Cumene Hydroperoxide
[80-15-9]

Review of Toxicological Literature

Prepared for

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EXECUTIVE SUMMARY

Cumene hydroperoxide was identified by the National Institute of Environmental Health Sciences as a possible candidate for toxicity and carcinogenicity testing.

Cumene hydroperoxide is produced at ~130°C via the oxidation of cumene with air in the presence of aqueous sodium carbonate as a catalyst and is analyzed by gas chromatography/mass spectrometry. Thirteen companies have been identified as producers of cumene hydroperoxide in the United States. In 1985, ~1.1 million pounds (514,000 kg) were produced in the United States. About 7 billion pounds of cumene hydroperoxide are consumed yearly in the United States, indicating that the majority of cumene hydroperoxide used is imported. No current data on import volumes were located. Over 95% of the cumene hydroperoxide produced in this country is used in the production of acetone and phenol. Additional applications include use as a catalyst, a curing agent, and as an initiator for polymerization.

Cumene hydroperoxide may enter the environment from industrial discharges and spills, and also as a by-product of fuel oil slicks exposed to ultraviolet (UV) light. It has been found in rivers and wastewaters in Illinois, Delaware, and Pennsylvania at levels as high as 0.25 ppm. Cumene can react with alkylperoxy radicals and oxygen in natural waters to form cumene hydroperoxide which is readily decomposed by UV light.

Human exposure to cumene hydroperoxide can occur in the workplace through contact with emissions produced during its use in acetone and phenol production. Only two cases of human workplace exposure were found in the literature; neither case positively linked cumene hydroperoxide to any adverse effects. No data were found regarding exposure to cumene hydroperoxide in the general public or the toxicity of cumene hydroperoxide in humans.

The Occupational Safety and Health Administration (OSHA) has not set workplace exposure limits for cumene hydroperoxide; however, the U.S. Department of Transportation (DOT) has classified it as a hazardous material subject to the packaging, storage, and transportation regulations in 49 CFR Parts 171 through 177.

No data on the chemical disposition and toxicokinetics of cumene hydroperoxide were found. Cumene hydroperoxide is reduced by glutathione (GSH) to cumenol.

The oral LD_{50} is approximately 382 mg/kg (2.51 mmol/kg) in rats and 400-800 mg/kg (2.63-5.26 mmol/kg) in mice. Symptoms of acute exposure in mice and rats include sluggishness, unsteadiness, hemorrhages of the lungs, congested kidneys, and blood-filled bladders. Animals exposed dermally exhibited toxic symptoms including skin necrosis, anesthesia, dark eyes, and lethargy. Death occurred anywhere from 1 to 24 hours after exposure. Guinea pigs experienced moderate to strong skin irritation when exposed to cumene hydroperoxide dermally (dose not provided) for 24 hours.

In rats, the short-term and subchronic effects of exposure included decreased body weight, respiratory difficulty, inflammation of the nasal turbinates, tremors, and coarse fur. Deaths occurred after intraperitoneal (i.p.) and oral exposure to 19 mg/kg (0.12 mmol/kg) 3 times/week for seven weeks. No data on the chronic effects of cumene hydroperoxide were found.

Cumene hydroperoxide can cause cytotoxic effects including intracellular oxidative stress and cell necrosis at doses as low as 1 μM. It initially prevented the growth of *Neuorspora crassa*; however, with increasing exposure duration, cells proceeded to grow at a subnormal, but steady rate. At concentrations in excess of 0.5 mM (76 μg/mL), cumene hydroperoxide was cytotoxic to human adenocarcinoma (A549) cells. When isolated rat hepatocytes were exposed to cumene hydroperoxide (50-200 μM), enhanced lipid peroxidation, decreased glucose-6-phosphatase activity, decreased cytochrome P450 content, and stimulation of aminopyrene demethylation were seen. Cumene hydroperoxide was also shown to inhibit lipoprotein secretion in the Golgi apparatus of rat hepatocytes.

Few data were found on the reproductive and teratological effects of cumene hydroperoxide. In one study, the presence of cumene hydroperoxide accelerated the activation of cytochrome $P450_{scc}$ and inhibited NADPH-dependent lipid peroxidation. In addition, cumene hydroperoxide was shown to inhibit progesterone biosyntheses which may lead to preeclampsia (development of hypertension during pregnancy).

In an investigation of its carcinogenicity, one subcutaneous (s.c.) sarcoma in addition to 11 malignant lymphomas were observed in 50 mice administered cumene hydroperoxide s.c. (50 μM; 7.6 μg/mL) (treatment duration not provided, control mice not included in the study), and one fibrosarcoma versus none in the controls was present in 30 mice treated s.c. with 3.3 mg (0.021 mmol) once a week for 76 weeks. In another mouse study, dermal application 3 times weekly (treatment duration not provided) of 1% cumene hydroperoxide did not induce papillomas or carcinomas. In rats, s.c. injection of 100 mg (0.657 mmol) induced no sarcomas. Cumene hydroperoxide is hypothesized to be an active promoter in the initiation-promotion mouse epidermis model due to its ability to generate free radicals.

Cumene hydroperoxide is genotoxic, inducing DNA damage and mutations in prokaryote and eukaryote systems. DNA single strand breaks (SSBs) were induced in isolated rat hepatic nuclei but single or double strand breaks were not induced in the DNA of lysed human adenocarcinoma (A549) cells. Cumene hydroperoxide enhanced asbestos-induced damage in calf thymus DNA.

Cumene hydroperoxide was usually mutagenic in *Salmonella typhimurium* strains TA97a, TA97, TA98, TA100, TA102, TA104, BA9, BA13, and strains of the TA7000 series, but usually not mutagenic in strains TA1535 and TA1537. It was mutagenic in *Escherichia coli* strain WP2 (pkM101) and WP2 uvrA (pkM101), and

positive for SOS induction at the *sox* and *soxRS* loci of ZA201, ZA480 (*mutM*), and ZA700 (*soxRS*) *E. coli* strains. Cumene hydroperoxide has been shown to increase the mutagenicity of other mutagens in *S. typhimurium* strain TA98.

In the yeast *Saccharomyces cerevisiae*, cumene hydroperoxide increased the frequency of intrachromosomal recombination in a dose-dependent manner.

In the single *in vivo* rodent study located, cumene hydroperoxide administered i.p. to male mice was negative in the dominant lethal assay for the single week of matings performed.

In the single immunotoxicity study located, cumene hydroperoxide depressed concanavalin A stimulation of splenocytes from Fischer 344 rats, but without affecting their viability.

The mechanism of free radical production from cumene hydroperoxide involves the homolytic cleavage of the peroxide O O bond by P450 to produce the cumoxyl radical. Trapping of free radicals has been shown to inhibit the cytotoxic effects of cumene hydroperoxide. Oxygen radicals may attack DNA at either the sugar or the base possibly leading to sugar fragmentation, base loss, and strand breaks.

Analysis of structure-activity relationships suggest that within the class of peroxides, alkyl hydroperoxides, such as cumene hydroperoxide, are of moderate to high concern in terms of potential carcinogenicity because they are more stable than other peroxides and have a better chance of remaining active.

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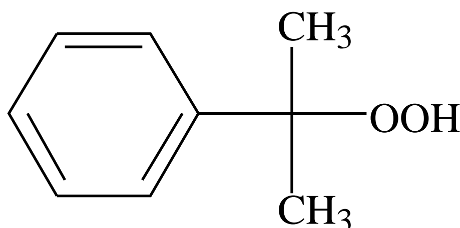
1.0 BASIS FOR NOMINATION

Cumene hydroperoxide was identified by the National Institute of Environmental Health Sciences (NIEHS) as a possible candidate for toxicity and carcinogenicity testing.

2.0 INTRODUCTION

Cumene Hydroperoxide

[80-15-9]



2.1 Chemical Identification

Cumene hydroperoxide ($C_9H_{12}O_2$; mol. wt. = 152.19) is also called:

alpha,alpha-Dimethylbenzylhydroperoxide
alpha-Cumene hydroperoxide
alpha-Cumyl hydroperoxide
Cumenyl hydroperoxide
Cumyl hydroperoxide
Hyperiz
Isopropylbenzene hydroperoxide
Kayacumene H
1-Methyl-1-phenylethylhydroperoxide
2-Phenyl-2-propyl hydroperoxide
Percumyl h
R 239A
Trigonox K 80
Trigonox K 239R
(HSDB, 1997; Chemfinder, 1998)

2.2 Physical-Chemical Properties

Property	Information	Reference
Physical state	colorless to pale yellow liquid	Lewis (1993)
Odor	sharp, aromatic	Radian Corporation (1991)
pH	~ 4	Radian Corporation (1991)
Melting point (°C)	< -40	Radian Corporation (1991)
Boiling point (°C)	100-101@ 8 mm Hg	Radian Corporation (1991)
	Decomposes at 127	HSDB (1997)
Freezing point (°C)	-9	Radian Corporation (1991)
Density	1.024 g/mL @ 20 °C, 1.03 g/mL @ 25 °C	Radian Corporation (1991)
Vapor pressure	0.24 mm Hg @ 20 °C	HSDB (1997)
Specific gravity	1.05 units	HSDB (1997)
% Volatile (by volume)	100%	Radian Corporation (1991)
Flash point	175 °C	HSDB (1997)
Flammability	0.9-6.5%	HSDB (1997)
Evaporation rate (butyl acetate=1)	0.1	Radian Corporation (1991)
Heat of combustion	-7400 cal/g	HSDB (1997)
Heat of decomposition	-475 cal/g	HSDB (1997)
Liquid surface tension	25 dynes/cm @ 25 °C	HSDB (1997)
Liquid/water interfacial tension	30 dynes/cm @ 25 °C	HSDB (1997)
Refractive index	1.5210 @ 20 °C	Aldrich (1996-1997)
Solubility (mg/mL @18°C)	water: <0.1 95% ethanol: $\geq$ 100 acetone: $\geq$ 100 dimethyl sulfoxide (DMSO): decomposes	Radian Corporation (1991)
Other solubilities	water (also given as): 1.39×10^4 mg/L @ 25 °C alcohol: soluble esters: soluble hydrocarbons (HCs): soluble chlorinated HCs: soluble	HSDB (1997) Lewis (1993)
Corrosion:	Reactive with metal-containing materials	HSDB (1997)

Cumene hydroperoxide reacts violently with reducing agents (e.g., sulfur dioxide), certain oxidizing agents (such as chlorine and ferric chloride), copper, lead, and copper or lead alloys, cobalt, and mineral acids (HSDB, 1997). Cumene hydroperoxide decomposes rapidly at temperatures above 150°C (HSDB, 1997), yielding acrid smoke and fumes (Lewis, 1993). At temperatures exceeding 125°C, the principal hazard is phenol formation (HSDB, 1997). Other potential decomposition products are 2-phenyl, 2-hydroxypropane, and acetophenone (HSDB, 1997). While the flammable limits of vapor-air mixture, and the ignition temperature are not known, all organic peroxides are highly flammable and have the potential to be explosive.

