

SUMMARY OF DATA FOR CHEMICAL SELECTION

***tert*-Butyl hydroperoxide**

75-91-2

BASIS OF NOMINATION TO THE CSWG

tert-Butyl hydroperoxide (*t*-BuOOH) is brought to the attention of the CSWG as a relatively stable alkyl hydroperoxide that has high production volume due to its use as a polymerization initiator.

Although *t*-BuOOH has an annual production volume exceeding a million pounds, its toxicity is not being evaluated by industry under the US Environmental Protection Agency (EPA) High Production Volume (HPV) Challenge Program. Available information on *t*-BuOOH was evaluated in the OECD/SIDS (Screening Information Data Set) program, and a report on human and environmental exposure has been issued by this group. OECD/SIDS noted that a test on inherent biodegradability, a semichronic toxicity test, and an *in vivo* chromosome aberration test should also be carried out.

As a selective reagent for the introduction of the peroxy group into organic substrates, *t*-BuOOH is important in the evaluation of new tester strains for detection of DNA damage or mutagenicity. This has led to a substantial database of mechanistic data, although uses of these data are curtailed by the lack of chronic carcinogenicity testing and the limited information on *t*-BuOOH as a tumor promoter. *t*-BuOOH has also not been evaluated in any transgenic animal models.

INPUT FROM GOVERNMENT AGENCIES/INDUSTRY

Dr. John Walker of the Interagency Testing Committee (ITC) provided information on previous ITC actions involving tertiary peroxides and on production volumes of *t*-BuOOH. The ITC did not move forward on these peroxides because of their inherent instability and their use primarily in closed industrial systems. The ITC selection criteria do not consider testing to fill significant research gaps on potentially carcinogenic chemicals.

Dr. Edwin Matthews, Director of Computational Toxicology, Center for Drug Evaluation Research, Food and Drug Administration (FDA) provided a structure-activity assessment of simple organic peroxides to assist in the selection of compounds from this class. The assessment, based on the MCASE Software Program developed under a Cooperative Research and Development Agreement between FDA and Multicase, Inc, predicted that *t*-BuOOH is probably carcinogenic. This prediction involved a high degree of uncertainty because of the paucity of data on the carcinogenicity of organic peroxides.

SELECTION STATUS

ACTION BY CSWG: 7/26/01

Studies requested:

- Carcinogenicity

Priority: High

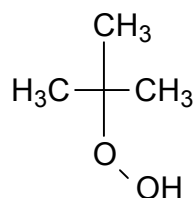
Rationale/Remarks:

- Relatively stable alkyl hydroperoxide
- High production volume chemical used as a polymerization initiator, but primary uses may involve closed systems, thus limiting worker exposure
- Significant gap in research knowledge; A:T base-pair mutagen used as a positive control that has not been tested for carcinogenic activity
- Consider including mechanistic studies, since role of iron and metabolism to *t*-butyl alcohol may be relevant to evaluation of carcinogenic activity
- Consider skin painting as a possibly appropriate route of administration
- NTP should coordinate testing with EPA's HPV Program

CHEMICAL IDENTIFICATION

<u>CAS Registry Number:</u>	75-91-2
<u>CAS Name:</u>	1,1-Dimethylethyl hydroperoxide (9CI)
<u>Synonyms and Trade Names:</u>	<i>t</i> -Butyl hydroperoxide; Cadox TBH; 2-hydroperoxy-2-methylpropane; Luperox TBH70; Perbutyl H; Slimicide DE-488; Trigonox a-75; Trigonox AW70; UN 2093; UN 2094
<u>Structural class:</u>	Alkyl hydroperoxide

Structure, Molecular Formula and Molecular Weight:



$\text{C}_4\text{H}_{10}\text{O}_2$

Mol. wt.: 90.12

Chemical and Physical Properties:

<u>Description:</u>	Water-white liquid (Lewis, 1993)
<u>Melting Point:</u>	6EC (CRC Press, 1996); 4.0-4.5EC (Sanchez & Myers, 1996); -8EC (Merck, 2000)
<u>Boiling Point:</u>	75EC (Lewis, 1993); 89EC @ 76 mm Hg (decomposes at 36EC) (CRC Press, 1996)
<u>Solubility:</u>	Sol. in water, ethyl ether, ethanol, carbon tetrachloride (CRC Press, 1996); sol. in chloroform (NTP, 2001); very sol. in organic solvents and alkali-metal hydroxides, decomposes at 75EC (Lewis, 1993)
<u>Vapor Density:</u>	0.896 (20/4EC) (Lewis, 1993; Merck 2000)
<u>Flash Point:</u>	130EF (54.4EC) (90%) (Lewis, 1993); 109EF (70 wt % in water) (Sigma-Aldrich, 2001a)
<u>O/W Partition Coefficient:</u>	$\text{Log } P_{o/w} = 0.7$ @ 25 EC (OECD, 2001)

Reactivity:

Combustible, oxidizer, moderate fire risk (Lewis, 1993); risk of explosion if heated under confinement (Fluka Chemicals, 2001); incompatible with strong acids and bases, strong reducing agents, organic materials, and finely powdered metals (Fluka Chemicals, 2001)

Liquid stable to 75EC (Merck, 2000); decomposition increases markedly with increased temperature and may be accelerated by the presence of Cu, Co, and Mn salts (Sigma-Aldrich, 2001b; Merck, 2000; Sanchez & Myers, 1996)

Technical Products and Impurities: Commonly available *t*-BuOOH is supplied as a 70% solution in water; 80% solutions are also available, and *t*-BuOOH can be purchased as 5-6 M solutions in nonane or decane (Sigma-Aldrich, 2001a).

