8.02	Ecetoc	4	Eco 4a
5.9.02	Ecetoc	8	Selection team meeting

12.9.02	Ecetoc	4	Eco 2a
13.9.02	Brixham	8	Eco 2a
20.9.02	Ecetoc	4	RLT meeting arrangements
11.10.02	Amsterdam	8	Eco 4
14.10.02	Ecetoc	8	RLT meeting
15.10.02	Ecetoc	8	RLT meeting
25.10.02	Ecetoc	4	RLT minutes
8.11.02	Ecetoc	8	Eco 1a
12.11.02	Ecetoc	2	Slides LRI Annual workshop
20.11.02	Brussels	4	LRI Annual workshop
21.11.02	Brussels	8	LRI Annual workshop
4.12.02	Ecetoc	2	Eco 1b RfP
6.12.02	Ecetoc	4	Arrangements RLT planning meeting Jan.

Table XXV: Primary DNA-Damage in Mammalian Cells - Induction of Sister Chromatid Exchanges (SCE)

Test system	Metabolic activation	Exposure time (h)	Concentration (µM)	Cytotoxicity (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 (10 µM) 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 (40 µM and 160 µM, respectively)	Tucker <i>et al</i> , 1989
V79 cells	No	3	10-80	20-40	Induction of SCE (20 µM)	Speit, 1986
Whole human blood or human purified lymphocytes	No	24	20-2,000	Unknown	SCE induction in purified lymphocytes (20 µM) but not in whole blood culture	Mehnert <i>et al</i> , 1984a
Human purified lymphocytes	No	2	80-200	Unknown	SCE induction in purified lymphocytes (80 µM) but not in whole blood culture	Mehnert <i>et al</i> , 1984a
Human purified lymphocytes	Catalase or peroxidase or S9-mix	2	80-200	Unknown	Reduction of H ₂ O ₂ -induced SCEs	Mehnert <i>et al</i> , 1984a
D98/AH2 human cells	No	24	15-60	> 60	3-fold SCE induction at 60 µM	Estervig and Wang, 1984
CHO cells	No	2	0.1-100 mM	10 mM	Slight increase of the SCE frequency (0.5 mM)	Wilmer and Natarajan, 1981

Table XXV (Continued): Primary DNA-Damage in Mammalian Cells - Induction of Sister Chromatid Exchanges (SCE)

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

Table XXVI: Chromosomal Aberrations in Cultured Mammalian Cells

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

a PBS, Phosphate-buffered saline

b MEM, Eagles Modified Minimal Essential Medium

Table XXVI (Continued): 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 (200 μM)	Tsuda, 1981
V79 cells	Chromosomal aberrations	3	No	100-1,000	Concentration-related increase of chromatid breaks (200 μM)	Tsuda, 1981
Syrian hamster cells	Chromosomal aberrations	3	No	100-1,000	Concentration-related increase of chromatid breaks (200 μM)	Tsuda, 1981
Balb/c mouse cells	Chromosomal aberrations	3	No	10-100	Concentration-related increase of chromatid breaks (50 μM)	Tsuda, 1981
CHO cells	Chromosomal aberrations	2	No	100-100,000	Only positive at 10,000 μM	Wilmer and Natarajan, 1981
CHO cells	Chromosomal aberrations	3	No	100-1,000	Concentration related increase of chromosome aberrations (100 μM), toxic at concentrations higher than 300 μM	Stich <i>et al</i> , 1978
Don-6 cells (Chinese hamster cells)	Chromosomal aberrations	3	No	500-2,000	Dose related increase of chromosome aberrations (1,000 μM)	Sasaki and Sugimura, 1980

H₂O₂ (3% solution) did not induce sex-linked recessive lethal mutations in *Drosophila melanogaster* following treatment of the larvae (Di Paolo, 1952).

Micronuclei were evaluated in male and female C57BL/6NCrlBr mice exposed to 6,000 mg H₂O₂/l drinking water for 14 days. H₂O₂ did not induce an increase in micronuclei. No statistically significant increases in the frequency of micronucleated polychromatic erythrocytes were observed and no statistically significant decreases in the proportion of young polychromatic erythrocytes to mature normochromatic erythrocytes were observed (Ross, 1995).

Swiss OF₁ mice received H₂O₂ i.p. at doses of 250, 500 and 1,000 mg/kgbw. The PE/NE ratio was statistically significantly lower ($P < 0.05$) from that of the respective vehicle control group, at the 3 doses of the 24-hour harvest and at 250 and 1,000 mg/kgbw at the 48-hour harvest, showing that the treatment effectively influenced the bone marrow. H₂O₂ did not induce cytogenetic damage to the bone marrow cells (Molinier, 1995).

7.2.4.3 Evaluation

The *in vitro* and *in vivo* genotoxic potential of H₂O₂ is summarised in Table XXVII.

On an *in vitro* basis, bacteria and other cells in culture that come into direct contact with H₂O₂ exhibit a genotoxic effect. However, in the presence of exogenous metabolic agents or catalase, the effect appears to be reduced or abolished. Since decomposition products of H₂O₂ like hydroxyl radicals and singlet oxygen (Section 7.1.1.3) are capable of damaging DNA directly, the genotoxic potential of H₂O₂ may depend on accessibility of the reactive hydroxyl radical to its target DNA. On the other hand, when H₂O₂ was tested for systemic genotoxic action in the mouse micronucleus assay by either the oral or i.p. routes, there was no evidence of *in vivo* mutagenicity. Apparently, in the whole animal many factors contribute to the reduction or elimination of systemic genotoxic potential.

Table XXVII: Evaluation of Genotoxicity (ECETOC, 1993)

End-point	Test system	Results
<i>IN VITRO</i>		
Gene mutation	<i>Salmonella typhimurium</i>	+ve without activation decrease of the genotoxic potential in presence of exogenous S9 or catalase
	<i>Escherichia coli</i>	+ve without activation
	<i>Saccharomyces cerevisiae</i>	+/-ve without activation
	<i>Bacillus subtilis</i>	+ve without activation
	Mammalian cells	+ve without activation
Primary DNA-damage		
DNA-repair	<i>Salmonella typhimurium</i>	+ve without activation
	<i>Escherichia coli</i>	+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
<i>IN VIVO</i>		
Gene mutation	<i>Drosophila melanogaster</i>	-ve
	<i>Salmonella typhimurium</i> (host mediated assay in mice)	+ve
Chromosomal aberration		
Micronucleus or metaphase analysis	Mice	-ve
	Rat	-ve
	Tumour cells (host mediated assay in mice)	+ve

7.2.5 Chronic Toxicity and Carcinogenicity

7.2.5.1 Chronic Toxicity (Table XXVIII)

Several long-term studies were conducted in rats, mice and rabbits with H₂O₂ 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 0.005-50 mg H₂O₂/kgbw/d 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 gastrointestinal mucosa and focal fatty changes in the hepatocytes were observed on autopsy. At 5 mg/kgbw/d the same effects occurred, but no decrease in body weight gain, catalase activity of the liver or histopathological changes in the stomach were observed. The lower doses showed only changes in haematology and enzyme activity. The NOEL for H₂O₂ was considered to be 0.1 mg/l (0.005 mg/kgbw/d) by the author (Antonova, 1974). The results should be considered with caution because of the lack of information on the methods used, experimental details and incomplete reporting of the results.

Aoki and Tani (1972) found focal necrosis of the liver, and thickening of the stomach wall of dd strain male mice administered 0.15% H₂O₂ in drinking water for 16 weeks, but not at 13 weeks. At 22 weeks, there were histological changes in the gastrointestinal 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 H₂O₂ to mice caused pathological changes in the liver, kidney, gastrointestinal tract and spleen at a concentration of 0.15% in drinking water, while no effect on body weight gain was observed. Ito *et al* (1982) examined the occurrence of gastrointestinal lesions in 3 strains of mice administered 0.4% H₂O₂ in drinking water for periods up to 210 days. 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 90 days. Gastric lesions were not observed in the other strains until 150 days. 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.

