6.3 TERRESTRIAL PLANTS

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

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

6.4 SOIL ORGANISMS

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

6.5 WILDLIFE

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

6.6 ECOSYSTEMS

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

6.7 EVALUATION

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

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

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

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

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

SECTION 7. KINETICS AND METABOLISM

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

7.1 ABSORPTION

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

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

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

7.1.1 Mechanism of Absorption

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

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

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

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

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

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

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

7.2 ENDOGENOUS FORMATION

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

7.3 METABOLISM (Figure 3)

7.3.1 Enzymatic Metabolism

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

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

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

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

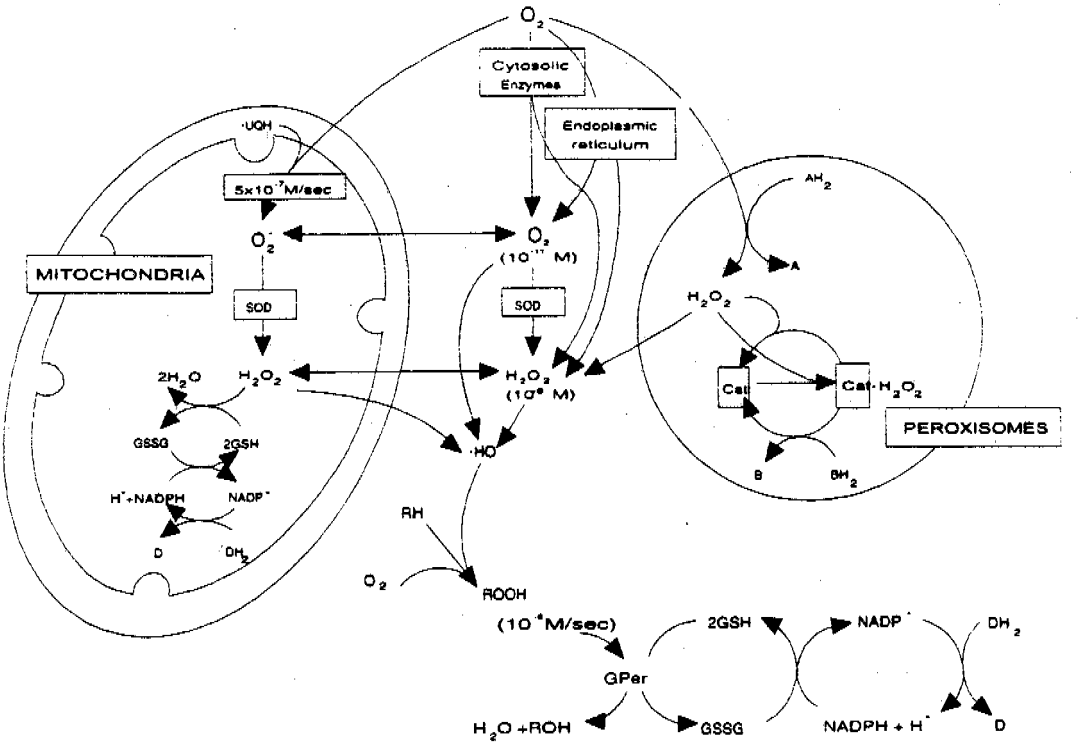
In mammalian tissue, the highest catalase activity is observed in the duodenum, liver, spleen, kidney, blood, mucous membranes and other highly vascularised tissues; the lowest catalase activity occurs in the brain, thyroid, testes and connective tissues (e.g. Table 20, controls) (Matkovics and Novak,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

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

FIGURE 3

METABOLISM OF HYDROGEN PEROXIDE

(Chance *et al*, 1979)



Concentrations and formation rates of oxygen metabolites are estimated.

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

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

TABLE 16

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

(Ito *et al*, 1984)

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

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

TABLE 17

GASTRO-INTESTINAL CATALASE LEVELS IN VARIOUS SPECIES

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

Number of samples used to calculate the mean not stated.

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

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

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

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

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

Peroxidases. Peroxidases decompose H_2O_2 through the reaction:

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

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

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

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

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

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

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

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

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

Distribution in the body of enzymes that form and eliminate H_2O_2

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

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

7.3.2 Hydrogen Peroxide Intake and Enzyme Activity

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

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

TABLE 18

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

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

* Not detectable.