2.3 Commercial Availability

Thirteen companies have been identified as current producers of cumene hydroperoxide in the United States: Aristech Chemical Corp. (Haverhill, OH); Allied Chemical Corporation (Philadelphia, PA); Hercules Incorporated (Gibbstown, NJ); Henkel, Inc. (Teaneck, NJ); Chevron U.S.A., Inc. (Richmond, CA); U.S. Steel Corp. (Ironton, OH); Thorson Chemical Corporation (New York, NY); SPS Technologies, Inc. (Montgomery, PA); Sumitomo Chemical America, Inc. (New York, NY); Montedison USA (New York, NY); Getty Refining & Marketing (El Dorado, KS); Reichhold Chemicals, Inc. (Pensacola, FL); and Clark Oil & Refining Corp. (Blue Island, IL) (TSCAPP database, 1998; HSDB, 1997). In 1988, Aristech Chemical Corp. opened a cumene hydroperoxide plant with a production capacity of 25 million pounds per year (CPI, 1988). Cumene hydroperoxide is commercially available with a technical purity of 80 to 95 percent. Impurities include cumene (9.6-16.8%), dimethyl phenylcarbinol (2.9-4.6%), and acetophenone (0.3-0.8%) (HSDB, 1997).

3.0 PRODUCTION PROCESSES AND ANALYSES

Cumene hydroperoxide is produced at approximately 130°C via the oxidation of cumene with air in the presence of aqueous sodium carbonate as a catalyst (Lewis, 1993; CEH, 1998). Cumene hydroperoxide is collected on activated charcoal, desorbed with trichlorofluoromethane, and analyzed by gas chromatography/mass spectrometry (GC/MS) (Cocheo et al., 1983). It is a captive intermediate in the production of acetone and phenol (Lewis, 1993).

4.0 PRODUCTION AND IMPORT VOLUMES

In 1985, about 1.1 million pounds (514,000 kilograms) of cumene hydroperoxide were produced in the United States (CEH, 1998). However, approximately 7 billion pounds are consumed yearly in the United States for phenol production (CEH, 1998) indicating that the majority of cumene hydroperoxide used is imported. Over 95% of the cumene hydroperoxide produced in the United States is from the oxidation of cumene. The cumene hydroperoxide

formed is then cleaved to form acetone and phenol (HSDB, 1997; Grayson, 1985). No recent data were located on production or import volumes for cumene hydroperoxide.

5.0 USES

Cumene hydroperoxide is used primarily in the production of acetone and phenol (Lewis, 1993). Several copper and iron salts are effective catalysts for the conversion of cumene hydroperoxide into phenol. Under mild conditions (0.1 mmol $FeCl_3$ in 5 mL of acetone at room temperature), 1 mmol of cumene hydroperoxide is converted into phenol with 100% yield in less than 5 minutes (Barton and Delanghe, 1997). Cumene hydroperoxide is also used as a catalyst for rapid polymerization, especially in redox systems, as a curing agent for unsaturated polyester resins, an initiator for polymerization of styrene and acrylic monomer, and a chemical intermediate for the cross-linking agent, dicumyl peroxide (Lewis, 1993; CEH, 1998).

6.0 ENVIRONMENTAL OCCURRENCE AND PERSISTENCE

Cumene hydroperoxide may enter the environment via industrial discharges and spills, and also as a by-product of fuel oil slicks exposed to ultraviolet (UV) light (Callen and Larson, 1978;Victorin and Stahlberg, 1988). A measurement of total oxidants in rivers and wastewaters in Illinois, Delaware, and Pennsylvania found cumene hydroperoxide at levels as high as 0.25 ppm (Larson et al., 1981). Small quantities of cumene hydroperoxide might also be formed *in situ* in aquatic systems by the reaction of cumene with alkylperoxy radicals and oxygen (Mill et al., 1980).

Hydroperoxides, such as cumene hydroperoxide, are decomposed abiotically via redox reactions utilizing multivalent metal ions, hydrolysis (acid catalyzed), attack by free radicals, and photodissociation (Grayson, 1985). Hydroperoxides are sensitive to multivalent metal ions to the extent that trace exposures could result in explosive reactions (Grayson, 1985). Cumene hydroperoxide is readily decomposed by UV light (113 nm) (Graedel, 1979).

With regard to environmental toxicity, acute toxic effects (e.g., the death of animals and plants; inhibited plant growth) generally appear 2-4 days after wildlife or plants come into

contact with this substance. Although cumene hydroperoxide has moderate acute effects on aquatic life forms, there is insufficient evidence to adequately assess the short-term effects of its environmental release (EPA, 1988). Long-term effects of the release of this substance into the environment may include shortened life span, reproductive and fertility problems, and behavioral effects (EPA, 1988).

Although there is insufficient data to predict the extent of environmental persistence, data suggest that cumene hydroperoxide will be moderately persistent in water, with an estimated half-life of 20-200 days (EPA, 1988).

7.0 HUMAN EXPOSURE

Exposure to cumene hydroperoxide can occur in occupational settings from contact with emissions produced during its use in phenol production (Grayson, 1985; Lewis, 1993). Exposure can also occur during its transport, storage, or use as a polymerization catalyst.

Only two cases of human exposure to cumene hydroperoxide were found in the literature. In one case, trace amounts of cumene hydroperoxide were detected in the extrusion area of an electrical cable insulating plant, where 5 employees worked. A concentration range of 0-60 $\mu g/m^3$ (n=10 samples) of cumene hydroperoxide was detected in ambient air by trapping cumene hydroperoxide on the activated charcoal of a personal sampler. Cumene hydroperoxide was apparently generated as a thermal degradation product of the vulcanizing agent dicumyl peroxide (Cocheo et al., 1983). In a second case, a former employee of Hercules Incorporated suffered a chemical burn from dermal exposure to cumene hydroperoxide. Subsequently, the employee had a history of skin rashes which might have been related to the chemical exposure (Anonymous, 1994).

8.0 REGULATORY STATUS

U.S. government regulations pertaining to cumene hydroperoxide are summarized in **Table 1**. No occupational exposure limits have been established in the United States for cumene hydroperoxide.

Table 1. Regulations Relevant to Cumene Hydroperoxide

	Regulation	Summary of Regulation/Other Comments
FDA	21 CFR 175.105	Cumene hydroperoxide is classified as an indirect food additive for use only as a component of adhesives.
OSHA	29 CFR 1910.1020	Under the Occupational Safety and Health Administration's (OSHA) standard, Access to Employee Exposure and Medical Records, employees exposed to toxic substances, such as cumene hydroperoxide, have a right to examine or copy relevant exposure and medical records, including sampling results.
EPA	40 CFR 60.489	Atmospheric Standards (ASTD): This standard requires all newly constructed, modified, and reconstructed (after January 5, 1981) Synthetic Organic Chemical Manufacturing Industry (SOCMI) process units to use the best demonstrated system of continuous emission reduction for equipment leaks of volatile organic compounds (VOC), considering costs, non-air quality health and environmental impact and energy requirements. Cumene hydroperoxide is a VOC covered under this rule.
	40 CFR 261.5	Generators of small quantities of cumene hydroperoxide may qualify for partial exclusion from EPA hazardous waste regulations.
	40 CFR 261.33	RCRA Requirements: When cumene hydroperoxide is a commercial chemical product, or manufacturing chemical intermediate, an off-specification commercial chemical product or a manufacturing chemical intermediate, it must be managed as a hazardous waste.
	40 CFR 302.6	CERCLA Reportable Quantities: Persons in charge of vessels or facilities are required to notify the National Response Center (NRC) immediately when there is a release of cumene hydroperoxide in an amount equal to or greater than its reportable quantity of 10 lb or 4.54 kg.
	40 CFR Part 716	TSCA Requirements: Under the Health and Safety Data Reporting Rule, manufacturers, importers, and processors of cumene hydroperoxide and mixtures are required to submit to EPA copies and lists of unpublished health and safety studies.
DOT	49 CFR 171.2	Cumene hydroperoxide must be properly classed, described, packaged, marked, and labeled for transportation and storage as required by 49 CFR 171-177.
	49 CFR Part 172	Cumene hydroperoxide is listed as a hazardous material by the U.S. Department of Transportation and is assigned a DOT number of UN 2116.

9.0 TOXICOLOGICAL DATA

9.1 General Toxicology

9.1.1 Human Data

No data on the toxicity of cumene hydroperoxide in humans were located.

9.1.2 Chemical Disposition, Metabolism, and Toxicokinetics

No data on the chemical disposition and toxicokinetics of cumene hydroperoxide were located. The only metabolism-related study located showed that cumene hydroperoxide is reduced by glutathione (GSH) to cumenol (Ketterer, 1988).

9.1.3 Acute Exposure

LC_{50} and LD_{50} values for cumene hydroperoxide are presented in **Table 2**. The details of studies discussed in this section are presented in **Table 3**.

Table 2. Acute Toxicity Values for Cumene Hydroperoxide

Route	Species (sex and strain)	$LD_{50}/LC_{50}/LT_{50}$	Reference
dermal	rat (sex and strain n.p.)	LD_{50}: 0.126 mL/kg (0.795 mmol/kg)	Union Carbide (1975)
	rat (sex n.p., Carworth Farm E (CFE))	LD_{50}: 0.5 to 1 mL/kg (3-6 mmol/kg) (occluded) and 1.13 to 1.43 mL/kg (7.10-9.00 mmol/kg) (unoccluded)	Brown et al. (1975)
inhalation	mouse (M, albino Swiss)	4-hour LC_{50}: 200 ppm (1,240 mg/m^3; 8.17 mmol/m^3)	Floyd and Stokinger (1958)
	rat (M, albino Wistar)	4-hour LC_{50}: 220 ppm (1,370 mg/m^3; 9.00 mmol/m^3)	Floyd and Stokinger (1958)
	rat (sex and strain n.p.)	LT_{50}: 6.7 hours	Union Carbide (1975)
i.p.	rat (M, albino Wistar)	LD_{50}: 95 mg/kg (0.62 mmol/kg)	Floyd and Stokinger (1958)
oral	mouse (sex and strain n.p.)	LD_{50}: 400-800 mg/kg (2.63-5.26 mmol/kg)	Eastman Kodak (1964)
	rat (sex and strain n.p.)	LD_{50}: 400-800 mg/kg (2.63-5.26 mmol/kg)	Eastman Kodak (1964)
	rat (M, albino Wistar)	LD_{50}: 382 mg/kg (2.51 mmol/kg)	Floyd and Stokinger (1958)
	rat (sex and strain n.p.)	LD_{50}: 1.3 mL/kg (8.18 mmol/kg)	Union Carbide (1975)

Abbreviations: i.p. = intraperitoneal injection; M = male; n.p. = not provided; LD_{50} = lethal dose for 50% of test animals; LC_{50} = lethal concentration for 50% of test animals; LT_{50} = lethal time for 50% of test animals.