Table XXVIII: Chronic Toxicity Studies

Species (strain)	Concentration in solution (%)	n	Dose (mg/kgbw/d)	Duration (months)	NOEL (%)	LEL (%)	Reference
Gavage							
Rabbit	0.00001, 0.00001	45	0.005, 0.05, 0.5, 5, 50	6	0.00001	-	Antonova, 1974
Rat	0.0001, 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 <i>et al</i> , 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 <i>et al</i> , 1984
Mouse (B6C3F ₁)	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)	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 2.0 ml solution and 13 g mouse

d Fresh dosing solution prepared every other day; stability of H₂O₂ in solution was analytically determined

e 35 weeks

f 210 days

In an additional study (Table XXIX) conducted by Ito *et al* (1982), the incidence and type of gastrointestinal lesions in C57BL mice given 0.4% H₂O₂ were examined before and after cessation of H₂O₂ administration. Mice were treated for up to 180 days with H₂O₂, and then given distilled water for 10, 20 or 30 days. Administration of H₂O₂ for 140 days 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 H₂O₂ administration followed by distilled water resulted in a decreased incidence of erosions and nodules within 20 days, and by 30 days of distilled water treatment, all duodenal erosions and nodules had resolved. H₂O₂ administration for 150 or 18 days followed by distilled water administration for 3 days also resulted in a regression of stomach lesions and duodenal plaques. The total incidence of duodenal nodules of mice treated for 150 or 18 days, then given distilled water for 3 days, also declined. However, the average number of nodules per mouse either remained the same or decreased.

Glandular stomach lesions (Section 7.2.5.2) were also found in mice after administration of 0.1% and 0.4% H₂O₂ in their drinking water in a carcinogenicity study by Ito *et al* (1981a,b).

Table XXIX: Effect of H₂O₂ Withdrawal on Gastro-duodenal Lesions^a

(after Ito *et al*, 1982)

Administration (days) of		Stomach			Duodenum		
H ₂ O ₂	distilled water	Lesions	Erosions	Nodules	Lesions	Plaques	Nodules
150	-	78	33 (1.3) ^b	56 (1.2)	89	67 (2.2)	67 (2.2)
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 Explained in Section 7.2.5.1

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

7.2.5.2 Carcinogenicity

Oral carcinogenicity studies have been reported by 2 groups of Japanese investigators. The study designs are shown in Table XXX.

Table XXX: 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 <i>et al</i> , 1981a, b
Mouse, C57BL/6N	0, 0.4 ^b	138	104 ^b	Ito <i>et al</i> , 1982
Rat, Fischer 344	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 H₂O₂ 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 H₂O₂

An increase in duodenal tumours was observed in male and female C57BL/6J mice administered H₂O₂ (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 H₂O₂ treated groups were comparable to those of control mice, except for a slight decrease in body weight of 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 H₂O₂ (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 gastrointestinal erosions was increased in treated mice (Table XXXI). There was no inflammatory response within the oral cavity, forestomach or distal intestinal tract. Erosions within the glandular stomach found at the antrum (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 H₂O₂, spontaneous disease, e.g. gastrointestinal amyloidosis, or both. Given the

incidence of erosions, an effect on mortality and on body weights would have been expected as observed with other gastrointestinal 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 (Ito *et al*, 1981a, b).

Table XXXI: Incidence of Gastrointestinal Erosions in C57BL/6J Mice
(Ito *et al*, 1981a, b)

Concentration in drinking water (%)	Glandular stomach				Duodenum			
	Males		Females		Males		Females	
0	2	(4%)	2	(4%) ^a	0	(0%)	2	(4%)
0.1	13	(25%)	7	(14%)	0	(0%)	1	(2%)
0.4	19	(38%)	23	(47%)	2	(4%)	2	(4%)

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

The incidence of hyperplasia, adenomas and carcinomas found by Ito *et al* (1981a,b) is shown in Table XXXII. In the stomach, hyperplasia within the glandular part occurred with similar frequency in all groups; 1 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 exhibit a dose-dependent distribution. The incidence of duodenal carcinomas was higher in the females of the 0.4% group compared to the controls, and 1 carcinoma was observed in 1 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* (1981a,b) 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% H₂O₂. 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 H₂O₂ is unstable under alkaline conditions.

(Ito et al, 1981a, b)

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

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

In another study by Ito *et al* (1982), male and female C57BL/6N mice were administered 0.1% or 0.4% H₂O₂ (30% food grade) in drinking water for up to 740 days. 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 gastrointestinal lesions (Table XXXIII). 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 adeno-carcinomas. By day 740, there was a concentration-dependent increase in the incidence of gastrointestinal lesions, consisting of erosions, adenomas and carcinomas.

Table XXXIII: Frequency of Gastro-duodenal Lesions in C57BL/6N Mice Orally Given 0.4% H₂O₂ (Ito *et al*, 1982)

Period (d)	No of mice tested	Stomach			Duodenum		
		Lesions	Erosions	Nodules	Lesions	Plaques	Nodules
30	7	29 (2.0) ^a	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)

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

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% H₂O₂ was administered in drinking water for 6 or 7 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 H₂O₂-treated mice with the greatest incidence occurring in mice having low catalase activity (Table XXXIV, VII). The incidence of duodenal tumours in mice with low catalase activity was comparable to the incidence in mice exhibiting hypocatalasemia (C3H/Csb).

Table XXXIV: Incidence of Duodenal Tumours in 4 Strains of Female Mice Treated with 0.4% H₂O₂ in Drinking Water (Ito *et al*, 1984)

Strain ^a	n	Number of mice with tumours (% incidence)	Total number of tumours
C3H/HeN	18	2 (11.1%)	2
B6C3F ₁	22	7 (31.8%)	8
C57BL/6N	21	21 (100%)	82
C3H/C	24	22 (91.7%)	63

a C3H/HeN, high catalase activity; B6C3F₁, F₁ hybrid of C3H and C57BL, with "normal" catalase activity; C57BL/6N, low catalase activity; C3H/C, mice with hypocatalasemia

H₂O₂ (30%) was administered to Fischer 344 rats in drinking water at concentrations of 0%, 0.3% or 0.6% for 78 weeks followed by a 6 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 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, H₂O₂ was not carcinogenic to Fischer 344 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 H₂O₂ in drinking water (Section 7.2.5.3).

Sencar mice (n=60) were treated topically twice a week with a 15% solution of H₂O₂ (0.2 ml) 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 H₂O₂ (0.2 ml) for up to 51 weeks. Control mice in each study were treated dermally with acetone (0.2 ml). 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, H₂O₂ 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.

7.2.5.3 Initiation-Promotion by Hydrogen Peroxide

Several investigators have examined the potential of H₂O₂ 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 XXXV.