Commercial free radical polymerizers are described by their decomposition rates, which are generally defined as the 10-hour half-life temperature for polymer processing. These rates depend on both the organic peroxide and the solvent system. The widely used Atofina products, Luperox TBH70 and Luperox TBH70X have 10-hr half-life temperatures of 117 and 172EC (Brandrup *et al.*, 1999; Atofina, 2001).

The primary impurity in most commercial *t*-BuOOH products is *t*-butyl alcohol. Other impurities may include dialkyl peroxides, hydroperoxides, and other organic compounds (OECD, 2001).

EXPOSURE INFORMATION

Production and Producers:

Manufacturing Process. *t*-BuOOH has been produced commercially by mixing either *tert*-butyl alcohol or isobutylene with sulfuric acid followed by reaction with hydrogen peroxide (Sanchez & Myers, 1996). *t*-BuOOH can also be prepared from *t*-butyl alcohol and 30% hydrogen peroxide in the presence of sulfuric acid or by oxidation of *tert*-butylmagnesium chloride (NLM, 2001d). *t*-BuOOH is manufactured in a closed system (OECD, 2001).

Producers and Importers. More than 100 commercially available organic peroxides are used in over 200 formulations. With the exception of the peroxyacids, the polymer industry consumes approximately 90% of all commercial peroxides. Akzo-Nobel and Elf Atochem are the largest producers of organic peroxides, each holding about 30 percent of the global market. They are followed by Laporte, which holds 15 percent of the market through its US subsidiary, Aztec Peroxides, and its German subsidiary, Peroxid Chemie. In the US, CK Witco, Norac, and Hercules are also important producers (Boswell, 2000; Sanchez & Myers, 1996).

Fourteen US producers or distributors of *t*-BuOOH are listed by Chemical Sources International (2001). Sources of *t*-BuOOH listed by the American Chemical Society include Acros-USA, Alfa Aesar, ICN, Lancaster, and Pfaltz & Bauer (Chemical Abstracts, 2001). Other producers or distributors include Akzo Nobel, ARCO Chemical Co., Elf Atochem North America, Sigma-Aldrich, Fine Chemicals Sithean Corporation, TRW Enterprises, and Witco Corp. (Chemyclopedia Online, 2001).¹

Production/Import/Export Level. *t*-BuOOH is a high production volume compound; according to EPA, production in 1994 was over 1 million pounds (EPA, 2001). US production was estimated to be probably greater than 4.54×10^5 g in 1972 and 2.27×10^5 g

¹According to the Atofina website, on June 19, 2000, Elf Atochem changed its name to Atofina Chemicals, Inc., reflecting a reorganization following the merger of TotalFina and Elf Aquitaine.

in 1975 (NLM, 2001d). *t*-BuOOH is listed in the EPA Toxic Substances Control Act (TSCA) Inventory (NLM, 2001b).

t-BuOOH is listed as a chemical of commerce in the U.S. International Trade Commission (USITC) publication *Synthetic Organic Chemicals, US Production and Sales* for the years 1983 to 1993 (USITC, 1984 - 1994). No production or sales quantities were disclosed. According to the USITC, separate statistics were not published to avoid disclosure of individual operations; however the USITC reporting guidelines specify that each company's report of a chemical represents production of \$30,726 kg [10,000 lbs] or sales \$\$17,314.

The Port Import/Export Reporting Service (PIERS) reported *t*-BuOOH exports of 879,378 lbs over the 4-year period from March 11, 1997 to May 15, 2001. Exporting manufacturers included Atofina Chemicals and Lyondell Petrochemical (DIALOG Information Services, 2001a).

Imports of 106,654 lbs were reported over the 11 month period from July 19, 2000 to June 1, 2001. Imports were primarily from Japan and the Netherlands (DIALOG Information Services, 2001b).

Use Pattern:

t-BuOOH is one of the most widely used cationic emulsion polymerization initiator systems, and it is also used as a catalyst in polymerization reactions to produce polyethylene, PVC, unsaturated polyesters, and related products. Typical applications include bulk, solution, and suspension polymerization and emulsion polymerization. Its use as a thermal polymerization initiator is limited because of a high 10-hour half life (125-172EC) and extreme sensitivity to radical-induced decompositions and transition-metal activation (Atofina, 2000; OECD, 2001; Sanchez & Myers, 1995; Worldplas.com, 2001).

A significant outlet for *t*-BuOOH is the molybdenum-complex catalyzed production of propylene oxide. The reported US capacity in 1991 was 0.55×10^6 t/yr (Hobbs, 1995). Other minor uses of *t*-BuOOH may include bleaching and deodorizing (Lewis, 1993).

A small fraction of *t*-BuOOH is used as a reagent (OECD, 2001). In research, it is used to examine the effects of oxidant stress on Ca^{2+} -dependent signal transduction in vascular endothelial cells (NLM, 2001b). *t*-BuOOH is becoming a standard for toxicity testing because it inflicts serious damage on cell membranes, readily detected as cytosolic enzyme leakage, via lipid peroxidation (Modrianský *et al.*, 2000). *t*-BuOOH use to study the effects of oxidative stress on mitochondria is also favored because it is not metabolized by catalase, a frequent contaminant in mitochondrial preparations (Kennedy *et al.*, 1992).