In an acute toxicity study, mice inhaled 4,103 ppm (25,539 mg/m^3; 167.8 mmol/m^3) cumene hydroperoxide for 4 hours (Floyd and Stokinger, 1958). Symptoms of exposure were labored breathing and excitability after 10 minutes. Porphyrin deposition in the nostrils was also observed.

Several acute toxicity studies have been conducted on rats. In a percutaneous toxicity study, Brown et al. (1975) exposed two groups of 10 rats (5 female and 5 male) dermally (occluded and unoccluded) to cumene hydroperoxide (up to 2 mL) for 1 minute. During the 10 day observation period, both occluded and unoccluded rats exposed to 1 mL or more of cumene hydroperoxide experienced hematuria. Rats in the occluded skin test experienced convulsions and slight to severe burns.

Rats treated with 0.5 to 2.0 mL/kg (0.48-1.9 g/kg; 3.1-13 mmol/kg) cumene hydroperoxide by gavage were observed for 5 minutes to 24 hours (Union Carbide, 1975). All five rats in the high dose group died within 24 hours. Signs and symptoms of exposure included sluggishness, unsteady gait, and prostration. Gross pathology revealed hemorrhages of the lungs, mottled livers and spleens, liquid-filled and burned stomachs, blood-filled bladders, and congested kidneys. In another study, rats given 400 mg/kg (2.63 mmol/kg) cumene hydroperoxide by gavage experienced extensive urinary bleeding (Floyd and Stokinger, 1958). All deaths (number n.p.) occurred within 5 days. Single gavage administration of 0.7 to 2.0 g/kg (4.60-13.1 mmol/kg) cumene hydroperoxide caused marked weight loss with lethality at the high (2.0 g/kg; 13.1 mmol/kg) and intermediate (1.0 g/kg; 6.57 mmol/kg) doses within 7 days (Dow Chemical, 1952).

In inhalation studies conducted on rats, the main signs and symptoms of exposure were loss of coordination (Union Carbide, 1975; Gage, 1970; Eastman Kodak, 1964), tremors (Gage, 1970; Eastman Kodak, 1964; Floyd and Stokinger, 1958), pneumonia (Union Carbide, 1975), nasal irritation (Dow Chemical, 1952), and congested lungs and kidneys (Gage, 1970).

Rats given a single i.p. injection of 200 mg/kg (1.31 mmol/kg) cumene hydroperoxide experienced porphyrin deposition in the nostrils and coarse pelage during the 4-week observation period (Floyd and Stokinger, 1958).

Guinea pigs exposed dermally to cumene hydroperoxide (dose n.p.) for 24 hours experienced moderate to strong skin irritation (Eastman Kodak, 1964).

Rabbits exposed dermally to 0.1 to 2.0 mL/kg (0.63-13 mmol/kg) cumene hydroperoxide experienced skin necrosis, lethargy, and prostration after 24 hours, and dark eyes after 1 hour. Two rabbits in the high dose group died after 24 hours; while 1 rabbit in the low dose group and 4 in the mid dose group died after 48 hours (Union Carbide, 1975). Rabbits exposed dermally to 100, 10, 1, and 0.1% solutions suffered slight to severe hyperemia, necrosis, weight loss, and scaliness (Dow Chemical, 1952). In ocular tests on rabbits, 100, 10, and 1% solutions caused slight pain, severe to moderate irritation, and transient corneal damage.

Table 3. Acute Exposure to Cumene Hydroperoxide

Species Strain, and Age	Number and Sex of Animals	Chemical Form and Purity	Route/Dose	Exposure/ Observation Period	Results/Comments	Reference
Mice						
Mice, Albino Swiss, age n.p.	10 M/dose	cumene hydroperoxide, 73% minimum purity	inhalation: 4,103 ppm (25,539 mg/m^3; 167.8 mmol/m^3)	4 h exposure, observation period n.p.	Labored breathing and excitability after 10 min of exposure, porphyrin deposition in nostrils.	Floyd and Stokinger (1958)
Rats						
Rats, Carworth Farm E (CFE), 12-14 weeks.	Occluded group: 5 F, 5 M Unoccluded group: 5 F, 5 M	cumene hydroperoxide, 23% active material stabilized with sodium bicarbonate	dermal: up to 2 mL	1 min exposure, 10 d observation period	Hematuria in both groups, convulsions and slight to severe skin burns in occluded group.	Brown et al. (1975)
Rats, strain and age n.p.	Group 1: 5, sex n.p. Group 2: 5, sex n.p. Group 3: 3, sex n.p.	cumene hydro-peroxide, purity n.p.	gavage: Group 1: 2.0 mL/kg (1.9 g/kg; 13 mmol/kg) Group 2: 1.0 mL/kg (0.96 g/kg; 6.3 mmol/kg) Group 3: 0.5 mL/kg (0.48 g/kg; 3.1 mmol/kg)	single treatment, 5 min to 24 h observation period	Group 1: Rubbing of mouth on bottom of cage immediately after treatment, sluggishness after 5 min, prostration after 1 h, death after 24 h. Group 2: Sluggishness after 5 min, unsteady gait after 20 min, 1 death after 4 h. Group 3: Sluggishness, no deaths.	Union Carbide (1975)
Rats, Albino Wistar, age n.p.	5 M/dose	cumene hydro-peroxide, 73% minimum purity	gavage: doses n.p. but included 400 mg/kg (2.63 mmol/kg)	single exposure, 4 wk observation period	Extensive urinary bleeding, death within 5 d, no weight loss. It was stated that four oral dose levels were given; however, specific doses were not reported, only as “four geometrically spaced dosage levels”. The study mentioned effects at a dose of 400 mg/kg.	Floyd and Stokinger (1958)

Table 3. Acute Exposure to Cumene Hydroperoxide (Continued)

Species Strain, and Age	Number and Sex of Animals	Chemical Form and Purity	Route/Dose	Exposure/ Observation Period	Results/Comments	Reference
Rats, strain and age n.p.	3/group, sex n.p.	cumene hydro-peroxide, purity n.p.	gavage: Group 1: 0.7 g/kg (4.60 mmol/kg) Group 2: 1.0 g/kg (6.57 mmol/kg) Group 3: 2.0 g/kg (13.1 mmol/kg)	single exposure, 7 day observation period	Marked weight loss. 1/3 deaths in group 2 and 3/3 deaths in group 3.	Dow Chemical (1952)
Rats, strain and age n.p.	Group 1: 6, sex n.p. Group 2: 6, sex n.p.	cumene hydro-peroxide, purity n.p.	inhalation: dose n.p.	Group 1: 4 h exposure Group 2: 8 h exposure Observation periods n.p.	Group 1: Slight loss of coordination after 35 min, poor coordination after 180 min, 1 survivor developed pneumonia. Group 2: Fair coordination after 25 min, eyes closed and poor coordination after 170 min, 4 deaths after 24 h, 1 survivor developed pneumonia.	Union Carbide (1975)
Rats, strain and age n.p.	2 F	cumene hydro-peroxide, purity n.p.	inhalation: 50 ppm (311 mg/m^3; 2.0 $mmol/m^3$)	4 h (3 times), observation period n.p.	Incoordination, tremor, narcosis, 1 death. Histological examination revealed congested lungs and kidneys.	Gage (1970)
Rats, strain and age n.p.	n.p.	cumene hydro-peroxide, purity n.p.	inhalation: 800 ppm (4,980 mg/m^3; 32.7 $mmol/m^3$)	6 h exposure, 14 d observation period	Loss of coordination, tremors. Returned to normal after 14 d.	Eastman Kodak (1964)
Rats, Albino Wistar, age n.p.	6 M/dose	cumene hydro-peroxide 73% minimum purity	inhalation: 4,103 ppm (25,539 mg/m^3; 1.67.8 $mmol/m^3$)	4 h exposure, observation period n.p.	Head and neck tremors, weakness in extremities, prostration, 1/6 deaths 7 d later.	Floyd and Stokinger (1958)
Rats, strain and age n.p.	3, sex n.p.	cumene hydro-peroxide, purity n.p.	inhalation: saturated atmosphere at 25°C	7 h, observation period n.p.	Slight, temporary weight loss, nasal irritation.	Dow Chemical (1952)

Table 3. Acute Exposure to Cumene Hydroperoxide (Continued)

Species Strain, and Age	Number and Sex of Animals	Chemical Form and Purity	Route/Dose	Exposure/ Observation Period	Results/Comments	Reference
Rats, Albino Wistar, age n.p.	5 M/dose	cumene hydro-peroxide, 73% minimum purity	i.p.: doses n.p. but included 200 mg/kg (1.31 mmol/kg)	single exposure, 4 wk observation period	Porphyrin deposition in the nostrils, coarse pelage, no weight loss seen. Cumene hydroperoxide was diluted to 10% in propylene glycol for easier intraperitoneal administration. It was stated that four i.p. dose levels were given; however, the range of doses were not reported in the study, only reported as "four geometrically spaced dosage levels". The study only mentioned the affects of a 200 mg/kg dose.	Floyd and Stokinger (1958)
Guinea Pigs						
Guinea pigs strain and age n.p.	n.p.	cumene hydro-peroxide, purity n.p.	dermal: dose n.p.	24 h, observation period n.p.	Moderate to strong skin irritation.	Eastman Kodak (1964)
Rabbits						
Rabbits, strain and age n.p.	Group 1: 4, sex n.p. Group 2: 4, sex n.p. Group 3: 4, sex n.p.	cumene hydro-peroxide, purity n.p.	dermal: Group 1: 0.1 mL/kg (95.9 mg/kg; 0.63 mmol/kg) Group 2: 0.2 mL/kg (192 mg/kg; 1.26 mmol/kg) Group 3: 2.0 mL/kg (1.9 g/kg; 13 mmol/kg)	48 h, observation period n.p.	Group 1: Skin necrosis, very lethargic after 24 h, 1 death after 48 h. Group 2: Skin necrosis, prostration after 24 h, 4 deaths after 48 h. Group 3: Skin necrosis, eyes dark after 1 h, 2 deaths after 24 h.	Union Carbide (1975)

Table 3. Acute Exposure to Cumene Hydroperoxide (Continued)

Species Strain, and Age	Number and Sex of Animals	Chemical Form and Purity	Route/Dose	Exposure/ Observation Period	Results/Comments	Reference
Rabbits, strain and age n.p.	Number and sex n.p.	cumene hydro-peroxide, purity n.p.	dermal: 100, 10, 1 and 0.1% solutions; amounts applied n.p.	1 to 10 applications to ear or abdomen (frequency n.p.), observation period n.p.	Slight to severe hyperemia, necrosis, weight loss, scaliness.	Dow Chemical (1952)
Rabbits, strain and age n.p.	Number and sex n.p.	cumene hydro-peroxide, purity n.p.	ocular: 100, 10, and 1% solutions; amount applied n.p., 2 groups/dose (washed and unwashed)	exposure n.p., observation period n.p.	Slight pain, severe to moderate irritation, transient corneal damage.	Dow Chemical (1952)

Abbreviations: d = day(s); F = female; h = hour(s); M = male; min = minutes; n.p. = not provided; wk = week(s)

9.1.4 Short-Term and Subchronic Exposure

The details of studies discussed in this section are presented in **Table 4**.