TABLE 19

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

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

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

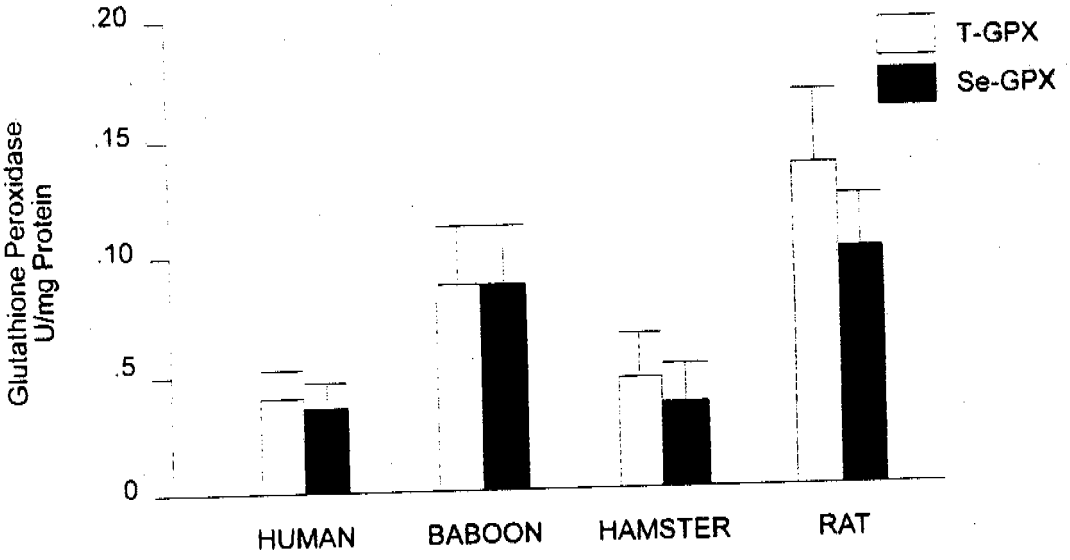
b Significantly different from other species, $P < 0.05$.

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

FIGURE 4

LUNG GLUTATHIONE PEROXIDASE (GSH-Px) ACTIVITY

(Bryan and Jenkinson, 1987)



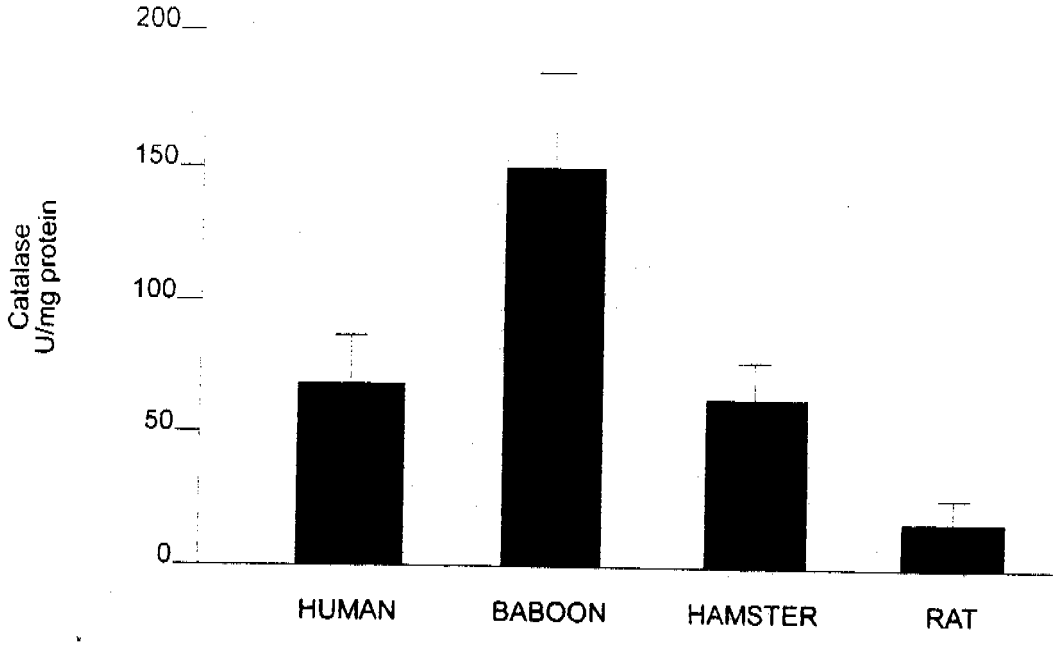
Units are µmol of reduced nicotinamide adenine dinucleotide phosphate oxidised /min/mg protein. Values are means ± SD for 6 animals/group and 5 humans/group. T-GPx is total GSH-Px activity and was measured using cumene hydroperoxide as substrate. Se-GPx is selenium-dependent GSPx acivity and was measured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