Human Exposure:

Occupational Exposure. The National Occupational Exposure Survey (NOES), which was conducted by the National Institute for Occupational Safety and Health (NIOSH) between 1981 and 1983, estimated that 12,168 workers (of which 4,787 are female) in 266 facilities representing 8 industries were potentially exposed to *t*-BuOOH in the workplace. The NOES database does not contain information on the frequency, level, or duration of exposure to workers of any chemical listed there in (DIALOG Information Services, 2001c).

Occupational exposure may occur through dermal contact or inhalation of this product at workplaces where it is used or produced (NLM, 2001d). One estimate of workplace exposure was available. Peak concentration (limited access) was 6 ppm; “worst case” at access points on walkways was 0.39 ppm with a mean of <0.1 ppm. The “worst case” expected human exposure was 1.44 mg/m^3 (0.39 ppm) with expected human exposure of 0.37 mg/m^3 (0.1 ppm) (OECD, 2001).

Environmental Exposure. Production and use of *t*-BuOOH as a chemical intermediate may result in its release to the environment through various waste streams (NLM, 2001d).

Plant emissions to air from one facility in the US and one facility in the Netherlands, as a percent of production, have been estimated to be 0.0413 percent of 14,000 T/yr and 0.016 percent of 17,000 T/y, respectively (OECD, 2001). If released into air, *t*-BuOOH will exist solely as vapor in the atmosphere and would be degraded (half life = 5 days at 25EC) (NLM, 2001d).

Plant emissions to water were estimated to be 0.002 percent of production capacity of 14,000 T/yr (OECD, 2001). If released into water *t*-BuOOH is not expected to adsorb to suspended solids and sediments. An estimated bioconcentration factor (BCF) of 3 suggests that the potential for bioconcentration in aquatic organisms is low. In soil *t*-BuOOH is expected to have high mobility. *t*-BuOOH should react with organic matter in soil and water and be rapidly decomposed by metal ions, being converted to the corresponding alcohol (NLM, 2001d).

Consumer Exposure. Consumer exposure might occur from minor uses of *t*-BuOOH as a bleaching or deodorizing agent. Consumer exposure might also occur if *t*-BuOOH is a component of the uncoated or coated food-contact surface of material used in manufacturing, packing, processing, preparing, treating, transporting, or holding foods.

Total daily intake, calculated via crops, milk, drinking water, and inhalation of air was estimated to be 0.803 $\mu\text{g}/\text{m}^3$ for persons living within 100 meters from plants using or producing *t*-BuOOH (OECD, 2001).

Environmental Occurrence: *t*-BuOOH has not been reported to occur naturally. Although *t*-BuOOH may enter the environment via industrial discharges and spills, because of the inherent instability of organic peroxides, *t*-BuOOH would not be expected to be a persistent environmental pollutant (NLM, 2001d).

Regulatory Status:

No standards or guidelines have been set by NIOSH or OSHA for workplace allowable levels of *t*-BuOOH. *t*-BuOOH is listed in Appendix A to the 1992 OSHA *Process Safety Management of Highly Hazardous Chemicals; Explosives and Blasting Agents* (29 CFR 1910.119) as a highly hazardous chemical which presents a potential for a catastrophic event at or above the threshold quantity of 5,000 pounds (57 *FR* 6356) (OSHA, 1992).

t-BuOOH was not on the American Conference of Governmental Industrial Hygienists (ACGIH) list of compounds for which recommendations for a Threshold Limit Value (TLV) or Biological Exposure Index (BEI) are made.

The Food and Drug Administration (FDA) has approved the use of *t*-BuOOH in adhesives, rubber articles intended for personal use, and in polymers for inclusion in food packaging (FDA, 2001).

Organic peroxides, including *t*-BuOOH are subject to various shipping regulations for dangerous materials. *t*-BuOOH (90%) is classified as Organic peroxide type C, liquid; Division 5.2; UN 3103 (NTP, 2001).

EVIDENCE FOR POSSIBLE CARCINOGENIC ACTIVITY

Human Data: No epidemiological studies or case reports investigating the association of exposure to *t*-BuOOH and cancer risk in humans were identified in the available literature.

Animal Data:

Acute Toxicity. The following information on the acute lethality of *t*-BuOOH was obtained from the Registry of Toxic Effects of Chemical Substances and the *IRPTC Data Profile for t-Butyl Hydroperoxide*: oral, rat, LD₅₀ = 370 mg/kg and 560 mg/kg; skin, rat LD₅₀ = 790 mg/kg; inhalation, rat, LC₅₀ = 500 ppm (4-hr); oral, mouse, LD₅₀ = 320 mg/kg; inhalation, mouse, LC₅₀ = 350 mg/kg; and skin, rabbit, LD₅₀ = 628 mg/kg (DIALOG, 2001c; OECD, 2001).

Carcinogenicity. No 2-year carcinogenicity studies of *t*-BuOOH were identified in the available literature.