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 H₂O₂ in acetone for 25 weeks. As the concentration of H₂O₂ decreased, the incidence of papillomas increased to a maximum of 10% (H₂O₂ 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% H₂O₂ 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 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 H₂O₂ was painted onto the left buccal pouch of 4 groups of male Syrian golden hamsters twice weekly for 19 or 22 weeks. Animals in Group A were painted 2 x/week with a 0.25% solution of DMBA in heavy mineral oil. Animals in Group B were painted 2 x/week with DMBA and 2 x/week (on days other than the DMBA painting) with 3% H₂O₂. Group C animals were painted in exactly the same way as Group B animals except that the concentration of H₂O₂ used was 30%. Group D animals were painted 2 x/week with 30% H₂O₂ alone. Cheek pouches from animals which had not been painted and from animals which had been painted 2 x/week with only the mineral oil vehicle served as controls. Six of 11 hamsters (55%) treated with DMBA and 3% H₂O₂ developed epidermoid carcinomas by 22 weeks, whereas all 5 hamsters treated with DMBA and 30% H₂O₂ developed epidermoid carcinomas by 22 weeks. No carcinomas were observed in hamsters treated with 30% H₂O₂ alone, but 3/7 (43%)

Table XXXV: Tumour Initiation-Promotion Studies^a

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

a Animals treated topically; control animals treated with acetone

b DMBA, Dimethylbenzanthracene; MNNG, N-methyl-N'-nitro-N-nitrosoguanidine

c DMBA administered once, topically, in acetone

d H₂O₂ administered topically in 0.2 ml acetone, 1 week before DMBA; concentrations measured

e DMBA painted onto one buccal pouch 2 x/week; H₂O₂ (3 or 30%) painted on opposite buccal pouch 2 x/week (on days other than DMBA painting)

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

g 0.2 ml H₂O₂ administered once

h TPA administered 2 x/week

i H₂O₂ administered in drinking water

j MAM given 3 x/week; H₂O₂ administration continued during MAM treatment interval except for 2 days following injection

k At end of 8 weeks, tap water given to one group and H₂O₂ (1.5%) administration continued in second group for an additional 13 weeks

of the hamsters treated with DMBA alone developed carcinomas. Only 1 carcinoma was observed in a hamster treated with DMBA and 30% H₂O₂ 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% H₂O₂ 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% H₂O₂ (Weitzman *et al*, 1986).

A group of Sencar mice was tested for tumour initiation by topical administration of H₂O₂ followed by twice weekly applications of 12-O-tetradecanoylphorbol-13-acetate (TPA). The authors found no significant increase in the incidence of dermal tumours and concluded that H₂O₂ was not a tumour initiator (Klein-Szanto and Slaga, 1982).

Oral Studies

Takahashi *et al* (1986) examined the potential of H₂O₂ 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, the water of one group being supplemented with 1% H₂O₂, for 7 weeks *ad libitum* after which the animals were maintained on normal feed and tap water. A third group (n=10) was not given MNNG or a sodium chloride supplemented diet, but was administered 1% H₂O₂ 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 XXXVI). In rats treated with MNNG and H₂O₂, there was no enhancement in the number of gastrointestinal tumours, although all treated animals exhibited forestomach papillomas; these also occurred in rats treated only with H₂O₂ 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 H₂O₂ 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.

Table XXXVI: Effect on Gastro-duodenal Carcinogenesis Induced by MNNG
(Takahashi *et al*, 1986)

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

a Adenomatous hyperplasia

b Number of rats with tumours (% incidence)

Hirota and Yokoyama (1981) examined the tumour promotion potential of 1.5% H₂O₂ in drinking water in the duodenum and jejunum of Fischer 344 rats. After 4 weeks of administration, methylazoxymethanol acetate (MAM) was administered i.p.; H₂O₂ administration was continued except for 2 days following injection. At the end of 8 weeks, one group of rats continued on H₂O₂ whereas a second group was given tap water to drink for an additional 5 weeks. A third group of 3 rats received only H₂O₂ 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 H₂O₂, although duodenal and upper jejunal hyperplasia were noted in the latter group. The authors concluded that H₂O₂ 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 of Oral Studies

These studies confirm that H₂O₂ induces inflammatory changes in tissues following topical application. Furthermore H₂O₂, at higher 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 gastrointestinal tract are observed similar to these found in longer-term studies by several investigators, e.g. Ito *et al* (1981a,b). Promotion of initiated gastrointestinal tumours by H₂O₂ has not been proven. Overall, H₂O₂ concentrations of < 1% do not appear to have gastrointestinal tumour promoting potential.

Anti-tumour Effects of H₂O₂

Several investigators have examined the potential anti-cancer effects of H₂O₂ (studies in which H₂O₂ was injected are not reviewed).

Regression of implanted Walker 256 adenocarcinomas in 6 rats treated with 0.45% H₂O₂ in the drinking water was observed by Holman (1957). The time to complete tumour regression was approximately 15-60 days, depending on tumour size. No other details were provided.

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% H₂O₂ in their drinking water. All rats died by 21 days and no tumour regression was observed. Similarly, Ghadially and Wiseman (1958) implanted Walker tumours or dibenzanthracene derived RD3 sarcomas into rats (n=29 or 35). Five days after implantation, the rats were administered 0.45% H₂O₂ in drinking water for up to 136 days; controls received tap water. No differences in survival time or tumour weight were observed between the treated rats and controls. These authors concluded that H₂O₂ 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 H₂O₂.

7.2.5.4 Evaluation

Details needed to fully evaluate the chronic toxicity or carcinogenic potential of H₂O₂ are lacking. Several studies reviewed show that long-term oral administration of 0.1-0.15% H₂O₂ causes an inflammatory response in gastroduodenal 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 H₂O₂ administration causes a regression of lesions induced by prolonged (up to 18 days) administration of H₂O₂ 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, H₂O₂ induced only papillomas; no malignant tumours of the forestomach were seen, even at nearly lethal concentrations (1-1.5% H₂O₂ in drinking water). Initiation-promotion studies suggest that H₂O₂ 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 7.2.4.1) would indicate that a genotoxic mechanism for tumour induction is feasible for H₂O₂, 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 H₂O₂ came directly into contact with the tissues and that the tumours were associated with persistent local inflammation supports a non-genotoxic mechanism for the gastrointestinal tract tumours. Non-genotoxic mechanisms have been discussed in detail by many authors, among others the interaction of H₂O₂ with bile (ECETOC and IPCS, 1991).

The US Food and Drug Administration 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).

7.2.6 Reproductive Toxicity

7.2.6.1 Male Fertility

The spermicidal activity of H₂O₂ was investigated both *in vivo* and *in vitro*. Washed and unwashed sperm from bull, rabbit, ram, fowl, dog and mouse was exposed to solutions containing 0, 0.3, 3, 30, 300 and 3,000 mg/l H₂O₂. Spermatozoa were evaluated for mobility *in vitro* following washing and when added directly to diluent. There were large species differences in the tolerance to H₂O₂. Rabbit spermatozoa were the most resistant and bull and fowl were the least resistant to the immobilising effect of H₂O₂. Although 3 mg/l had small, but significant effects on bull and fowl spermatozoa, concentrations 10 to 100 x greater were required to immobilise rabbit spermatozoa. In general, washing increased the toxicity of H₂O₂, 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 H₂O₂.

Male albino mice were given 0.33%, 1.0% or 3.0% H₂O₂ 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 21 days on H₂O₂. 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 had no significant spermicidal action in mice at concentrations up to 1% in solution (Wales *et al*, 1959).

7.2.6.2 *In Vitro Embryotoxicity*

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

7.2.6.3 *Reproductive Organs*

Male and female rats were administered H_2O_2 daily by gavage at doses of 1/10-1/5 LD_{50} (which is not specified) for 45 days. At the high dose, females showed modifications of the oestrus cycle and males reduced mobility of spermatozoa, without effects on the weight of the testicles. In a second experiment male and female rats received daily doses of 0.005, 0.05, 0.5, 5 and 50 mg H_2O_2 /kgbw by gavage for 6 months and were mated. The signs of maternal toxicity have been described in Section 7.2.5.1. Variations of the oestrous cycle in females were observed at 50 and 0.5 mg H_2O_2 /kgbw, but not at 5 mg/kgbw. Reduced mobility of spermatozoa in males were observed at 50 mg H_2O_2 /kgbw. No changes were observed in the morphology and weight of the testes. Among the high dose females, only 3/9 produced litters, compared to 7/9 in the control group. In addition litter size and bodyweight gain of the offspring of the high dose females was reduced relative to those of control females (Antonova, 1974). The results of this study should be considered with caution because the authors did not describe the experimental details of the reproductive phase of the study and their reporting of the results was incomplete.