A subchronic toxicity study by Watanabe et al. (1979) concluded that inhalation of cumene hydroperoxide vapor is irritating to mucous membranes and the respiratory system. The 3-month study was conducted on male and female Fischer 344 rats exposed for 6 hours/day, five days/week at concentrations ranging from 1 to 124 mg/m^3 (0.16-20 ppm; 0.007-0.815 $mmol/m^3$). There were no recognized dose-related effects on hematology, urinalysis, clinical chemistry, body weights, organ weights, or pathology in rats exposed to up to 31 mg/m^3 (4.98 ppm; 204 $\mu mol/m^3$) Rats exposed to 124 mg/m^3 (20 ppm; 0.815 $mmol/m^3$) showed decreased body weight, difficulty breathing, and eye and nose irritation. Changes such as thymic atrophy, decreased circulating white blood cells, and decreased lipid content of the liver were also seen in high-dose animals; however, the authors concluded that these signs may have been stress-related.

A group of six female rats subjected to seven 5-hour exposures of 31.5 ppm (1.29 $mmol/m^3$) cumene hydroperoxide exhibited salivation, respiratory difficulty, tremors, hyperemia of the ears and tail, and weight loss (Gage, 1970). Autopsy revealed lung effects including emphysema and thickening of the alveolar walls. Another group of six female rats were subjected to twelve 4.5-hour exposures of 16 ppm (0.66 $mmol/m^3$) cumene hydroperoxide. Signs of exposure were salivation and nose irritation. All organs appeared normal following histological examination.

Floyd and Stokinger (1958) conducted subchronic tests to study possible cumulative effects of i.p. and oral doses of cumene hydroperoxide in rats. Rats were given i.p. injections of 1/5 the intraperitoneal LD_{50} (19 mg/kg; 0.12 mmol/kg) three times/week for 7 weeks. All five rats gained weight normally throughout the test period; however, one rat died during the 7-week period. In some rats, the fur was noticeably coarse; no other symptoms were reported. Five male rats given cumene hydroperoxide (19 mg/kg; 0.12 mmol/kg) orally 3 times a week for seven weeks showed weight loss and coarse pelage; four of the rats died during the 7-week period.

Table 4. Short-Term and Subchronic Exposure to Cumene Hydroperoxide

Species Strain, and Age	Number and Sex of Animals	Chemical Form and Purity	Route/Dose	Exposure/ Observation Period	Results/Comments	Reference
Rats, Fischer 344, age n.p.	10 F, 10 M in each group and control	cumene hydroperoxide, purity n.p.	inhalation: 1, 6, 31, and 124 mg/m^3 (0.16, 0.96, 4.98, and 20 ppm; 0.007, 0.04, 0.20, and 0.815 mmol/m^3)	Exposed 6 h/d, 5 d/wk for 90 d, observation period n.p.	Decreased body weight, stress, ulcerations and inflammation of the cornea, nasal turbinates, and stomach lining, and death at 124 mg/m^3. No significant effects were produced at 1, 6, and 31 mg/m^3.	Watanabe et al. (1979)
Rats, strain and age n.p.	Group 2: 6 F Group 3: 6 F	cumene hydroperoxide, purity n.p.	inhalation: Group 2: 31.5 ppm (196 mg/m^3; 1.29 mmol/m^3) Group 3: 16 ppm (100 mg/m^3; 0.66 mmol/m^3)	Group 2: 5 h (7 times) Group 3: 5.4 h (12 times) observation periods n.p.	Group 2: Salivation, respiratory difficulty, tremors, hyperemia of ears and tail, weight loss. Histological examination revealed emphysema and thickening of alveolar walls. Group 3: Salivation, nose irritation, organs normal.	Gage (1970)
Rats, Albino Wistar, age n.p.	5 M	cumene hydroperoxide, 73% minimum purity	i.p.: 19 mg/kg (0.12 mmol/kg), 3 times/wk	7 wk, observation period n.p.	One death, coarse pelage, normal weight gain.	Floyd and Stokinger (1958)
Rats, Albino Wistar, age n.p.	5 M	cumene hydroperoxide, 73% minimum purity	oral: 19 mg/kg (0.12 mmol/kg), 3 times/wk	7 wk, observation period n.p.	Four deaths, weight loss, coarse pelage.	Floyd and Stokinger (1958)

Abbreviations: d = day(s); d/wk = days per week; F = female; h = hour(s); h/d = hours per day; M = male; min = minute(s); n.p. = not provided; ppm = parts per million; wk = week(s).

9.1.5 Chronic Exposure

No data on the effects of chronic exposure to cumene hydroperoxide were located.

9.2 Cytotoxicity of Cumene Hydroperoxide

The details of studies discussed in this section are presented in **Table 5**.

Cell metabolism of cumene hydroperoxide can produce cytotoxic effects such as intracellular oxidative stress and cell necrosis (Persoon-Rothert et al., 1992).

9.2.1 Lower Eukaryotic Systems

Cumene hydroperoxide at sublethal concentrations (28-2800 µM; 4.3-426.1 µg/mL) initially prevented the growth of *Neurospora crassa* mycelia. However, with increasing exposure duration, cells proceeded to grow at a subnormal, but steady rate as the cells gain resistance (Munkres and Colvin, 1976). Mycelia cells appeared to develop a physiological resistance to the toxicity of cumene hydroperoxide through the induction of antioxygenic enzymes (e.g., superoxide dismutase, glutathione peroxidase, glutathione reductase). The addition of nordihydroguaiaretic acid (NDGA), a free radical scavenger, decreased the toxicity of cumene hydroperoxide by inhibiting lipid peroxidation (Munkres and Colvin, 1976).

9.2.2 *In Vitro* Mammalian Systems

The effects of cumene hydroperoxide (0.01-0.2 mM; 1.5-30 mg/mL) on the plating efficiencies of human adenocarcinoma (A549) cells were studied; concentrations in excess of 0.5 mM (76 µg/mL) were cytotoxic (Baker and He, 1991).

Koster et al. (1981) investigated the effect of cumene hydroperoxide on isolated perfused rat heart. Perfusion of rat hearts with cumene hydroperoxide at 0.1 and 0.5 mM (15 and 76 µg/mL) led to the formation and release of malonaldehyde (one of the final products of lipid peroxidation) and protein indicating the induction of cell damage. There was also evidence in mitochondria of lipid peroxidation. In a related study by Persoon-Rothert et al. (1992), exposure of neonatal rat heart cells to cumene hydroperoxide (50 µM; 7.6 µg/mL) resulted in a calcium

overload which led to a deterioration of cell membrane phospholipids by lipid peroxidation and cell death. Prior treatment with Trolox C (a vitamin E analogue) successfully inhibited the calcium disturbance and subsequent cell injury (Persoon-Rothert et al., 1992).

Dowjat et al. (1996) found that the induction of peroxidase deficiency in Chinese hamster fibroblasts increased their resistance to the cytotoxic effects of cumene hydroperoxide (10-50 µM; 1.5-7.6 µg/mL). The LD_{50} values calculated for the Ni-2 cells (10 µM; 1.5 µg/mL) were significantly higher than the corresponding value for the CH-1 cells (5.8 µM; 0.88 µg/mL). These results suggested that the cytotoxic effects of cumene hydroperoxide may be mediated in part by free radicals generated during peroxidase-catalyzed reactions (Dowjat et al., 1996).

The addition of cumene hydroperoxide to rat liver microsomes or to isolated rat hepatocytes caused several metabolic changes. When Chiarpotto et al. (1984) exposed isolated rat hepatocytes to 50-200 µM (7.6-30.4 µg/mL) cumene hydroperoxide, effects including enhanced lipid peroxidation, decreased glucose-6-phosphatase activity, decreased cytochrome P450 content, and stimulation of aminopyrene demethylation were seen. Cumene hydroperoxide also had an inhibitory effect on lipoprotein secretion, leading the authors to postulate possible damage to the Golgi apparatus (Chiarpotto et al., 1984).

A study using isolated hepatocytes from rats deficient in selenium and vitamin E demonstrated that these micronutrients play a role in the toxicity of cumene hydroperoxide (Hill and Burk, 1984). Hepatocytes from selenium-deficient rats treated with 0.5 mM (76 µg/mL) cumene hydroperoxide died within 4 hours, while 58% of hepatocytes from control rats remained viable. Hepatocytes from vitamin E-deficient rats died after 2 hours. This led to the conclusion that cells from animals deficient in selenium and vitamin E had increased susceptibility to cumene hydroperoxide. The micronutrient-deficient cells also showed a marked decrease in intracellular glutathione when compared with untreated hepatocytes. The authors noted that lipid peroxidation was not increased in treated cells, which suggested that cumene hydroperoxide injures cells through a mechanism other than lipid peroxidation (Hill and Burk, 1984).

Vimard et al. (1996) used pheochromacytoma cells (PC12) to further investigate the cytotoxic effects of cumene hydroperoxide and mechanisms of cell death. At concentrations

above 1 µM (0.2 µg/mL), cumene hydroperoxide caused significant cell death that reached a maximum (89-90% toxicity) at 100 µM (15.2 µg/mL). Cell death was preceded by a decrease in cellular adenosine triphosphate (ATP) content, suggesting that cell mitochondria were the primary target of hydroperoxide action. Also, there was a dose-dependent increase in lactate dehydrogenase (LDH), which was presumed to interfere with the ability of the cells to divide. Vreogop et al. (1995) also used neuronal cells to assess the extent and location of cellular damage induced by cumene hydroperoxide (1-1000 µM; 0.2-152.2 µg/mL). Based on a dose-dependent decline in -amino-isobutyric acid (AIB) uptake and only a slight alteration in mitochondrial function at the highest concentration of cumene hydroperoxide tested, the authors concluded that the primary site of action occurred at the cell plasma membrane. The inactivation of glucose transport as cumene hydroperoxide was localized in the plasma membrane supported this conclusion (Vreogop et al., 1995).