In skin carcinogenicity studies by Van Duuren and coworkers (1967) and Hoshino and coworkers (1970), *t*-BuOOH applied (in benzene²) to the skin of mice either at 3% three times a week for 74 weeks or at 16.6% six times a week for 45 weeks had no tumorigenic effect (Watts, 1985).

Tumor Promotion. In contrast, 13 tumors were produced, nine malignant, among 38 mice painted with 16.6% *t*-BuOOH in benzene for 45 weeks after treatment with the initiator, 4-nitroquinoline 1-oxide (Hoshino *et al.*, 1970). The assessment of this result is complicated by the lack of a control group treated with the initiator followed by benzene alone and by the fact that 16.6% *t*-BuOOH had been described by the same investigators as having an irritant effect when applied repeatedly (Watts, 1985).

²The use of benzene as the solvent would not represent currently acceptable standard procedure because of the known carcinogenic effects of benzene.

In another experiment, papillomas were induced in Sencar mice using a two-stage initiation-promotion protocol and in SKH-1 mice using UVB radiation. *t*-BuOOH or 12-*O*-tetra-decanoylphorbol-13-acetate (TPA) was then applied topically to evaluate effects on malignant conversion. Continued application of *t*-BuOOH was nearly twice as effective as continued application of TPA in Sencar mice after a total of 44 weeks on test, and it was equally effective in SKH-1 mice after 51 weeks (Athar *et al.*, 1989).

Dietary administration of *t*-BuOOH to aflatoxin B₁-initiated trout enhanced hepatocarcinogenesis. Ten months after initiation, liver tumor incidences were 1.6 and 1.9 times control values in low dose (500 ppm) and high dose (1,500 ppm) fish, respectively. Multiple tumors were also observed more frequently in the peroxide-treated fish (Orner *et al.*, 1993).

Short-Term Tests: *t*-BuOOH has become a standard for testing the effectiveness of new bacterial strains in detecting oxidative damage. As such, considerable information on the genotoxicity of *t*-BuOOH has been generated in the scientific literature. Representative data are presented below in Table 1.

t-BuOOH is believed to be an SOS-dependent mutagen that damages A:T base pairs specifically (Levin & Ames, 1986). Ohta and coworkers (2000) found that *t*-BuOOH induced predominantly G:C6A:T, G:C6T:A, and A:T6T:A mutations in *Escherichia coli* tester strains in the WP3101P-WP3106P series and the *Salmonella typhimurium* tester strains TA7002, TA7004, and TA7005.

Table 1. Genotoxicity studies of t-butyl hydroperoxide.

Tests for Gene Mutation			
Test	Strain/Conditions	Results	References
Mammalian microsome test	<i>Salmonella</i> TA98, TA100 & TA1537 w S-9	positive	EPA Document No. 88-8300479, cited in NLM, 2001d
	<i>Salmonella</i> TA98, TA100, TA1535, TA1537 & TA1538 wo S-9; TA1535 & TA1538 w S-9	negative	EPA Document No. 88-8300479, cited in NLM, 2001d
	<i>Salmonella</i> TA102 wo S-9	positive	Hageman <i>et al.</i> , 1988; Levin <i>et al.</i> , 1982; Mersch-Sunderman & Krämer, 1993; Minnunni <i>et al.</i> , 1992; Ruiz-Rubio <i>et al.</i> , 1985
	TA98 & TA100 w S-9	positive	Yamaguchi & Yamashita, 1980
	TA104 wo S-9	positive	Levin & Ames, 1986
	TA2638 wo S-9	positive	Levin <i>et al.</i> , 1982; Watanabe <i>et al.</i> , 1998a,b
	TA7002, TA7004 & TA7005	positive	Ohta <i>et al.</i> , 2000
Tryptophan reversion test	<i>E. coli</i> IC203; IC206; OC207; IC208; IC5184; IC5185; IC5059; IC5064	positive	Blanco <i>et al.</i> , 1998
	<i>E. coli</i> IC3821	positive	Blanco <i>et al.</i> , 1995
	<i>E. coli</i> WP2/pKM101; WP2 <i>uvrA</i> /pKM101	positive	Blanco <i>et al.</i> , 1998; Watanabe <i>et al.</i> , 1998a,b
	<i>E. coli</i> <i>mutM</i> , <i>mutY</i> , or <i>SoxRS</i> mutants of WP2s	positive	Kato <i>et al.</i> , 1994
	<i>E. coli</i> IC188 w S-9 (WP2 Mutoxitest)	negative	Martínez <i>et al.</i> , 2000
	<i>E. coli</i> IC188 wo S-9 & IC203 w/wo S-9	positive	Martínez <i>et al.</i> , 2000
Arginine reversion test to detect oxidative mutagens	<i>E. coli</i> MX100	positive	Kranendonk <i>et al.</i> , 1996
Forward mutations/small deletions	<i>Neurospora crassa</i>	positive (high mortalities)	Dickey <i>et al.</i> , 1949, cited in Watts, 1985
Mutations at TK locus	Mouse lymphoma assay w/wo S-9	positive	EPA Document No, 88-8300479 cited in NLM, 2001d
SLRL test	<i>Drosophila</i> exposed as adults	equivocal - positive	NTP, 2000; Vogel <i>et al.</i> , 1999; Woodruff <i>et al.</i> , 1985