7.2.6.4 *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 3 days. 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.

7.2.6.5 Evaluation

Data on the teratogenic potential and reproductive toxicity are limited and do not allow a complete evaluation. However, such effects are not expected at concentrations which do not exert systemic toxicity.

7.3 EFFECTS ON HUMANS

7.3.1 Short-term Exposure

In a retrospective study of all poison reports in a regional poison control centre over a 3-year period, 0.34% of the cases (325 of 95,052) were due to H_2O_2 exposures. Ingestion was the most common route of exposure, accounting for 83% of the cases, followed by ocular (8.0%), dermal (7.7%), inhalation (0.6%) and rectal (0.6%) exposures. Children under 6 years old were the most common age group of accidental exposures accounting for 60.6% of all cases, followed by individuals older than 17 years with 20.9% of the cases and children and teens from 6 to 17 years of age with 10% of the cases. The remainder of the cases did not have age reported. By severity, the most common outcome of the cases was minor effect in 36.0% of the cases and no effect in 29% of the cases. Moderate and major effects were the outcomes in 1.5% and 0.3% of the cases, respectively. There was one case which resulted in mortality (0.3% of the cases). The remainder of the cases (33.0%) had an unknown outcome. The most common symptoms of orally-exposed individuals were nausea (29%); vomiting (31%), respiratory difficulties (9%), sore throat (4.7%) and abdominal distension (4.7%). Cases in which exposure occurred to concentrations of H_2O_2 less than 10% had less severe outcomes compared to concentrations greater than 10%. Ocular and dermal exposures to dilute solutions resulted in transient symptoms without permanent effects. The most common ocular symptoms included burning (65%), redness (50%) and blurry vision (19%). The 3 major dermatological findings were paresthesias (60%), whiteness (56%) and blistering (16%) (Dickson and Caravati, 1994).

7.3.1.1 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.

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 41 mg/m^3 . 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 exposed to 41 mg/m^3 (1 measurement only) 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 (Kaelin *et al*, 1988).

Slight nasal irritation and skin bleaching upon contact were observed in factory workers involved in drum and tank filling at a H_2O_2 production facility. The maximum mean exposure level during the operations (1 h) was 3.5 mg/m^3 (CEFIC, 1996).

Pulmonary function testing including FVC, FEV, PEFR and FEF 25-57 performed on all employees of a H_2O_2 production facility for a period of 3-5 years showed no evidence of adverse effects from occupational exposure. In the past, at the same plant there were reports of hair bleaching, nose bleeds and eye or respiratory irritation. However, there have been very few reported incidents since operating procedures have been improved and local exhaust or dilution ventilation installed leading to typical exposure levels ranged from not detectable to 0.79 mg/m^3 (8-h TWA) (CEFIC, 1996).

Bleaching has been known to occur with levels of 0.5-1 ppm (0.7-1.4 mg/m^3) vapour (FMC, 1990), but it is uncertain whether bleaching was due to vapour exposure or to transfer of H_2O_2 from the hands to hair.

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

7.3.1.2 Oral Exposure

A 1-year old child died of respiratory failure within 1 hour after ingesting an unknown quantity of a 30% H₂O₂ 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 gastrointestinal mucosa.

A 2-year old boy ingested an unknown amount of a 3% H₂O₂ solution. He was foaming around the mouth and had vomited 3 x. A gas embolism was visualised in the portal venous system. It was concluded that liberation of oxygen from H₂O₂ 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% H₂O₂ 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 16 hours after exposure showed erosion of the cardiac stomach and erythema of the lower oesophageal sphincter and a gastric burn. Follow-up endoscopy 12 days later showed normal oesophageal and duodenal mucosa with minimal hyperaemia 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% H₂O₂ 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 4-year old friend was in critical condition with oropharyngeal burns after accidentally ingesting a solution of 35% H₂O₂ (Thompson, 1989).

Five non-fatal poisonings in adults were reported following consumption of about 25-100 ml of H₂O₂ (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 100 ml of the H₂O₂ solution. Symptoms were considered to be due to microemboli of generated oxygen. All victims recovered completely in 2 to 3 weeks (Budagovskija *et al*, 1971).

A near-fatality occurred when a 33 year old woman unintentionally ingested the contents of a 1 pint bottle (< 500 ml) of 35% H₂O₂. 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).

There are a number of unofficial reports that some individuals self-administer H₂O₂ 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 H₂O₂. A 39-year old man was admitted to a hospital emergency room in haemolytic crisis 24 hours after alleged i.v. injection of H₂O₂ (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).

Humans are exposed to H₂O₂ (0.75%) in dentifrice products, as commercially available tooth pastes. Monitoring of adverse reactions by the Cosmetic, Toiletry and Fragrance Association (CTFA, 1994) indicates that the incidence of adverse reactions is extremely low. Between 1991 and 1994 40.6 million units of these dentifrices with H₂O₂ were sold in the United States. Only 421 adverse reactions (i.e. 1 reaction for every 100,000 units sold) were reported. Reported reactions were primarily burning mouth and oral irritation. Symptoms generally subsided with cessation of the product use. The incidence and types of reactions were typical of other dentifrices on the market without H₂O₂.

Evaluation

Young children are particularly vulnerable to accidental ingestion of H₂O₂. Deaths have been reported from ingestion of unknown quantities of H₂O₂ solutions of 30-40% concentration.

7.3.1.3 Dermal Exposure

A characteristic temporary whitening of the skin occurs after dermal application of 20% to 30% H₂O₂. The whitening is due to oxygen bubbles acting as microemboli in the capillaries and blocking circulation (Hauschild *et al*, 1958). H₂O₂ is used topically to bleach hair at concentrations of 3% to 6%. No clear data are available on the skin irritation effects of various H₂O₂ solutions in human beings (see also Section 7.3.2.1).

7.3.2 Irritation, Sensitisation and Immunotoxicity

7.3.2.1 Skin Sensitisation

A single case report observed skin sensitisation reactions from 2 women who had been exposed to H_2O_2 as an ingredient in commercial hair dyes. Both women tested positive to 3% H_2O_2 and numerous other ingredients in the hair dyes. The authors reported that 156 other hairdressers patch tested with a hair dresser series tested negatively to 3% H_2O_2 . This report indicates that some individuals may be extremely sensitive to the ingredients in hair dyes. In general, the data do not provide adequate evidence that H_2O_2 is a skin sensitizer in man (Aguire *et al*, 1994)

7.3.2.2 Acute Effects from Irrigating and Cleansing Solutions

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 6 subjects over a 5-day use period with 2 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 3 to 5 x/d as a mouth rinse resulted in mucosal irritations in 2 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 mouth wash and dipped their toothbrushes into the solution before brushing their teeth. Application of the H_2O_2 was 2-3 x/d 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).

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 1 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-hour 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 20 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 3 paediatric 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).

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 2 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 3 to 5 x/d 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 hyperaemia (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 800 mg/l were tested; none affected the physiological integrity of the eye. An initial conjunctival hyperaemia occurred with the 800 and 400 mg/l levels but not with 200 mg/l and below. Levels of H_2O_2 in excess of 100 mg/l were associated with an initial subjective stinging response. Subjective effects, such as stinging were first noticed between 50 and 100 mg/l; the NOEL was 50 mg/l. 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 247 mg/l 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 50-60 mg/l after neutralisation; this is close to the natural background level of H_2O_2 in the eye lens (Section 7.1.3.2).