Table 5. Cytotoxicity of Cumene Hydroperoxide

Test System or Species, Strain, and Age	Biological Endpoint	Chemical Form, Purity	Dose	Endpoint Response	Reference
9.2.1 Lower Eukaryotic Systems					
Neurospora crassa, RL 1256A (74A8) strain	cell growth	cumene hydroperoxide, purity n.p.	28-2800 µM (4.3-426.1 µg/mL)	Inhibited growth, cells able to develop resistance possibly by induction of antioxygenic enzymes.	Munkres and Colvin (1976)
9.2.2 In Vitro Mammalian Systems					
Human adeno-carcinoma cells (A549)	plating efficiency	cumene hydroperoxide, purity n.p.	0.01-0.2 mM (1.5-30 µg/mL)	Decreased plating efficiency at >0.05 mM.	Baker and He (1991)
Isolated perfused heart of M Wistar rats	malonaldehyde formation and lipid peroxidation, protein release	cumene hydroperoxide, purity n.p.	0.1-0.5 mM (15-76 µg/mL)	Malonaldehyde formed and protein released at 0.3 mM.	Koster et al. (1981)
Heart myocytes from 2 day old Wistar rats, sex n.p.	Calcium homeostasis, cell death, cellular -hydroxybutyrate dehydrogenase (-HBDH)	cumene hydroperoxide, purity n.p.	50 µM (7.6 µg/mL)	Exposure resulted in a calcium overload which led to a deterioration of cell membrane phospholipids by lipid peroxidation, causing cell death. Pretreatment with Trolox C inhibited calcium disturbance and cell injury.	Persoon-Rothert et al. (1992)
Chinese hamster (CHE & CH-1) fibroblasts	cell viability	cumene hydroperoxide, purity n.p.	10-50 µM (1.5-7.6 µg/mL)	Induction of peroxidase deficiency increased resistance to the cytotoxic effects of cumene hydroperoxide.	Dowjat et al. (1996)
Liver hepatocytes isolated from M Wistar rats	metabolic alterations: cytochrome P450 content, aminopyrene demethylation, glucose-6-phosphatase activity	cumene hydroperoxide, reagent grade	50, 100, 150, and 200 µM (7.6, 15.2, 22.8, and 30.4 µg/mL)	Decreased cytochrome P450 content, stimulation of aminopyrene demethylation, decreased glucose-6-phosphatase activity, enhanced lipid peroxidation, and inhibition of lipoprotein secretion.	Chiarpotto et al. (1984)

Table 5. Cytotoxicity of Cumene Hydroperoxide (Continued)

Test System or Species, Strain, and Age	Biological Endpoint	Chemical Form, Purity	Dose	Endpoint Response	Reference
Liver hepatocytes from vitamin E and selenium-deficient M Sprague-Dawley rats	cell viability, glutathione synthesis and release, lipid peroxidation	cumene hydroperoxide, purity n.p.	0.5 mM (76 µg/mL), 4-h incubation	All cells from selenium-deficient rats lost viability within 4 h; increase in thiobarbituric acid (TBA)-reactive substances; cells from vitamin E-deficient rats lost viability after 2 h, decrease in intracellular glutathione in cells from selenium-deficient rats; lipid peroxidation did not increase in treated cells.	Hill and Burk (1984)
Pheochromacytoma cells (PC 12)	cell viability, ATP production	cumene hydroperoxide, purity n.p.	1-100 µM (0.2-15.2 µg/mL)	>1 µM: significant cell death, decrease in cellular ATP. 100 µM: 89-90% toxicity. Increase in lactate dehydrogenase, disruption in mitosis.	Vimard et al. (1996)
N18-RE-105 (N18) neuronal cells from mouse neuro-blastoma and a Fischer rat embryonic neural retina	cytotoxicity	cumene hydroperoxide, purity n.p.	1-1000 µM (0.2-152.2 µg/mL)	Dose-dependent decline in -amino-isobutyric acid (AIB) uptake, slight alteration in mitochondrial function at highest concentration, inactivation of glucose transport as cumene hydroperoxide was localized at the cell plasma membrane.	Vroegop et al. (1995)

Abbreviations: -HBDA = -hydroxybutyrate dehydrogenase; ATP = adenosine triphosphatase; h = hour(s); M = male; n.p. = not provided.

9.3 Reproductive and Teratological Effects

No data on the reproductive and teratological effects of cumene hydroperoxide were found except for one *in vitro* study suggesting that cumene hydroperoxide can inhibit progesterone biosynthesis, and therefor potentially contribute to the development of preeclampsia (proteinuric hypertension). Klimek et al. (1998) studied the modulating effect of hydroperoxide-dependent formation of free radicals on progesterone biosynthesis. The idea that natural and synthetic organic hydroperoxides may stimulate the formation of thiobarbituric acid-reactive substances (TBARS) was confirmed by this study. The addition of 1 µM (0.2 µg/mL) cumene hydroperoxide to a suspension of human placental mitochondria resulted in the formation of TBARS. The presence of cumene hydroperoxide accelerated the activation of cytochrome $P450_{scc}$ and inhibited NADPH-dependent lipid peroxidation. Low levels of lipid hydroperoxides are necessary for normal pregnancy.

9.4 Carcinogenicity

The details of these studies are presented in **Table 6**.

9.4.1 Mice

A study by Kotin and Falk (1963) observed one subcutaneous sarcoma in addition to 11 malignant lymphomas in 50 mice administered s.c. doses of cumene hydroperoxide (50 µM; 7.6 µg/mL). In mice, 1% cumene hydroperoxide applied three times weekly (duration n.p.) onto clipped dorsal skin did not induce papillomas or carcinomas (Van Duuren et al., 1965). However, a subcutaneous (s.c.) injection of 3.3 mg (0.22 mmol) once a week for 76 weeks in the left axillary area of mice induced one fibrosarcoma, while no tumors were present at the injection site among control mice (Van Duuren et al., 1966).

9.4.2 Rats

One study was found on the carcinogenicity of cumene hydroperoxide in rats. Cumene hydroperoxide (100 mg; 0.657 mmol) administered s.c. induced no sarcomas (Van Duuren et al.,

1967).

Table 6. Carcinogenicity of Cumene Hydroperoxide

Species, Strain, and Age	Number and Sex of Animals	Chemical Form and Purity	Dose/Route	Exposure/ Observation Period	Results/Comments	Reference
9.4.1 Mice						
C57B1, age n.p.	50, sex n.p.	cumene hydroperoxide, purity n.p.	50 µM (7.6 µg/mL) given by s.c. injection	n.p.	Treated mice had 1 sarcoma and 11 malignant lymphomas. A control group was not mentioned in the study.	Kotin and Falk (1963)
Swiss-Millerton, 8-wk-old	30 F	cumene hydroperoxide, ~99% pure	1% in benzene applied three times weekly onto clipped dorsal skin with brush	n.p.	Treatment induced no papillomas or carcinomas, similar to the control group (60 animals given benzene alone).	Van Duuren et al. (1965)
ICR/Ha Swiss, 8-wk-old	30 F	cumene hydroperoxide, purity n.p.	3.3 mg (0.022 mmol) cumene hydroperoxide in 0.05 mL (66 mg/mL) tricaprylin given by s.c. injection once a wk in the left axillary area	76 wk exposure, observation period n.p.	Treated mice had 0 benign tumors and 1 malignant tumor (fibrosarcoma).	Van Duuren et al. (1966)
9.4.2 Rats						
Sprague-Dawley, 6-wk-old	20 F	cumene hydroperoxide, purity n.p.	100 mg (0.657 mmol) cumene hydroperoxide in 0.1 mL (1000 mg/mL) tricaprylin given by s.c. injection once a wk in the left axillary area	77 wk exposure, observation period n.p.	No tumors induced at treatment site.	Van Duuren et al. (1967)

Abbreviations: F = female; n.p. = not provided; s.c. = subcutaneous; wk = week(s).

9.5 Initiation/Promotion Studies

In an overview of the relationship between oxidative stress and chemical carcinogenesis, Trush and Kensler (1991) hypothesized that cumene hydroperoxide falls into the category of tumor promotors which activate exogenous sources of reactive intermediates. Free radical signals have been detected in keratinocytes following incubation with cumene hydroperoxide. Cumene hydroperoxide is believed to be an active promoter in the initiation-promotion model of the mouse epidermis due to its ability to generate free radicals.

9.6 Genotoxicity

Studies discussed in this section are presented in **Table 7**.

9.6.1 Acellular Systems

DNA single strand breaks (SSBs) were induced by cumene hydroperoxide (100 µM; 15.2 mg/mL)-hematin (20 µM; 13 mg/mL) in hepatic nuclei isolated from male Sprague-Dawley rats and treated *in vitro* (Cohen et al., 1984). Uric acid (500 µM; 79.1 mg/mL) inhibited the cumene hydroperoxide-hematin induction of DNA damage, suggesting that hydroperoxyl free radicals were the primary toxic species in this system. However, cumene hydroperoxide alone was not active in this assay system. Cumene hydroperoxide (0.01-0.2 µM; 1.52-30.4 µg/mL) did not induce single or double strand breaks in DNA of lysed human adenocarcinoma (A549) cells (Baker and He, 1991). Also, 200 µM (30.4 µg/mL) did not induce SSBs in either lysed DNA samples or intact nuclei from human myeloid leukemia cells (Guidarelli et al., 1997).