Second chromosome lethal mutation test	<i>Drosophila</i> exposed as embryos or adults	positive (embryos); negative (adults)	Altenberg, 1954
Tests for Chromosome Damage			
Test	Strain/Conditions	Results	Reference
Chromosomal aberrations (<i>in vivo</i>)	Outbred white rat bone marrow cells (exposure for 2.5 or 4 months)	positive	Katosova <i>et al.</i> , 1977
	Sprague-Dawley rat bone marrow cells (10-100 ppm up to 5 days)	negative	EPA Document No. 888300479 (cited in NLM, 2001d)
Dominant lethal test for chromosome damage	ICR/Ha Swiss mice; 15 or 75 mg/kg ip; mated 8 wk	negative	Epstein <i>et al.</i> , 1972
	CFT-Swiss mice; 30 µmol/kg ip; mated 5 wk	sig. incr. in dead implants for 4 wk	Kumar, 1999
	Hybrid mice; 2.05, 17.28, or 107.15 mg/m ³ for 2.5 or 4 months	positive	Katosova <i>et al.</i> , 1977
Reciprocal chromosomal translocation test	<i>Drosophila</i>	negative	Vogel <i>et al.</i> , 1999; Woodruff <i>et al.</i> , 1985
Tests for Aneuploidy			
Test	Strain/Conditions	Results	Reference
Aneuploidy in mammalian cells	Chinese hamster V79 lung cells	disturbances of spindle function (c-mitosis)	Önfelt, 1987
Other Indicators of Genetic Damage or Mutagen Exposure			
Test	Strain/Conditions	Results	References
SOS chromotest to identify DNA damage	<i>E. coli</i> PQ37 wo S-9	weak positive	Mersch-Sundermann <i>et al.</i> , 1994; Müller & Janz, 1992
	<i>E. coli</i> PQ300 wo S-9	positive	Müller & Janz, 1992
	<i>E. coli</i> PQ37, PM21, & GC4798	positive	Eder <i>et al.</i> , 1989
Rec-lac test for SOS gene expression	<i>E. coli</i> KY946 (DNA repair-deficient deriv. of GE94)	positive	Nunoshiba & Nishioka, 1991

Cell transformation assay	C3H/10T1/2 mouse cells	negative	EPA Document No. 88-8300479, cited in NLM, 2001d
DNA repair synthesis	Unscheduled incorporation of tritiated thymidine by UV-irradiated human fibroblasts	positive	Stich <i>et al.</i> , 1981
Mitotic gene conversion tests in yeast	<i>Saccharomyces cerevisiae</i>	positive	Callen & Larsen, 1978
Induction of prophage λ cI _{ts} 857	<i>E. coli</i> w S-9	positive	Ho & Ho (1979)
Metabolic cooperation assay for tumor promoting agents	Chinese hamster V79 lung fibroblasts	negative for gap-junction inhibition at noncytotoxic doses	Bohrman <i>et al.</i> , 1988

CA = chromosomal aberrations; ip = intraperitoneal; SCE = sister chromatid exchanges; SLRL = sex-linked recessive lethal; UDS = unscheduled DNA synthesis; w/wo = with/without

Metabolism: *t*-BuOOH is metabolized to *t*-butyl alcohol by a two-electron (non-radical) reduction process mediated by glutathione peroxidases. It is also degraded by catalytically active metal ions (heme proteins, such as cytochrome P450) to free radical intermediates (Iannone *et al.*, 1993; O'Brien, 1988).

Free radical intermediates formed via P450 isoenzymes and peroxidases are potential mediators of a broad spectrum of chemical toxicities. These free radicals are thought to produce lipid peroxidation, which is associated with alkali-labile DNA single-strand breaks, DNA-protein cross-links, and decreased nucleic acid synthesis in cell cultures (O'Brien, 1988; Timmins & Davis, 1993).

Lipid peroxidation can also be initiated by peroxy radicals formed by oxygen addition to highly reactive carbon radicals; these peroxy radicals can epoxidize xenobiotics and may be responsible for DNA strand breakage (O'Brien, 1988).

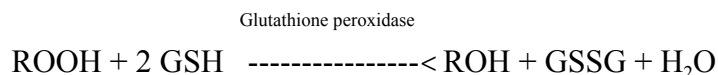
When nonrespiring liver mitochondria from male Sprague-Dawley rats were treated with *t*-BuOOH, methyl, *tert*-butoxyl, and *tert*-butylperoxy radicals were observed. A significant increase in methyl radical production was observed in respiring mitochondria.

Studies with iron chelators suggested that *t*-BuOOH interacts with a tightly liganded metal (e.g., heme iron) to form these free radicals (Kennedy *et al.*, 1991).

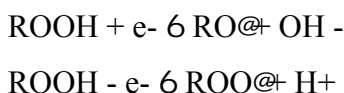
Cytochrome P450 catalyzes the formation of hydrocarbons from hydroperoxides via hydroperoxide-dependent nicotinamide adenine dinucleotide (phosphate) (NADPH) oxidation. For *t*-BuOOH oxidation in rabbit liver microsomes, P450 3A was the most active cytochrome followed by P450 2, whereas cytochromes 3B, 3C, 4, and 6 were much less active. With P450 2 and excess NADPH, when the reaction was allowed to go to completion, the hydroperoxide was completely consumed; acetone and methane in roughly a 1:1 molar ratio were determined to be the products. This reductive β -scission reaction is not the sole pathway with the less active cytochromes 3B, 3C, 4 and 6; for these cytochromes, increased formation of *t*-butyl alcohol from *t*-BuOOH would be expected (Vaz *et al.*, 1990).