7.3.3 Long-term Exposure

7.3.3.1 Chronic Toxicity and Carcinogenicity

No data are available.

7.3.3.2 Reproductive Toxicity

Human sperm was exposed to solutions containing 0, 0.3, 3, 30, 300 and 3,000 mg/l 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 300 mg/l (Wales *et al*, 1959). Removal of endogenous catalase by washing increased the toxicity of H_2O_2 to spermatozoa.

7.3.3.3 Neurotoxicity

No information is available.

7.3.4 Evaluation

Occupational exposure to H_2O_2 aerosol concentrations of 12 to 41 mg/m^3 resulted in skin, eye and throat irritation.

The irritant threshold to the lungs of human volunteers was considered to be 10 mg/m^3 for with a NOEL of 5 mg/m^3 for exposure periods up to 4 hours.

Slight nasal irritation was observed in factory workers at short-term exposure up to 3.5 mg/m^3 .

Pulmonary function testing reported no evidence of adverse effects from occupational exposure during several years at levels ranging from not detectable to 0.79 mg/m^3 (8-h TWA).

Gradual bleaching of hair is a common occurrence among factory workers and has been reported from exposure levels of 0.7-1.4 mg/m^3 vapour, but it is uncertain whether bleaching was due to vapour exposure or to transfer of H_2O_2 from the hands to hair.

Ingestion is the most common route of accidental exposure, although the incidence is very low (reported at 0.34% of all accidental exposures in one survey). Outcomes are generally minor

(concentrations less than 10%); however, deaths have been reported from ingestion of unknown quantities of 30-40% solutions by children. The effects seen were related to a corrosive action of the gastrointestinal tract and to rapid generation of large volumes of oxygen. Complete recovery has occurred in near fatal cases within 2-3 weeks with medical care.

Dermal exposure to dilute solutions has been reported to result in transient symptoms without permanent effects, such as paresthesias, whiteness and blistering.

Accidental exposure to H_2O_2 has occurred from its use in household topical disinfectants and cosmetic/personal care products generally involving exposure to the 3% solution.

The incidence of adverse reactions reported to dentifrices containing 0.75% H_2O_2 is extremely low (one in 100,000 units sold) and does not differ from the incidence for similar products without H_2O_2 as an ingredient.

H_2O_2 solutions of 1-3% have been used as anti-bacterial agent in the eye and as mouth washes without causing significant injury. Accidental ocular exposure cases are generally a small percent (reported at 8.0% of all accidental exposures in one survey) of all accidental exposure to H_2O_2 . Symptoms of burning, redness and blurry vision have been reported. In the eye, H_2O_2 solutions of > 200 mg/l caused hyperaemia; pain and stinging occurred with concentrations of ≥ 100 mg/l.

8. GROUPS AT EXTRA RISK

Acatalasemic individuals are more susceptible to H_2O_2 exposure because of a hereditary disorder in their H_2O_2 metabolising enzymes, i.e. the blood catalase activity level is below normal (hypocatalasemia). Acatalesemia is a rare (frequency 0.2-0.4%) genetic defect occurring particularly in the Orient (Ogata, 1991) (Section 7.1.4.1, *Catalase*).

Another group of individuals more sensitive to H_2O_2 exposure are persons with glucose-6-phosphate dehydrogenase (G6PD) deficiency (Section 7.1.4.1, *Glutathione Peroxidase*).

9. GAPS IN KNOWLEDGE AND ONGOING RESEARCH

9.1 ACUTE TOXICITY

There is no formal datagap for acute toxicity.

9.2 IRRITATION AND SENSITISATION

There is no datagap on sensitisation or irritation/corrosion.

9.3 REPEATED EXPOSURE

There is no OECD standard (sub)chronic toxicity study. Such study could confirm the local nature of irritative effects and possibly conclude about the possible systemic effects on haematology, liver, kidney and spleen suggested by limited observations in the literature.

A 3-months drinking water study is being conducted by the CEFIC Peroxygen Sector Group to establish a NOEL for local and systemic effects.

Given the irritating properties of H₂O₂ and in absence of further evidence of systemic effects, the lack of a repeated exposure study by the inhalation route is not considered to be a datagap.

9.4 CARCINOGENICITY

There is no formal datagap for carcinogenicity. Based on the information about the possible local and/or systemic effects that will be gathered from the results of the (sub)chronic and genotoxicity studies, the need of such a study should be reevaluated.

9.5 GENOTOXICITY

In vitro, H₂O₂ is genotoxic in the absence of a metabolic activation system. Addition of such an activation system (S9) reduces or, more often, abolishes the genotoxic response. *In vivo*, H₂O₂ is present as a natural metabolic product against which effective defence mechanisms (catalase, GSH peroxidase) are active. Exogenous H₂O₂ was not genotoxic in the *in vivo* mouse micronucleus test following acute i.p. and subacute oral administration.

Further data would be useful to better evaluate the *in vivo* genotoxic potential of H₂O₂ of exogenous origin in target tissues. The Peroxygen Sector Group in its study programme, is considering an evaluation of the *in vivo* genotoxic potential after subacute oral dosage in circulating lymphocytes, gut and skin chromosome aberration, and DNA damage in single cell gel assays as genetic end points. However, presently most of these tests are poorly validated in the potential target tissue (gut, skin). Other studies (e.g. dominant lethal, germ cells) should be considered only in case of significant positive results in the above *in vivo* tests.

9.6 DEVELOPMENTAL TOXICITY/TERATOGENICITY

There are no standard studies on developmental toxicity/teratogenicity for H₂O₂ but in the absence of firm evidence of systemic toxicity it is presently not considered that this lack of standard studies constitutes a datagap.

Depending on the outcome of the subchronic study on the evidence of systemic effect the need of such information should be reconsidered.

9.7 OCCUPATIONAL EXPOSURE AND EPIDEMIOLOGY

Information available about measured occupational levels and industrial hygiene record studies on H₂O₂-exposed workers is limited. It seems that few hygiene problems are encountered.

10. EXISTING OCCUPATIONAL EXPOSURE LIMITS

The national regulations and guidelines on occupational exposure limits for H₂O₂ are listed in Table XXXVII. In general, the time-weighted average concentration over an 8-hour working day which should not be exceeded is 1 ppm (1.4 mg/m³); this level should not be higher than 2-3 ppm (2.8-4.2 mg/m³) for any short period of exposure (5-15 min).

Documentation supporting the existing occupational exposure limits is only available from the US American Conference of Governmental and Industrial Hygienists (ACGIH, 1986). The TLV-TWA of 1 ppm of H₂O₂ vapour is recommended to minimise the likelihood of irritation and bleaching of hair. The basis of their recommendation is the study by Oberst *et al*, 1954 which reported skin irritation and hair bleaching in dogs exposed to 7 ppm H₂O₂ (10 mg/m³).

Table XXXVII: Occupational Exposure Limit Values

Country	TWA (mg/m ³) ^a	STEL (mg/m ³) ^a	Ceiling value (mg/m ³) ^a	Reference
Austria	1.4	3 (5 min)	-	DFG, 1995
Belgium	1.4	-	-	ACGIH, 1995
Denmark	1.4	-	-	Arbejdstilsynet, 1988
Finland	1.4	4.2	-	ILO, 1991
France	1.5	-	-	INRS, 1993
Germany	1.4	3 (5 min)	-	DFG, 1995
Italy	1.4	-	-	ACGIH, 1995
Netherlands	1.4	-	-	Arbeidsinspectie, 1995
Norway	1.4	-	-	Arbejdstilsynet, 1995
Sweden, 1991	1.4	3	-	AFS, 1993
Switzerland	1.4	-	-	CNA, 1987
UK	1.4	3 (10 min)	-	UK HSE, 1995
USA				
- OSHA	1.4	-	-	OSHA, 1989b
- ACGIH	1.4	-	-	ACGIH, 1995

TWA Time-weighted average concentration (8-hour working period)

STEL Short-term exposure limit (15 minutes, unless specified otherwise)

a Official values; some countries use different conversion factors and/or other ambient temperature

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 75 ppm (106 mg/m³); this concentration is thought not to cause any impairment or irreversible health effects "immediately dangerous to life and health" (NIOSH, 1994).