9.6.2 Prokaryotic Systems

When tested for mutagenic activity in *Salmonella typhimurium* strain TA102 (Levin et al., 1984), cumene hydroperoxide (100 µg/plate; 0.66 µmol/plate) was positive with and without metabolic activation (S9). Cumene hydroperoxide was also positive in the L-arabinose forward mutation assay in *S. typhimurium* strains (Levin et al., 1982, cited by Ruiz-Rubio et al., 1985), BA9 (42-125 nmol/plate; 6.4-19.0 µg/plate) and BA13 (8.3-42 nmol/plate; 1.3-6.4 µg/plate)

(Ruiz-Rubio et al., 1985). Cumene hydroperoxide (0.03-147 µg/plate; 0.2-966 µmol/plate) induced *his* gene mutations in *S. typhimurium* strains TA100 and TA98 with, but not without, induced rat and hamster S9; no mutagenic activity was detected in TA1535 and TA1537, with or without S9 (Mortelmans et al., 1986). In a plate incorporation study conducted in the absence of S9 (Kensese and Smith, 1989), cumene hydroperoxide was mutagenic in *S. typhimurium* TA97, TA102, and TA1537 (1.4 mmol/plate; 210 mg/plate) and in TA100 (0.7 mmol/plate; 100 mg/plate); negative results were obtained in TA98 and TA1538. However, in a preincubation assay with TA97, TA102, TA1537, TA100, TA98, and TA1538 tested without S9 (Kenese and Smith, 1989), cumene hydroperoxide was positive only in TA1537 (1.35 µmol/plate; 205 µg/plate); the addition of catalase or dismutase superoxide completely abolished the positive response. Additional mutagenicity studies in *S. typhimurium* gave the following results: positive in TA102 in the absence of S9 (30-200 µg/plate; 0.2-1.31 µmol/plate) (Wilcox et al., 1990); positive in TA104 in the absence of oxygen radical scavengers (107 nmol/plate; 16.3 µg/plate) (de Kok et al, 1992); positive in TA97a and TA102, with and without S9 (50-200 µg/plate; 0.33-1.31 µmol/plate) (Wilcox et al., 1993); positive in TA102 without S9 (0.197-0.986 µmol/plate; 30.0-150 µg/plate) (Kranendonk et al., 1996); positive in TA97a, TA100, TA102, and TA104 with S9 (1.67-500 µg/plate; 0.012-3.28 µmol/plate) (Dillon et al., 1998); positive in TA97a and TA102 without S9 (Dillon et al., 1998); equivocal in TA100 without S9 (Dillon et al., 1998). Recently, cumene hydroperoxide (1-100 µg/mL; 0.007-0.7 µmol/mL) was used in a validation study of *S. typhimurium* strains in the TA7001-TA7006; strains TA98 and TA1537 were also used (Gee et al., 1998); positive results were obtained in all the TA7000 strains except TA7003, and in TA98 and TA1537.

Cumene hydroperoxide (30-200 µg/plate; 0.2-1.31 µmol/plate) was mutagenic at the *trpE* locus in *Escherichia coli* WP2 (pkM101) and WP2 *uvrA* (pkM101) in the absence of S9 (Wilcox et al., 1990). Cumene hydroperoxide (1-33 µg/mL; 0.007-0.22 µM) increased -galactosidase activity in a dose-dependent manner in DNA repair-deficient *E. coli* strain KY946 [*uvrA*], but not in the repair deficient strains KY945 [*recA*] and KY943 [*lexA*] (Nunoshiba and Nishioka, 1991). In a comparative study with *E. coli* strains PQ300 and PQ37, cumene hydroperoxide

(1.0-264 µM; 0.15-40.2 µg/mL) induced an SOS response in PQ300 that was roughly three times greater than in PQ37 (Müller and Janz, 1992). Cumene hydroperoxide (1-10 µg/plate; 0.007-0.066 µmol/plate) induced Trp+ mutations in *E. coli* strain WP2s, and derivatives carrying the mutations *mutY*, *mutM*, *soxRS*, or combinations of these mutations; all strains were tested with and without S9 and incorporated pKM101 plasmid (Kato et al., 1994). In addition, cumene hydroperoxide (5-20 µg/mL; 30-130 µM) induced the SOS response in *mutM* and *soxRS* mutants, as well as in the parent strain. Cumene hydroperoxide (25 and 50 µg/plate; 0.16 and 0.33 µmol/plate) was tested for Trp+ reversion in *E. coli* strains IC3841 (*oxyRmut+*), IC2869 (*oxyR+mut+*), IC3894 (*oxyRmutY*), and IC3793 (oxyR+mutY) (Urios et al., 1995). Cumene hydroperoxide produced an increase in Trp+ revertants in IC3841, and IC3894 was more sensitive (about 3-fold) to SOS-independent mutagenicity than IC3793 (Urios et al., 1995). Cumene hydroperoxide (0.197-0.986 µmol/plate; 30.0-150 µg/plate) induced Trp+ reversions in *E. coli* strain MX100 (Kranendonk et al., 1996).

9.6.3 Lower Eukaryotic Systems

The cellular toxicity and effects on mitotic gene conversion of potential fuel oil photoproducts were investigated in *Saccaromyces cerevisiae,* strain D4 (Callen and Larson, 1978). Irradiated fuel oil (near-u.v. exposures up to 96 hours) at concentrations up to 20 ppt resulted in 50% cell death; gene conversion at *trp5* was significant up to 24 hours. Cumene hydroperoxide (0-0.56 mM; 0-85 µg/mL) was cytotoxic but not convertagenic. Brennan et al. (1994) investigated the effect of cumene hydroperoxide (30-50 µg/mL; 0.23-0.33 mM) on the frequency of intrachromosomal and interchromosomal recombination in *Saccharomyces cerevisiae*; a dose-dependent increase in the frequency of intrachromosomal recombination was observed.

9.6.4 *In Vitro* Mammalian Systems

Sandstrom (1991) investigated the induction and rejoining of DNA SSB in relation to cellular growth in human mesothelioma-derived P31 cells exposed to cumene hydroperoxide (0.1-10 mM; 0.02-1.5 mg/mL) at 0°C and 37°C. There was a 5-fold increase in the cytotoxicity of cumene hydroperoxide at 37°C in the presence of metabolic activation, compared to the level of cytotoxicity at 0°C in the absence of metabolic activation. The induction of SSB did not correlate well with cytotoxicity; for example, cumene hydroperoxide produced fewer SSB than t-butyl hydroperoxide despite having greater cytotoxicity at each temperature. The rejoining pattern of SSB did not change with temperature but the overall number of breaks was increased at 37°C.

Cumene hydroperoxide (30-300 µM; 4.6-45.7 µg/mL) induced SSB in a dose-dependent manner in cultured human myeloid leukemia U937 cells, but the amount of damage was significantly less than what was observed with similar concentrations of hydrogen peroxide or t-butyl hydroperoxide (Guidarelli et al., 1997).

9.6.5 *In Vivo* Mammalian Systems

Single doses of cumene hydroperoxide (34 and 90 mg/kg; 0.22 and 0.59 mmol/kg, respectively) were administered i.p. to 5 or 7 male mice, respectively, in the dominant lethal assay (Epstein et al., 1972). Male mortality was low (no deaths in the 34 mg/kg dose group and 1 of 7 males in the 90 mg/kg dose group). Early fetal deaths and preimplantation losses were within control ranges for female mice mated with treated males. However, because matings were only conducted for one week post-treatment, this study was adequate for measuring effects on mature sperm, but was inadequate for measuring induction of dominant lethal mutations over the entire course of spermatogenesis (approximately 6 weeks).

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Table 7. Genotoxicity of Cumene Hydroperoxide

Test System	Biological Endpoint	S9 Metabolic Activation	Chemical Form, Purity	Dose	Endpoint Response	Reference
9.6.1 Acellular Systems						
Hepatic nuclei from male Sprague-Dawley rats	DNA single strand breaks (SSB)	-	cumene hydroperoxide, purity n.p.	hematin (20 µM; 13 mg/mL) plus cumene hydroperoxide (100 µM; 15.2 mg/mL), with or without uric acid (500 µM; 79.1 mg/mL)	Positive for the combination of hematin and cumene hydroperoxide; addition of uric acid provided 80% reduction in DNA damage; cumene hydroperoxide alone did not induce DNA SSB.	Cohen et al. (1984)
DNA extracted from human adenocarcinoma cell line (A549)	SSB and double strand breaks (DSB)	-	cumene hydroperoxide, purity n.p.	0.01-0.2 µM (1.52-30.4 µg/mL)	SSB: negative DSB: negative	Baker and He (1991)
DNA and intact nuclei obtained from human myeloid leukemia (U937) cells	DNA SSB	-	cumene hydroperoxide, purity n.p.	200 µM (30.4 µg/mL)	negative	Guidarelli et al. (1997)
9.6.2 Prokaryotic Systems						
Salmonella typhimurium TA102	*his* gene mutations	+/-	cumene hydroperoxide, purity n.p.	100 µg/plate (0.66 µmol/plate)	positive	Levin et al. (1984)
S. typhimurium BA9 and BA13	ara gene mutations	n.p.	cumene hydroperoxide, purity n.p.	BA9: 42-125 nmol/plate (6.4-19.0 µg/plate) BA13: 8.3-42 nmol/plate (1.3-6.4 µg/plate)	positive	Ruiz-Rubio et al. (1985)
S. typhimurium strains TA100, TA1535, TA1537, and TA98	*his* gene mutations	+/-	cumene hydroperoxide, 78% pure (technical grade)	0.03-147 µg/plate (0.2-966 nmol/plate)	-S9 +S9 TA100 - + TA1535 - - TA1537 - - TA98 - +	Mortelmans et al. (1986)

Table 7. Genotoxicity of Cumene Hydroperoxide (Continued)

Test System	Biological Endpoint	S9 Metabolic Activation	Chemical Form, Purity	Dose	Endpoint Response	Reference
S. typhimurium strains TA97, TA98, TA100, TA102, TA1537, and TA1538	*his* gene mutations	-	cumene hydroperoxide, purity n.p.	Plate incorporation: Strains TA98, TA100, and TA1538: 0.7 mmol/plate (100 mg/plate) Strains TA97, TA102, TA1537: 1.4 mmol/plate (210 mg/plate) Preincubation assay: Strain TA100: 0.7 µmol/plate (106 µg/plate) Strains TA102, TA1537, TA1538: 1.35 µmol/plate (205 µg/plate) Strain TA97, TA98: 2.0 µmol/plate (304 µg/plate)	Plate incorporation: positive in TA97, TA100, TA102, and TA1537. Negative in TA98, TA1538. Preincubation assay: weakly positive in TA1537, and negative in all other strains. The positive effect was completely abolished by catalase or superoxide dismutase.	Kensese and Smith (1989)
S. typhimurium TA102	*his* gene mutations	-	cumene hydroperoxide, purity n.p.	30-200 µg/plate (0.2-1.31 µmol/plate)	positive	Wilcox et al. (1990)
S. typhimurium strain TA104	*his* gene mutations	-	cumene hydroperoxide, purity n.p.	107 nmol/plate (16.3 µg/plate)	positive Scavenging of generated free radicals caused a decrease in mutagenicity.	de Kok et al. (1992)
S. typhimurium strains TA97a and TA102	*his* gene mutations	+/-	cumene hydroperoxide, purity n.p.	50-200 µg/plate (0.33-1.31 µmol/plate)	positive	Wilcox et al. (1993)
S. typhimurium strain	*his* gene mutations	-	cumene	0.197-0.986 µmol/plate	positive	Kranendonk et

Table 7. Genotoxicity of Cumene Hydroperoxide (Continued)