Organic hydroperoxides alter mitochondrial function and cause mitochondrial membrane damage via the glutathione peroxidase-glutathione reductase system pathway as well as by radical pathways. In this nonradical pathway, *t*-BuOOH induces the hydrolysis of pyridine nucleotides and subsequent release of calcium from mitochondria (Bellomo *et al.*, 1984; Kennedy *et al.*, 1991; O'Brien, 1988).

Most hydroperoxide is metabolized to non-radical products via two-electron reduction by glutathione peroxidase enzymes, and the glutathione disulfide (GSSG) produced is then recycled.



As demonstrated in the equations below, however, hydroperoxides can generate free radicals via two major pathways, one-electron reduction to produce *t*-butoxyl and methyl radicals and one-electron oxidation to form *t*-butylperoxy radicals.



Detection of radical species suggests that degradation of organic peroxides via one-electron oxidation or reduction are significant pathways. Of the two processes that generate radicals, one-electron reduction is believed to be the more important under normal conditions (Iannone *et al.*, 1993; O'Brien, 1988; Timmins & Davis, 1993a).

If a free radical has a high one electron oxidation potential, it may be reactive enough to oxidize NADPH, glutathione (GSH), and polyunsaturated fatty acids. Free radicals formed by a peroxidase-catalyzed one electron oxidation can dismutate or undergo a further one electron oxidation to an electrophilic two electron oxidation product which can alkylate DNA bases (O'Brien, 1988).

Studies to Identify Mechanisms Leading to Tumor Promotion: *t*-BuOOH is an organic peroxide that shows activity in several short-term tests believed to be markers of tumor promotion, including hyperplasia, induction of dark basal keratinocytes, and induction of ornithine decarboxylase activity (Gimenez-Conti *et al.*, 1991). Perhaps of greatest relevance to existing studies of *t*-BuOOH tumor promotion, however, are data in the literature to suggest that the tumor-promoting activity of organic peroxides and hydroperoxides is related to metabolic activation to free radicals (Iannone *et al.*, 1993).

Free Radical Generation. Free radical generation in biological systems is usually studied using electron paramagnetic resonance (EPR) techniques and spin-trapping. This technique involves reaction of the initially-generated radical (itself either too short-lived or of too low a concentration to detect directly) with an added organic compound, known as a spin-trap, to generate stable radical adducts. The EPR spectra of these stable adducts provides information about the original radical (Timmins & Davis, 1993a,b).

Using spin trapping and EPR, it has been possible to demonstrate the formation of phenoxy and /or alkyl (methyl, phenyl) radicals in murine keratinocyte cultures treated with various organic peroxides. In 1987, Taffe and coworkers were able to generate radical adducts in keratinocyte isolates from Sencar mice in the presence of spin traps and

t-BuOOH. Timmins and Davis later extended this study to determine that production of ascorbyl radicals from *t*-BuOOH occurs in intact skin tissue; using the same technique with benzoyl peroxide and lauroyl peroxide did not produce measurable amounts of the ascorbyl radicals. Timmins and Davis concluded that free radical generation from tumor-promoting organic peroxides occurs in intact skin tissue through a one-electron reductive pathway and that the radical generation that takes place at the viable cells of the epidermis and/or dermis depends on the ability of the peroxide to penetrate the skin (Taffe *et al.*, 1987; Timmins & Davis, 1993b).

The capacity of freshly isolated and cultured keratinocytes from normal human skin (8 subjects) to activate *t*-BuOOH to a free radical intermediate has also been demonstrated by the spin-trapping technique (Iannone *et al.*, 1993).

It has been proposed that hemoglobin reacts with *t*-BuOOH to produce the *tert*-butylperoxyl radical which, through secondary reactions, produces *tert*-butoxy and methyl radicals that are able to oxidize thiols to their corresponding thiyl radicals (Hix *et al.*, 2000).

The *in vivo* metabolism of *t*-BuOOH was examined using EPR spin-trapping and DNA methylation studies in male Wistar rats given 1 mL/kg *t*-BuOOH by gavage. EPR signals were detected in the blood, bile, and organic extracts of the liver and stomach. Analysis of these signals demonstrated that *t*-BuOOH metabolism *in vivo* produces alkyl radicals, detected in the bile and liver and stomach extracts, and hemoglobin-thiyl radicals in the blood. 8-Methylguanine, a mutagenic lesion and typical product of methyl radical attack on DNA, was also detectable in both the liver and stomach of the treated rats (Hix *et al.*, 2000).

DNA Damage. DNA damage, primarily strand breaks have been reported by several authors in a number of *in vitro* test systems.