11. SUMMARY EVALUATION AND RECOMMENDATION FOR A SCIENTIFICALLY-BASED OCCUPATIONAL EXPOSURE LIMIT

11.1 SUBSTANCE IDENTIFICATION

Common name:	Hydrogen peroxide (H ₂ O ₂)
CAS name:	Hydrogen peroxide
CAS registry No.	7722-84-1
EEC No.	008-003-00-9
EEC classification:	Solution with a concentration $\geq 60\%$: corrosive (C) and oxidising (O) 20% $\leq$ concentration < 60%: corrosive (C) 5% $\leq$ concentration < 20%: irritant (Xi) Solution with a concentration < 5% not classified
EEC labelling:	5% $\leq$ concentration < 20%: R36/38, S28-36/39-45, nota B 20% $\leq$ concentration < 60%: R34, S28-36/39-45, nota B Concentration $\geq 60\%$: R8-34, S28-36/39-45, nota B
EINECS name:	Hydrogen peroxide
EINECS No.	231-765-0
Formula:	H ₂ O ₂
Structure:	H—O—O—H
Molecular mass:	34.0

11.2 OCCURENCE AND USE

11.2.1 Chemical and Physical Properties

Hydrogen peroxide (H_2O_2) is normally handled as an aqueous solution, in concentrations ranging from dilute (< 5%) to > 90% by weight.

H_2O_2 and its aqueous solutions are clear, colourless, weakly acid liquids (pH 3.5-4.5; boiling point: 100-125 °C) with a low vapour pressure (70% H_2O_2 : 1,470 Pa at 30 °C) and a distinctive and mildly pungent odour (over a concentrated solution). The concentration of H_2O_2 (90%) at vapour saturation is 3,049 mg/m³ at 25 °C.

Pure H_2O_2 solutions are stable. Small quantities of stabilisers (up to 0.25%) are added to the 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 gas are generated.

Conversion factors for H_2O_2 concentrations in air, calculated at 20 °C and 1,013 hPa are:

$$1 \text{ mg/m}^3 = 0.707 \text{ ppm}$$

$$1 \text{ ppm} = 1.414 \text{ mg/m}^3$$

11.2.2 Occurrence and Use

H_2O_2 occurs naturally as a consequence of physiological (Section 11.3) and photochemical processes. In water and soil it may also be formed by the Weiss mechanism (oxidation of iron and copper ions). H_2O_2 occurs naturally in air as a result of photolysis of ozone. During net deposition H_2O_2 is efficiently removed from the atmosphere, though extended dry periods lead to its accumulation. H_2O_2 is also naturally 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 from minutes to several hours depending on the mineral content and the concentration of microorganisms.

World-wide more than 1,450 kt H_2O_2 (100% basis) per year is used, of which 580 kt/y in Western Europe. The production in Western Europe has increased over the past decade and amounted to 600 kt in 1993. In Western Europe there are approximately 30 production sites (CEFIC, 1994, 1995).

Industrial emissions arise from H_2O_2 production and during its main uses in the production of chemicals, for bleaching of cellulose pulp and textiles and for other purposes such as waste water treatment.

Minor quantities of H_2O_2 are used in applications (food processing, disinfection, drinking water treatment and hair bleaching) which result in direct contact with man.

11.2.3 Exposure Levels at the Workplace

The predominant route of occupational exposure to H_2O_2 is by inhalation and by direct contact with skin and mucous membranes.

In general, exposure levels during a 4 to 8-hour workshift were below 1.4 mg/m^3 . During specific operations, like drum and tank filling and stabilisation short-term (15 min to 1 h) levels were measured up to 4.7 mg/m^3 . The highest exposure concentrations (around 6 mg/m^3) occurred during stabiliser make-up (CEFIC, 1996).

Incidental exposures may be higher. For example, in an unmanned pump house at a production site H_2O_2 concentrations were 10 and 2 mg/m^3 before and after reparation of a leaking seal box (ECETOC, 1991).

Limited data are available on exposure during use. In the packaging industry, where H_2O_2 solutions are used for disinfection, $< 0.20 \text{ mg H}_2\text{O}_2/\text{m}^3$ (range $0.21\text{--}1.2 \text{ mg/m}^3$) was recorded during start-up of a coffee cream packaging machine (Suenaka *et al*, 1984). H_2O_2 levels near a fruit juice packaging machine were $0.2\text{--}0.66 \text{ mg/m}^3$ (Tetra Pak, 1991). Kaelin *et al* (1988) reported an incident of high H_2O_2 exposure near a milk packaging machine where the levels were $12\text{--}42 \text{ mg H}_2\text{O}_2/\text{m}^3$ before and $1.5\text{--}4.5 \text{ mg/m}^3$ after installation of ventilation.

11.2.4 Exposure Levels in the Environment

Environmental levels of H_2O_2 originating from industrial and domestic emissions are limited due to its rapid removal in air and decomposition in waste water.

Natural background concentrations of H_2O_2 found in the atmosphere vary with the intensity of solar radiation, humidity and temperature as well as the presence of precursors and scavengers of free radicals. The tropospheric lifetime of H_2O_2 is 10–20 hours.

During day-time H_2O_2 concentrations range from 0.4-4 $\mu\text{g}/\text{m}^3$, falling to below 0.01 $\mu\text{g}/\text{m}^3$ at night (Das *et al*, 1983). H_2O_2 in the atmosphere tends to concentrate into cloud droplets (where concentrations can reach 8,000 $\mu\text{g}/\text{l}$) and is removed by rain-out (Kelly *et al*, 1985). During fog and severe smog, atmospheric concentrations may rise to 250 $\mu\text{g}/\text{m}^3$ (Bufalini *et al*, 1972). Surface waters generally contain less than 100 $\mu\text{g}/\text{l}$, dropping to below 1 $\mu\text{g}/\text{l}$ at 100 meters depth. Levels up to 3 $\mu\text{g}/\text{l}$ are found in ground water (Holm *et al*, 1987).

Humans may be exposed to H_2O_2 via food. In certain vegetables (e.g. potato tubers) naturally occurring levels of up to 8,000 $\mu\text{g}/\text{kg}$ have been found (Warm and Laties, 1982).

Environmental exposure by inhalation is less than the levels of H_2O_2 normally found in exhaled air (300-1,000 $\mu\text{g}/\text{m}^3$) (Williams *et al*, 1982), environmental exposure becomes significant only in foggy conditions.

No exposure data are available on medical or cosmetic uses of H_2O_2 .

11.2.5 Measuring methods

There are several analytical procedures to measure H_2O_2 in air. The most common method suitable for workplace air is based on colorimetry a paper strip containing the reaction product of H_2O_2 with acidic titanium chloride or titanium oxalate. This method is sensitive down to 10 ppb (14 $\mu\text{g}/\text{m}^3$) (Interox, 1991; Pilz and Johann, 1974). More sensitive methods for ambient air allow detection of concentrations as low as 3 ppt (4 ng/m^3) (Lazrus *et al*, 1985, 1986; Ferm, 1988).

Sensitive analytical methods to determine H_2O_2 in water and biological media are also available (Section 6.2 and 6.3, Table V and VI).

11.3 HEALTH SIGNIFICANCE

H_2O_2 produced during normal aerobic cell metabolism involves a number of enzymatic reactions especially by superoxide dismutase.