Test System	Biological Endpoint	S9 Metabolic Activation	Chemical Form, Purity	Dose	Endpoint Response	Reference
TA102			hydroperoxide, purity n.p.	(30.0-150 µg/plate)		al. (1996)
S. typhimurium TA97a, TA100, TA102 and TA104	*his* gene mutations	+/-	cumene hydroperoxide, purity n.p.	1.67-500 µg/plate (0.012-3.28 µmol/plate)	Positive in TA97a, TA102, TA104, +/- S9. W+ in TA100 +S9. Equivocal in TA100 without S9.	Dillon et al. (1998)
S. typhimurium TA7000 series (7001-7006), TA98, and TA1537	*his* gene mutations	+/-	cumene hydroperoxide, 80% pure (technical grade)	1-100 µg/mL (0.007-0.7 µmol/plate)	-S9 +S9 TA7001 - + TA7002 + - TA7003 - - TA7004 + + TA7005 + + TA7006 + + TA7007 + + TA7000 series mix + + TA1537 + + TA98 + -	Gee et al. (1998)
Escherichia coli WP2 (pkM101) and WP2 *uvrA* (pkM101)	*TrpE* gene mutations	-	cumene hydroperoxide, purity n.p.	30-200 µg/plate (0.20-1.31 µmol/plate)	positive	Wilcox et al. (1990)
E. coli strain GE94 and its DNA repair deficient derivatives KY946[*uvrA*], KY945[*recA*], and KY943[*lexA*]	-Galactosidase activity (SOS response)	+	cumene hydroperoxide, purity n.p.	1-33 µg/mL (0.007 - 0.22 µM)	Positive in KY946[*uvrA*] and GE94; negative in KY945[*recA*] and KY943[*lexA*]	Nunoshiba and Nishioka (1991)
E. coli PQ300 and PQ37	oxidative DNA damage, SOS response	-	cumene hydroperoxide, purity n.p.	1.0-264 µM (0.15-40.2 µg/mL)	positive	Müller and Janz (1992)
E. coli (±pKM101)-strains WP2s, ZA570 (*mutY*), ZA580 (*mutM*), ZA590 (*mutYmutM*), ZA700	Mutagenic activity, SOS response	-	cumene hydroperoxide, purity n.p.	Mutagenicity: 1, 3, 10 µg/plate (0.007, 0.02, 0.066 µmol/plate) SOS inducing activity:	Mutagenesis: positive SOS induction: positive	Kato et al. (1994)

Table 7. Genotoxicity of Cumene Hydroperoxide (Continued)

Test System	Biological Endpoint	S9 Metabolic Activation	Chemical Form, Purity	Dose	Endpoint Response	Reference
(*soxRS*), ZA770 (*mutYsoxRS*), and ZA780 (*mutMsoxRS*); ZA201, ZA480 (*mutM*), and ZA700 (*soxRS*)				5, 10, 20 µg/mL (30, 66, 130 µM)		
E. coli strains IC3821, (*oxyR30*) IC3789 (*oxyR+*), IC3841 (*oxyRmut+*), IC2869 (*oxyR+mut+*), IC3894 (*oxyRmutY*), and IC3793 (*oxyR+mutY*)	*Trp+* revertants in strains with and without SOS repair	-	cumene hydroperoxide, purity n.p.	25 and 50 µg/plate (0.16 and 0.33 µmol/plate)	positive; greater response in strains that had SOS repair capacity	Urios et al. (1995)
E. coli strain MX100	*argE3* gene mutations	-	cumene hydroperoxide, purity n.p.	0.197-0.986 µmol/plate (30.0-150 µg/plate)	positive	Kranendonk et al. (1996)
9.6.3 Lower Eukaryotic Systems						
Saccaromyces cerevisiae (D4)	mitotic gene conversion at *trp5* and *ade2*	-	cumene hydroperoxide, purity n.p., fuel oil irradiated with u.v. light, purity, n.p.	cumene hydroperoxide (0-0.56 mM; 0-85 µg/mL); fuel oil, (up to 20 ppt) with u.v. light (up to 96 hr)	Incubation of cells with irradiated fuel oil resulted in 50% cell death and significant levels of conversion. Cumene hydroperoxide induced dose-dependent cytotoxicity, but did not induce conversions.	Callen and Larson (1978)
S. cerevisiae (RS112)	intrachromosomal recombination	-	cumene hydroperoxide, purity n.p.	30-50 µg/mL (0.20-0.33 mM)	positive	Brennan et al. (1994)
9.6.4 In Vitro Mammalian Systems						
Human P31 cells	DNA SSB and cytotoxicity	+/-	cumene hydroperoxide, purity, n.p.	0.1-10 mM (0.02-1.5 mg/mL), at 0°C and 37°C	5-fold increase in cytotoxicity at 37°C (+S9) versus 0°C (-S9). SSB induction did not correlate well with degree of cytotoxicity. The rejoining pattern of SSB did not change with temperature; increase in the total amount of	Sandstrom (1991)

Table 7. Genotoxicity of Cumene Hydroperoxide (Continued)

Test System	Biological Endpoint	S9 Metabolic Activation	Chemical Form, Purity	Dose	Endpoint Response	Reference
					SSB at 37°C.	
Human myeloid leukemia (U937) cells	DNA SSB	-	cumene hydroperoxide, purity n.p.	30-300 µM (4.6-45.7 µg/mL)	positive	Guidarelli et al. (1997)
9.6.5 In Vivo Mammalian Systems						
ICR/Ha Swiss mice, male and female, 8-10 weeks old	Dominant lethal mutations	NA	cumene hydroperoxide, purity n.p.	Male mice were administered a single i.p. dose of cumene hydroperoxide, 34 or 90 mg/kg (0.22 or 0.59 mmol/kg) (LD_5 and LD_{50}), respectively.	Negative for the single week of matings that were performed (measuring mature spermatozoa). The study is inadequate to determine dominant lethality for other stages of spermatogenesis.	Epstein et. al. (1972)

Abbreviations: + = positive; - = negative; LD_5 = lethal dose in 5% of test animals; LD_{50} = lethal dose in 50% of test animals; IF = increase in the induction factor; NA = not applicable; n.p. = not provided; rev = number of revertants; SSB = single strand breaks; w = weak.

9.7 Cogenotoxicity

Studies discussed in this section are presented in **Table 8**.

9.7.1 Acellular Systems

The role of cumene hydroperoxide in enhancing asbestos-induced DNA damage was investigated in calf thymus DNA (Mahmood et al., 1994). The presence of cumene hydroperoxide (40 mM; 6.1 mg/mL) or asbestos (1 mg/mL) alone had little effect on DNA integrity, while the presence of both cumene hydroperoxide and asbestos enhanced several fold the susceptibility of the DNA to S1 nuclease hydrolysis (a marker for DNA SSB).

9.7.2 Prokaryotic Systems

Rueff et al. (1992) investigated the use of a novel biomimetic system to promote the *in vitro* activation of promutagens to active *Salmonella* mutagens. *S. typhimurium* strain TA98 was used along with 8 known *Salmonella* mutagens and various oxygen donors, including cumene hydroperoxide, in the presence of tetraphenylporphinatoiron(III) chloride (TPP). Neither cumene hydroperoxide (3.2 mM; 0.49 mg/mL) nor TPP (0.6 mM) were mutagenic in TA98 alone, but 6 of the 8 test compounds were converted to active mutagens in the presence of cumene hydroperoxide plus TPP. The strongest response was obtained with the heterocyclic amine, 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ).

9.7.3 Lower Eukaryotic Systems

The effect of cumene hydroperoxide on the activation of the promutagens aflatoxin B1, -naphthylamine, dimethylsulfoxide, ethyl carbamate, and dimethyl nitrosamine was investigated in the yeast *S. cerevisiae* D4 (Callen et al., 1978). The ability of all promutagens to induce gene conversion was increased in the presence of cumene hydroperoxide (1 mM; 0.2 mg/mL). The bioactivation was highest in cells containing the highest levels of cytochrome P450, measured by spectral analysis.

Table 8. Cogenotoxicity of Cumene Hydroperoxide

Test System	Biological Endpoint	S9 Metabolic Activation	Chemical Form, Purity	Dose	Endpoint Response	Reference
9.7.1 Acellular Systems						
Calf thymus DNA	DNA susceptibility to S_1 nuclease (a marker for SSB)	-	asbestos and cumene hydroperoxide, purities n.p.	DNA (1 mg/mL) was incubated with asbestos (1 mg/mL) and cumene hydroperoxide (40 mM; 6.1 mg/mL), alone and in combination	Cumene hydroperoxide alone did not induce SSB; with asbestos, cumene hydroperoxide induced SSB.	Mahmood et al. (1994)
9.7.2 Prokaryotic Systems						
S. typhimurium strain TA98	*his* gene mutations	+	cumene hydroperoxide, purity n.p.	3.2 mM (0.49 mg/mL) cumene hydroperoxide with 8 different chemicals requiring activation for a positive response in the *Salmonella* gene mutation assay	Cumene hydroperoxide by itself was negative; a dose related strong + with IQ (0.1-1.8 nmol/plate); w+ with B(a)P; + with methylcholanthrene, DMBA, and 2-AF; - with 2-AFF and quercetin.	Rueff et al. (1992)
9.7.3 Lower Eukaryotic Systems						
S. cerevisiae (D4)	mitotic gene conversion at *trp5* and *ade2*	-	cumene hydroperoxide, purity n.p.	dimethyl nitrosamine, aflatoxin B1, - naphthylamine, dimethylsulfoxide, or ethyl carbamate, in the presence and absence of cumene hydroperoxide (1 mM; 0.2 mg/mL)	Cumene hydroperoxide alone was nonconvertogenic. Promutagens with 1 mM cumene hydroperoxide were all positive.	Callen et al. (1978)

Abbreviations: 2-AF = 2-aminofluorene; 2-AFF = 2-acetylaminofluorene; B(a)P = benzo(a)pyrene; DMBA = 7,12-dimethylbenz[a]anthracene; IQ = 2-amino-3-methylimidazo[4,5-*f*]quinoline; NA = not applicable; N.D. = no data; n.p. = not provided; SSB = single strand break(s).

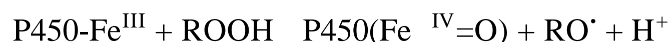
9.8 Immunotoxicity

Shimura et al. (1985) found that cumene hydroperoxide (10-1000 µM; 1.5-152.2 µg/mL) depressed concanavalin A stimulation of splenocytes from Fischer 344 rats, without affecting their viability. This action was attributed to radical species formation mediated by the hydroperoxyl part of cumene hydroperoxide, with resulting cross-linking of proteins.