- In rat hepatocytes, DNA damage by *t*-BuOOH appeared to be single rather than double DNA strand breaks. Oxidative DNA modifications were not detected (Latour *et al.*, 1995).
- Significant DNA damage (strand breaks) was observed at high concentrations in plasmid pAQ1 of *S. typhimurium* strains TA1535/pAQ1 but not in TA1978/pAQ1 after incubation with *t*-BuOOH. According to the authors, the DNA damage observed was not the result of a direct reaction, but appeared to be mediated by radicals, and repair after treatment was very rapid (Epe *et al.*, 1990).
- In reactions that required intracellular free iron, *t*-BuOOH caused more DNA strand breakage and more extensive poly ADP-ribose accumulation in a nonpromotable clone of mouse epidermal cells JB6 than in a promotable clone. The promotable clone exhibited constitutive *c-myc* expression while *c-fos* mRNA was very low in untreated cultures of both clones. Low concentrations of *t*-BuOOH induced *c-myc* and more strongly *c-fos* in the promotable clone. Both proto-oncogenes were strongly induced in the nonpromotable clone (Müehlmatter *et al.*, 1989).
- The treatment of murine hybridoma cells with low concentrations of *t*-BuOOH resulted in DNA base damage in their chromatin. However, high concentrations of this hydroperoxide inhibited DNA base damage, leading the investigators to conclude that oxidative stress imposed on the cells and not *t*-BuOOH exposure by itself may be the direct cause of the DNA base damage observed (Altman *et al.*, 1994).
- In human myeloid leukemia U937 cells, formation of DNA single strand breaks induced by *t*-BuOOH was prevented by iron chelators, was not affected by antioxidants or glucose omission before and during peroxide exposure, and was enhanced by prior catalase depletion. Lowering basal intracellular calcium concentration markedly reduced the DNA-damaging efficiency of *t*-BuOOH (Guidarelli *et al.*, 1997).

- T:A-A:T and G:C-A:T transversions were observed in the SOS-proficient *E. coli* strain IC3821 and G:C-T:A transversions were observed in SOS-independent *E. coli* IC3894 and IC3981 strains (Urios & Blanco, 1996).

Cytotoxicity. The cytotoxic effects of *t*-BuOOH include GSH depletion, lipid peroxidation, hemolysis and oxidative denaturation of hemoglobin, permeabilization of cell membranes and impaired ATP synthesis, and DNA damage. These effects have been attributed to both radical and non radical mechanisms (Hix et al., 2000).

There was a 10-fold increase in cytotoxicity for *t*-BuOOH under metabolizing conditions (37EC) in comparison to nonmetabolizing conditions (0EC) when human P31 cells were exposed for 60 minutes. Although DNA single strand breaks were also observed, there was no clear-cut relationship between induction of these breaks and cytotoxicity (Sandström, 1991).

Other Biological Effects:

Peroxidases are widely distributed among nonhepatic target tissues and all aerobic organisms must cope with reactive oxygen species, such as the hydroxyl radical, formed in respiring cells. Several enzymes protect cells from oxidative damage including superoxide dismutase, catalase, and glutathione peroxidase. Various enzymes also repair macromolecules damaged oxidatively (O'Brien, 1988). However, such damage has been seen in a number of organs, as described below.

Liver Damage. Damage to the isolated perfused livers of male Wistar rats was demonstrated by an increase in lactate dehydrogenase (LDH), glutamate-pyruvate transaminase (GPT), and glutamate dehydrogenase (GLDH); decreased oxygen consumption; and nearly complete suppression of bile flow after the addition of 0.5 mmol/L of *t*-BuOOH to the perfusate. Hepatic adenosine triphosphate (ATP) and reduced glutathione (GSH) concentrations were also lowered. Glycine added 30 minutes before the toxicant prevented the enzyme releases by about 80 percent and attenuated the

reduction of oxygen consumption but did not alter the bile flow suppression nor affect the decreases of ATP levels (Deters *et al.*, 1998).

Extrusion of excess water and reaccumulation of K⁺ were completely inhibited and production of malondialdehyde (MDA) was increased in tissue slices of liver and hepatomas 9618A and 3924A. Lower cell contents of cytochrome P450 affected MDA levels but did not reduce the deleterious effects of *t*-BuOOH (Borrello *et al.*, 1988).

Treatment of male Wistar rats with 100 mg/kg *t*-BuOOH led to moderate hepatotoxicity as evidenced by a rise in plasma activities of liver-specific enzymes (glutamate-pyruvate transaminase and sorbitol dehydrogenase) and an increase in hepatic calcium content. A 17% depletion of hepatic glutathione was observed. All these effects were antagonized by pretreatment with the iron chelator, deferoxamine, and potentiated by pretreatment with ferrous sulfate or experimental hemochromatosis. The authors noted the close relationship between *t*-BuOOH-induced lipid peroxidation and its hepatotoxicity (Younes & Wess, 1990).

Kidney. *t*-BuOOH induced oxidative injury to a suspension of rabbit renal proximal tubules. Initially, *t*-BuOOH increased tubular glutathione disulfide content and lipid peroxidation. Subsequently, there was an increase in ouabain-sensitive oxygen consumption, mitochondrial dysfunction, and a decrease in glutathione content. Cell death began between 30 and 60 minutes. Toxicity was dependent on iron-mediated free radical formation, since deferoxamine and several antioxidants prevented lipid peroxidation, mitochondrial dysfunction, and cell death (Schnellmann, 1988).

At 10 and 30 mg/kg bw/day given orally, *t*-BuOOH produced treatment-related changes in the form of tubular nephrosis in male rats. Multifocal, increased accumulation of tubular proteinaceous material was also observed in male rats. No effects were observed in female rats or in male rats at 3 mg/kg bw/day. The *IRPTC Data Profile* concluded that the effects were a male rat characteristic of low significance for human health (OECD, 2001).