H_2O_2 is converted 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, glutathione peroxidase activity is higher in the lungs of rats than in the lungs of baboons or hamsters. Lung catalase activity was higher in the baboon than in the hamster, and much higher than in the rat. Human lung antioxidant enzyme activity is more similar to that of the hamster (Bryan and Jenkinson, 1987).

In bacteria, catalase activity is induced by exposure to H_2O_2 (Chance *et al*, 1979; Ishida and Sasaki, 1981; Winquist *et al*, 1984). Such induction is less apparent in tissues of rats and mice (Matkovics and Novak, 1977; Kihlstrom *et al*, 1986).

In the absence of any enzymatic activity H_2O_2 can also be decomposed by transition metals (e.g. iron, copper) to highly reactive hydroxyl radicals (Halliwell and Gutteridge, 1984) capable of inducing various toxic effects (Roots and Okada, 1975; Birnboim, 1982; Aust and White, 1985; Troll and Wiesner, 1985; Kappus, 1987).

There is limited information concerning the absorption of H_2O_2 . The permeability of biological membranes to H_2O_2 is 0.04 - 0.2 cm/min, which is comparable to that of water, but less than that of oxygen (De Duve, 1965 as quoted in Chance *et al*, 1979; Dick, 1964; Nicholls, 1972).

Although significant amounts of topically applied H_2O_2 can penetrate the epidermis or mucous membranes, H_2O_2 undergoes rapid spontaneous or enzyme-catalysed decomposition to water and oxygen in the underlying tissue. This may lead to small gas emboli and reversible blanching of the exposed tissue area (Hauschild *et al*, 1958). Formation of larger volumes of gaseous oxygen can lead to detachment of cell layers and the rupture of tissues and organs (Sheehan and Brynjolfson, 1960; Ludewig, 1965; Urschel, 1967). Locally formed oxygen is removed by the blood. However, the increase in oxygen content in the blood results in a hyperbaric response. There is no evidence that H_2O_2 itself is absorbed in amounts sufficient to produce systemic effects (Dieter, 1988).

The major adverse effect of H_2O_2 are related to its irritating properties.

Concerning the skin, H_2O_2 solutions of less than 35% applied to the rabbit skin are not considered, according to EU classifications, as irritant (Aguinaldo *et al*, 1992). But solutions of 50% and higher are considered corrosive (Aguinaldo *et al*, 1992; Sarver, 1994).

The gastrointestinal tract is clearly more sensitive to H_2O_2 . In dogs, oedema, followed by destruction and sloughing of the cornified epithelial layer of the gingiva, was observed after continuous drip with a 1% H_2O_2 solution (Martin *et al*, 1968; Dorman and Bishop, 1970), whilst in human beings mouth washes with

up to 3% neutralised H_2O_2 did not cause mucosal irritation when used several times per day (Herrin *et al*, 1987; Rees and Orth, 1986; US FDA, 1983). Therapeutical/clinical use of 1-3% solutions in contact with the intestinal mucosa induced ulcerative colitis and inflammation (Pumphrey, 1951; Ludington *et al*, 1958; Sheehan and Brynjolfsson, 1960). In mice, long-term exposure to 0.1% H_2O_2 induced inflammation of the gastrointestinal tract (Aoki and Tani, 1972).

In contact with the eye, H_2O_2 solutions of 10% or more cause irreversible damage to the rabbit eye, including blindness (Weiner *et al*, 1990). Solutions less than 5% are considered, according to EEC classification criteria, as not irritant to rabbit eye (Du Pont, 1972b,c, 1973b). The first effects on the rabbit cornea are observed with 1% solutions (Koster, 1921 as quoted in Grant, 1986). In human beings, 1-3% solutions have been used for eye treatment without causing injury (Grant, 1986). However, solutions containing more than 200 mg/l cause hyperaemia and down to 100 mg/l pain and stinging (Paugh *et al*, 1988).

Effects of exposure to H_2O_2 by inhalation are limited to irritating effects of the respiratory tract and the eyes. Exposure to H_2O_2 vapour (338-427 mg/m^3) induces only signs of irritation in rodents (Comstock *et al*, 1954; Oberst *et al*, 1954). In mice a 50% reduction of the respiratory rate (RD_{50}) is observed at 665 $\text{mg H}_2\text{O}_2/\text{m}^3$ after 30 minutes of exposure (Janssen, 1995b). In rats, the 4-hour LOEC for respiratory mucosa was 60 $\text{mg H}_2\text{O}_2/\text{m}^3$ (Kondrashov, 1977). In human beings exposed for 4 hours the irritation threshold for the respiratory tract was 10 $\text{mg H}_2\text{O}_2/\text{m}^3$ and for the skin 20 mg/m^3 (Kondrashov, 1977). At these concentrations eye and throat irritation as well as gradual bleaching of hair have been reported (Kaelin *et al*, 1988). After prolonged exposure, in rats and dogs respiratory irritation and transient skin thickening have been observed at 1-10 $\text{mg H}_2\text{O}_2/\text{m}^3$ (Comstock *et al*, 1954; Oberst *et al*, 1954; Kondrashov, 1977).

The acute oral toxicity of H_2O_2 is moderate. The acute oral toxicity of H_2O_2 in experimental animals depends on the concentration of its solution; the lethal dose in rats varies from 800 to 2,000 mg/kgbw (Ito *et al*, 1976; FMC, 1983a, 1986, 1989a; Mitsubishi, 1981; Du Pont, 1996; Liarskii *et al*, 1983). In humans, death has been reported to result from the accidental ingestion of unknown quantities of 30-40% solutions (Giusti, 1973; Zecevic and Gasperec, 1979; Thompson, 1989). Toxic effects observed generally related to the corrosive action and the rapid generation of large volumes of oxygen in the gastrointestinal tract. There was complete recovery within 2-3 weeks even in near fatal cases (Budagovskija *et al*, 1971; Humberston *et al*, 1990).

By inhalation, brief exposure (5-15 minutes) to aerosols > 9,400 $\text{mg H}_2\text{O}_2/\text{m}^3$ (Punte *et al*, 1953) and 2 hour exposure to approximately 900 $\text{mg H}_2\text{O}_2/\text{m}^3$ was lethal to mice (Janssen, 1995a). After repeated exposure, mortality in mice was observed at 80 $\text{mg H}_2\text{O}_2/\text{m}^3$ (Comstock *et al*, 1954; Oberst *et al*, 1954).

Concerning effects of H_2O_2 after prolonged exposure, more detailed studies have been performed by administration in drinking water. Exposure to H_2O_2 above 1% in drinking water was lethal to mice and rats within weeks (Shapiro *et al*, 1960; Aoki and Tani, 1972). After prolonged exposure, decreased bodyweight gain was observed in mice with concentrations of 0.4% and in rats with 0.25% and above (Romanovski *et al*, 1960; Aoki and Tani, 1972) (other studies observe first effects at higher levels). In mice long-term exposure to 0.1% induced inflammation of the gastrointestinal tract (Ito *et al*, 1981a, b). 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 (Aoki and Tani, 1972).

The carcinogenic effects of H_2O_2 were studied in rats and mice. H_2O_2 induced an increase of duodenal tumours in mice chronically 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 (Ito *et al*, 1981a, b). Rats exposed to near lethal concentrations in their drinking water developed forestomach papilloma but no tumours in the glandular stomach and duodenum (Ishikawa and Takayama, 1984). The absence of tumours in these organs correlates well with the high catalase levels found and demonstrates the protective role of this enzyme (Ito *et al*, 1981a, b).