9.9 Other Data

9.9.1 Mechanism of Cumene Hydroperoxide Activity

The production of radicals from cumene hydroperoxide occurs via homolytic cleavage by the P450 (Barr et al., 1996). The reaction involves the homolytic scission of the peroxide O O bond to produce the cumoxyl radical. The following mechanism illustrates the process:



$$P450\text{-}Fe^{III} + ROOH \quad P450(Fe^{IV}{=}O) + RO^{\bullet} + H^{+}$$

Cumene hydroperoxide can reportedly support cytochrome P450-catalyzed reactions, even in the absence of oxygen, NADPH, and, cytochrome P540-NADPH oxido reductase (Cvrk and Strobel, 1998).

The role of free-radical trapping in the prevention of cell damage by cumene hydroperoxide was studied by Tsai et al. (1997). Polyhydroxylated C_{60} (fullerenol), a free-radical trapper, prevented cumene hydroperoxide-elicited damage in hippocampal slices from male Wistar rats *in vitro*. Cumene hydroperoxide (0.5-1.0 mM; 76-152 µg/mL) reversibly reduced the amplitudes of CA1-evoded population spikes in the hippocampal slices. The free-radical scavenging activity of polyhydroxylated C_{60} (0.1 mM) prevented reduction of the population spikes and also prevented the effects of cumene hydroperoxide on paired-pulse facilitation.

The production of free radicals during the metabolism of organic peroxides in normal human keratinocytes was investigated by Iannone et al. (1993). An electron spin resonance-spin trapping technique was used to capture the formation of methyl free radicals after one-electron

oxidation or reduction of cumene hydroperoxide in isolated and cultured normal human keratinocytes. Cumene hydroperoxide was cytotoxic at 25 mM (3.8 mg/mL) after 30 minutes of incubation, while a concentration of 1 mM (152 µg/mL) hydroperoxide did not affect cell survival. However, free radical production was detected over the entire concentration range.

Cumene hydroperoxide has been shown to produce free radicals in the presence of metals. Using electron spin resonance (ESR) spin trapping, incubation of 1 mM Cr(III) with 10 mM (10,000 µM) cumene hydroperoxide at physiological pH generated a spectrum characterizing the presence of cumene hydroperoxide-derived free radicals (Shi et al., 1993). Another study found Cr(VI) capable of generating free radicals from the hydroperoxide in the presence of thiols (i.e., cysteine and penicillamine) (Shi et al., 1994a), indicating that Cr(V) and the free radical intermediates may play an important role in the mechanism of Cr(VI) carcinogenicity.

In a study conducted by Athar et al. (1989), the addition of cumene hydroperoxide (40 mM; 6.1 mg/mL) to incubations of keratinocytes prepared from squamous cell carcinoma produced an ESR spectrum showing the presence of two radical adducts. These findings provided the first direct evidence that human carcinoma skin keratinocytes metabolize cumene hydroperoxide into oxygen-centered and carbon-centered radicals (Athar et al., 1989; cited by Kensler et al., 1995; Taffe et al., 1987). Once formed, the unstable free radicals undergo fragmentation, addition, hydrogen-abstraction, or substitution reactions leading to the modification of cellular molecules (e.g., protein oxidation or alkylation, lipid peroxidation, and/or DNA damage) (Kensler, 1989; Trush and Kensler, 1991; Kensler et al., 1995).

Timmins and Davies (1993; 1994) studied the production of free radicals from cumene hydroperoxide in isolated murine keratinocytes using electron paramagnetic resonance (EPR) spin-trapping. The addition of cumene hydroperoxide (1 mM; 0.2 mg/mL) to the keratinocyte cultures in the presence of the spin-trap 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO, 50 mM) produced several radical species with the signal from the alkoxyl radical adduct being very weak. This was expected, since the alkoxyl radical undergoes rapid -scission to give the methyl radical. Topical application of cumene hydroperoxide (0.1 mM; 15.2 µg/mL in acetone) to full thickness biopsies of murine skin produced an EPR spectrum showing the presence of the ascorbyl radical

species, indicating that cumene hydroperoxide reacts to form the species either intracellularly or in the extracellular matrix. The radical species then reacts with ascorbate in viable cells of the epidermis or dermis (Timmins and Davies, 1994).

Oxygen radicals may attack DNA at either the sugar or the base (Imlay and Linn, 1988). Attack at a sugar leads to sugar fragmentation, base loss, and a strand break with a terminal fragmented sugar residue. Attack at bases can produce ring-saturated thymines, hydroxymethyluracil, thymine fragments, and adenine ring-opened products (Imlay and Linn, 1988).

9.9.2 Metabolic Effects

Cumene hydroperoxide (0.05-0.5 mM; 7.6-76.1 µg/mL) has been shown to affect the metabolism of xenobiotics *in vitro*. A study of effects on the microsomal-dependent metabolism of benzo(a)pyrene using induced and uninduced liver microsomes from male Wistar rats indicated that total benzo(a)pyrene metabolism was dependent on cumene hydroperoxide concentration and was maximal at 0.15 mM (23 µg/mL) (Wong et al., 1986).

In the presence of cumene hydroperoxide (0.2 µM; 0.0304 µg/mL), microsomes prepared from rat liver catalyzed the disappearance of the carcinogen *N*-hydroxy-*N*-acetyl-2-aminofluorene (N-OH-AFF), as evidenced in optical difference spectra (Floyd, 1976). Upon completion of the reaction, addition of a new batch of the reactants provided very little sequential reaction; addition of larger amounts of cumene hydroperoxide and N-OH-AFF, together or separately, in varying concentrations did the same. This was speculated as cumene hydroperoxide inactivation of the system-perhaps oxidative reactions with N-OH-AFF acting as an electron donor.

10.0 STRUCTURE-ACTIVITY RELATIONSHIPS

Within the class of organic peroxides, alkyl hydroperoxides, such as cumene hydroperoxide, are of the highest in terms of concern for carcinogenicity (Lai et al., 1996). When compared to other organic peroxides (e.g., *p*-menthane hydroperoxide, diisopropyl benzene hydroperoxide, methyl ethyl ketone peroxide; di-*t*-butyl peroxide), cumene hydroperoxide

produced more malignant lymphomas (Kotin and Falk, 1963). Structure-activity relationship analysis shows that alkyl hydroperoxides exhibit mutagenicity and potential carcinogenicity probably because they are more stable than other peroxides and have a better chance of remaining active, or because they can generate small alkyl radicals (e.g., methyl radicals) that interact with DNA to initiate carcinogenesis (Lai et al., 1996). Also contributing to the concern for the potential carcinogenicity of cumene hydroperoxide is the fact that organic peroxides may lead to the formation of epoxides which have been shown to be carcinogenic (Kotin and Falk, 1963).

11.0 ONLINE DATABASES AND SECONDARY REFERENCES

11.1 Online Databases

Chemical Information System Files

SANSS (Structure and Nomenclature Search System)
TSCATS (Toxic Substances Control Act Test Submissions)
TSCAPP (TSCA Plant and Production Search System)

DIALOG Files

Chemical Economics Handbook (CEH) (February 1998, SRI International)

National Library of Medicine Databases

EMIC and EMICBACK (Environmental Mutagen Information Center)

STN International Files

BIOSIS
CANCERLIT
CAPLUS
CHEMLIST
EMBASE
HSDB
MEDLINE
Registry
RTECS
TOXLINE

TOXLINE includes the following subfiles:

Toxicity Bibliography	TOXBIB
International Labor Office	CIS
Hazardous Materials Technical Center	HMTC
Environmental Mutagen Information Center File	EMIC
Environmental Teratology Information Center File (continued after 1989 by DART)	ETIC
Toxicology Document and Data Depository	NTIS
Toxicological Research Projects	CRISP
NIOSHTIC7	NIOSH
Pesticides Abstracts	PESTAB
Poisonous Plants Bibliography	PPBIB
Aneuploidy	ANEUPL
Epidemiology Information System	EPIDEM
Toxic Substances Control Act Test Submissions	TSCATS
Toxicological Aspects of Environmental Health	BIOSIS
International Pharmaceutical Abstracts	IPA

Federal Research in Progress	FEDRIP
Developmental and Reproductive Toxicology	DART

In-House Databases

CPI Electronic Publishing Federal Databases on CD
Current Contents on Diskette 7

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APPENDIX A - UNITS AND ABBREVIATIONS

2-AF = 2-aminofluorene

2-AFF = 2-acetylaminofluorene

°C = degrees Celsius

µg/m^3 = micrograms per cubic meter

µg/mL = micrograms per milliliter

µM = micromolar

ADPRT = adenosine diphosphate ribosyl transferase

AIB = "-amino-isobutyric acid

ASTD = Atmospheric Standards

B(a)P = benzo(a)pyrene

cal/g = calories per gram

CERCLA = Comprehensive Environmental Response, Compensation, and Liability Act

CFR = Code of Federal Regulations

d = day(s)

DMBA = 7,12-dimethylbenz[a]anthracene

DMPO = 5,5-dimethyl-1-pyroline-N-oxide

DMSO = dimethyl sulfoxide

DOT = Department of Transportation

DSB = double stranded break

dynes/cm = dynes per centimeter

EPA = Environmental Protection Agency

EPR = electron paramagnetic resonance

ESR = electron spin resonance

F = female

FANFT = N-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide

FDA = Food and Drug Administration

g = grams

g/mL = grams per milliliter

GSH = glutathion

GSSG = oxidized glutathion

GST = glutathione-*S*-transferase

h = hour(s)

HC = hydrocarbon

Hg = mercury

i.p. = intraperitoneal injection

IQ = 2-amino-3-methylimidazo[4,5-*f*]quinoline

kg = kilograms

lb = pounds

LC_{50} = lethal concentration for 50% of test animals

LD_{50} = lethal dose for 50% of test animals

LDH = lactate dehydrogenase

LT_{50} = lethal time for 50% of test animals

M = male

Mg = metric tons

mg/kg = milligrams per kilogram

mg/m^3 = milligrams per cubic meter

mg/mL = milligrams per milliliter

min. = minutes

mL/kg = milliliters per kilogram

mm = millimeters

mM = millimolar

mmol = millimoles

mmols/kg = millimoles per kilogram

mo = month(s)

n = number of samples in the set

NA = not applicable

N.D. = no data

n.p. = not provided

nm = nanometers

nmols/plate = nanomoles per plate

NRC = National Response Center

N-OH-AFF = N-hydroxy-N-acetyl-2-aminoflourine

OSHA = Occupational Safety and Health Administration

ppm = parts per million

ppt = parts per trillion

RCRA = Resource Conservation and Recovery Act

s.c. = subcutaneous

SOCMI = Synthetic Organic Chemical Manufacturing Industry

SSB = single strand break(s) in DNA

TBARS = thiobarbituric acid reactive substances

TSCA = Toxic Substances Control Act

UV = ultraviolet

VOC = volatile organic compounds

wk = week(s)

yr = year(s)