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

FIGURE 5

CATALASE (CAT) ACTIVITY IN LUNG

(Bryan and Jenkinson, 1987)



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

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

7.3.3 Non-enzymatic Metabolism

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

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

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

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

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

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

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

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

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

7.4 TISSUE AND BODY FLUID LEVELS

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

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

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

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

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

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

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

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

7.5 SUMMARY

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

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

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

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

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

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

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

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

8.1 ACUTE TOXICITY

8.1.1 Oral (Table 21)

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

TABLE 21

ACUTE ORAL TOXICITY

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

8.1.2 Dermal (Table 22)

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

TABLE 22

ACUTE DERMAL TOXICITY

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

8.1.3 Inhalation (Table 23)

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

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

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

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

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

8.1.4 Evaluation

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

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

TABLE 23

ACUTE INHALATION TOXICITY

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

* Combined inhalation exposure and skin application.

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

8.2.1 Skin Irritation (Table 24)

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

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

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

TABLE 24

SKIN IRRITATION CLASSIFICATION IN RABBITS

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

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

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

c Scores indicated are for intact skin sites.

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

8.2.2 Mucous Membrane Irritation

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

8.2.3 Respiratory Tract Irritation

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

8.2.4 Eye Irritation (Table 25)

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

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

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

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

TABLE 25

EYE IRRITATION CLASSIFICATION

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

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

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

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

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

8.2.5 Sensitisation

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

8.2.6 Evaluation

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

8.3 SUBCHRONIC TOXICITY

8.3.1 Oral

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

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

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

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

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

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

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

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

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

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

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

8.3.2 Dermal

No information is available.

8.3.3 Inhalation (Table 26)

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

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

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

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

TABLE 26

SUBCHRONIC INHALATION STUDIES

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

Oberst *et al*, 1954).

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

8.3.4 Evaluation

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

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

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

8.4 CHRONIC TOXICITY AND CARCINOGENICITY

8.4.1 Chronic Toxicity (Table 27)

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

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

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

TABLE 27

CHRONIC TOXICITY STUDIES

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

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

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

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

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

8.4.2 Carcinogenicity

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

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

TABLE 28

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

(after Ito *et al*, 1982)

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

a Explanation in text, section 8.4.1

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

TABLE 29

DESIGN OF CARCINOGENICITY STUDIES

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

a Fresh dosing solution was prepared daily.

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

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

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

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

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

TABLE 30

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

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

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

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

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

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

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

TABLE 31

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

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

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

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

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

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

TABLE 33

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

(Ito *et al*, 1984)

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

C3H/HeN, high catalase activity; B6C3F1, F_1 hybrid of C3H and C57BL, with "normal" catalase activity; C57BL/6N, low catalase activity; $C3H/C^s_b$, mice with hypocatalasemia.

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

TABLE 32

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

(Ito *et al*, 1982)

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

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

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

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

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

8.4.3 Initiation-Promotion of Hydrogen Peroxide

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

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

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

TABLE 34

TUMOUR INITIATION-PROMOTION STUDIES[a]

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

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

a Animals treated topically; control animals treated with acetone.

b DMBA administered once, topically, in acetone.

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

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

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

f 0.2ml H_2O_2 administered once.

g TPA administered 2x/week.

h H_2O_2 administered in drinking water.

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

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

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

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

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

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

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

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

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

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

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

TABLE 35

EFFECT ON GASTRO-DUODENAL CARCINOGENESIS INDUCED BY MNNG

(Takahashi *et al*, 1986)

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

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

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

8.4.4 Evaluation

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

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

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

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

8.5 GENOTOXICITY

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

8.5.1 Gene Mutations

Gene mutations in bacteria and yeast

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

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

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

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

TABLE 36

GENE MUTATIONS IN *SALMONELLA TYPHIMURIUM* (AMES TEST)

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

TABLE 36 (cntd.)

GENE MUTATIONS IN *SALMONELLA TYPHIMURIUM* (AMES TEST)

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

TABLE 37

GENE MUTATIONS IN OTHER BACTERIA OR YEAST

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

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

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

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

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

Gene mutations in cultured mammalian cells (Table 39)

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

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

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

Gene mutations in *Drosophila melanogaster*

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

TABLE 38

THE OVERALL RESPONSE TO H_2O_2 IN BACTERIAL TESTS

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

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

TABLE 39

GENE MUTATIONS IN CULTURED MAMMALIAN CELLS

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