Chronic H_2O_2 exposure, at concentrations which induce significant inflammation of the exposed tissue, has been shown to give a weak tumour promoting effect. Repeated topical application of 15% H_2O_2 to Sencar mice did not induce skin tumours, demonstrating the absence of initiating properties (Klein-Szanto and Slaga, 1982).

Concerning mutagenicity, in *in vitro* tests, without metabolic activation H_2O_2 induced 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. However, addition of exogenous metabolic activation or catalase reduced or abolished the genotoxic response (references in Section 7.2.4, Table XVIII to XXVI).

In *in vivo* tests, no chromosomal aberrations or micronuclei were observed in the bone marrow of rats and mice after oral or i.p. administration. In contrast, in a host-mediated assay with mice gene mutations in bacteria and chromosomal aberrations in tumour cells were observed (Schöneich, 1967; Kawasaki *et al*, 1969; Keck *et al*, 1980; Ross, 1995; Molinier, 1995).

Reproduction toxicity data are limited. Spermatozoa in mice and rabbits were unaffected and male mice were fertile after treatment with 1-3% H₂O₂ in drinking water (Wales *et al*, 1959). Data on the teratogenic potential of H₂O₂ are limited and do not allow a complete evaluation (Hankin, 1958; Moriyama *et al*, 1982).

Occupational exposure to H₂O₂ aerosol concentrations of 12 to 41 mg/m³ resulted in skin, eye and throat irritation (Kaelin *et al*, 1988).

The irritant threshold to the lungs of human volunteers was considered to be 10 mg/m³ with a NOEL of 5 mg/m³ for exposure periods up to 4 hours (Kondrashov, 1977).

Slight nasal irritation was reported in factory workers at short-term exposure around 3.5 mg/m³ (CEFIC, 1996).

Pulmonary function monitoring showed no evidence of adverse effects from occupational exposure during several years at levels ranging from not detectable to 0.79 mg/m³ (8-h TWA) (CEFIC, 1996).

Gradual bleaching of hair is a common occurrence among factory workers and has been reported from exposure levels of 0.7-1.4 mg/m³ vapour (FMC, 1990), but it is uncertain whether bleaching was due to vapour exposure or to transfer of H₂O₂ from the hands to hair.

Dermal exposure to dilute solutions has been reported to result in transient symptoms without permanent effects, such as paresthesias, whiteness and blistering (Dickson and Caravati, 1994).

Ingestion is the most common route of accidental exposure, although the incidence is very low (0.34% of all accidental exposures in one survey) (Dickson and Caravati, 1994). Young children are particularly vulnerable.

Accidental exposure to H₂O₂ has occurred from its use in household topical disinfectants and cosmetic/personal care products generally involving exposure to the 3% solution. Accidental ocular exposure cases are generally a small percent (8.0% of all accidental exposures in one survey) of all accidental exposure to H₂O₂. Symptoms of burning, redness and blurry vision have been reported. In the eye, H₂O₂ solutions of > 200 mg/l caused hyperaemia; pain and stinging occurred with concentrations of ≥ 100 mg/l (Paugh *et al*, 1988; Dickson and Caravati, 1994).

The incidence of adverse reactions reported to dentifrices containing 0.75% H_2O_2 is extremely low (one in 100,000 units sold) and does not differ from the incidence for similar products without H_2O_2 as an ingredient (CTFA, 1994).

11.4 FINAL EVALUATION AND RECOMMENDATION

11.4.1 Hazard Identification

The main health hazard of H_2O_2 is related to its irritating properties.

In human volunteers the irritation threshold for the respiratory tract was 10 mg $\text{H}_2\text{O}_2/\text{m}^3$ and for the skin 20 mg $\text{H}_2\text{O}_2/\text{m}^3$. At concentrations in the range of 12 to 41 mg/ m^3 eye and throat irritation have been reported. Slight nasal irritation was reported during short-term exposure to H_2O_2 concentrations around 3.5 mg/ m^3 . In rats and dogs respiratory irritation and transient skin thickening have been observed at 1-10 mg $\text{H}_2\text{O}_2/\text{m}^3$ after prolonged exposure. In human beings, under conditions of occupational exposure, when respecting a limit value of 1.4 mg $\text{H}_2\text{O}_2/\text{m}^3$, no complaints of irritation have been reported.

Bleaching of hair has been known to occur with levels of 0.7-1.4 mg/ m^3 H_2O_2 , but it is uncertain whether bleaching was due to vapour exposure or to transfer of liquid H_2O_2 from the hands to the hair. Under controlled vapour exposure in humans or animals hair bleaching was seen from approximately 10 mg/ m^3 .

Any inflammatory response to H_2O_2 is related to the extent that enzymes (catalase, peroxidase) are able to convert H_2O_2 into water and oxygen. Saturation of the enzyme systems may lead to H_2O_2 decomposition by transition metals (e.g. iron, copper) to highly reactive hydroxyl radicals, which induce an inflammatory response.

No carcinogenicity studies by inhalation or epidemiological studies on H_2O_2 are available. When administered by drinking water H_2O_2 induced an increase of duodenal tumours in mice. The tumour incidence correlated with a specific inflammatory response (irritation) in this tissue.

The genotoxicity of H_2O_2 has been extensively studied. In *in vitro* tests without metabolic activation H_2O_2 induced gene mutations, primary DNA damage, chromosome aberrations and micronuclei in mammalian cells, as well as morphological cell transformations. As in the case of inflammation, an exogenous metabolic fraction of enzymes, like catalase, reduced or abolished the genotoxic response.

In vivo, 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. These results further illustrate the importance of the metabolic systems in converting H_2O_2 into water and oxygen.

In the absence of any evidence for a genotoxic potential of H_2O_2 *in vivo*, it is concluded that the irritating properties of H_2O_2 should form the basis for establishing an OEL.

11.4.2 Risk Assessment

The threshold for respiratory, eye or throat irritation is situated around 10 mg/m^3 and no such irritation is expected within the normal variation of exposure levels when respecting an 8-hour TWA OEL of 1.4 mg/m^3 . Moreover, no adverse systemic health effects have been reported when respecting this exposure limit. A STEL of 3 mg/m^3 could be considered to avoid nasal irritation during short-time exposure up to 15 minutes.

In setting any exposure limit for H_2O_2 it should be considered that humans may exhale up to 1 mg/m^3 H_2O_2 resulting from endogenous formation. Furthermore, it should be realised that a small portion of the population may be deficient in H_2O_2 metabolising enzyme systems (acatalasemia or glucose-6-phosphate dehydrogenase (G6PD) deficiency).

Although significant amounts of topically applied H_2O_2 can penetrate the epidermis or mucous membranes, this is followed by rapid spontaneous or enzyme-catalysed decomposition to oxygen and water in the underlying tissue. Following the "decision tree" proposed by ECETOC (1993b), no skin notation is necessary.

11.4.3 Recommendations

An occupational exposure limit value (OEL) of 1.4 mg/m^3 (1 ppm) as 8-hour TWA concentration is recommended for H_2O_2 . This is also the lowest OEL in EU member states.

Short-term exposure should not exceed 3 mg/m^3 (2 ppm) for 15 minutes (STEL). In the EU the same value is applied in Germany (5 minutes), in the UK (10 minutes) and Norway, Sweden and Finland (15 minutes).

As H_2O_2 is both produced and rapidly converted to oxygen and water by the human body no method for biological monitoring reflecting exogenous exposure can be developed.

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- Eka Nobel (1986c). Product information, hydrogen peroxide 50%. Eka Nobel, Surte, S.
- Eka Nobel (1988a). Varuinformation, vaeteperoxid 70%. Eka Nobel, Surte, S.

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12.3 DATABASES CONSULTED

This report has been based on an earlier literature collection (ECETOC, 1993a) and a EUCLID data sheet (Degussa, 1994).

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