Heart. Rat heart mitochondrial membranes exposed to *t*-BuOOH in the presence of Cu²⁺ undergo lipid peroxidation as evidenced by the accumulation of thiobarbituric acid reactive substances. Mitochondrial lipid peroxidation resulted in a marked loss of both cytochrome c oxidase activity and cardiolipin content. Various lipid-soluble antioxidants prevented the lipid peroxidation and associated loss of cytochrome c oxidase activity. Externally added cardiolipin also prevented the loss of cytochrome c oxidase activity (Paradies *et al.*, 1998).

Early Development. Early development of the embryo is thought to be very susceptible to reactive oxygen species capable of producing oxidative stress. The “two-cell blockage” of morula development of the mouse embryo *in vitro* has been attributed to oxidative stress due to an increase in hydrogen peroxide levels and a subsequent decrease in intracellular GSH concentration. Two-cell and morula/blastocyst embryos cultured for 15 minutes in the presence of 13.3 μM *t*-BuOOH showed decreased GSH concentration, by 75% for the two-cell embryo and 25% for the blastocyst stage (Dwivedi & Iannaccone, 1998).

Gardiner and Reed provided evidence that glutathione status may be extensively involved in the embryotoxicity of a number of chemicals, including *t*-BuOOH. Incubation of embryos with 6.6 μM or higher concentrations of *t*-BuOOH resulted in a significant decrease in GSH content of the embryos. Addition of *t*-BuOOH to culture media also significantly decreased the percentage of morula/blastocyst stage embryos that developed to blastocyst stages *in vitro* (Harris, 1997).

To test the proposal that concomitant generation of oxidative stress of oocytes with increasing maternal age may be a major factor responsible for the age-related increase in aneuploid conceptions, germinal vesicle (GV)-stage mouse oocytes from unstimulated ICR and (C57BLx CBA)F₁ hybrid female mice were matured in the presence of varying concentrations of *t*-BuOOH. *In vitro* exposure of mouse oocytes to *t*-BuOOH during meiosis reduced the length and width of meiosis spindles. This reduction was associated with an increase in the percentage of oocytes showing chromosome scattering and

clumping and of aneuploidy in metaphase II oocytes. However, *t*-BuOOH at the concentrations used, had only a minimal negative effect on the frequency of meiotic maturation. According to the authors, these results suggested that oxidative stress during meiotic maturation *in vitro* may induce chromosomal errors that are undetectable in the living oocyte and whose developmental consequences may become manifested after fertilization (Tarin *et al.*, 1996).

According to Tarin and coworkers (1996), their results are in agreement with previous studies showing an increased frequency of hyperhaploidy and c-mitosis in V79 Chinese hamster lung cells treated with *t*-BuOOH. The mechanism by which *t*-BuOOH modifies the organization of the meiotic spindles and induces aneuploidy is not known.

Structure-Activity Relationships:

From a class study of 72 alkyl hydroperoxides, dialkyl peroxides, and diacylperoxides, 5 structurally related alkyl peroxides with at least some information on genotoxicity, clastogenicity, DNA damage, tumor promotion, or carcinogenicity were selected. Information sources used to identify the above information included searches of the CCRIS (NLM, 2001a), Genetox (NLM, 2001c), and HSDB (NLM, 2001d) databases and a search of PHS-149 (Cancer Chem, 2000). An exhaustive search of all the toxicological literature was not attempted. The NLM databases were searched over a time period between October 2000 and April 2001. PHS-149 data was collected from Cancer Chem version 2 (1999). The information found is presented in Table 2.

It should also be noted that the metabolite of *t*-BuOOH, *t*-butyl alcohol has been tested for carcinogenic activity by the NTP and was found to cause tumors in the male rat renal tubule (NLM, 2001a), possibly via the process initiated by the irreversible binding of certain chemicals to alpha-2u-globulin. Cumene hydroperoxide has been submitted to NTP as a test nomination by the National Institute for Environmental Health Sciences; work has not commenced on cumene hydroperoxide as of this date.

Table 2. Information on genotoxicity, carcinogenicity, and tumor promotion of peroxides.

Compound	Genotoxicity/Clastogenicity	Carcinogenicity/Tumor Promotion
<p>t-Butyl hydroperoxide 75-91-2</p> $\begin{array}{c} \text{CH}_3 \\ \\ \text{H}_3\text{C}-\text{C}-\text{OOH} \\ \\ \text{CH}_3 \end{array}$	<p><i>Mutagenicity:</i> <i>S. typhimurium</i>, w/wo S-9, multiple strains, mixed response (CCRIS) Cultured L5178Y mouse lymphoma cells (+) (HSDB) <i>E. coli</i> WP2 & IC5064 (+) (CCRIS) <i>Neurospora crassa</i> (+) (Genetox)</p> <hr/> <p><i>Chromosome damage:</i> CA: rat bone marrow cells, (<i>in vivo</i>) inhalation, 2.5 or 4 mo) (+) (Katosova <i>et al.</i>, 1977) <i>Vinca faba</i> (+) (Genetox) Dominant lethals, mouse (+) (inhalation, 2.5 or 4 mo) (Katosova <i>et al.</i>, 1977) Sex chromosome loss & nondisjunction, <i>Drosophila melanogaster</i> (+) (NTP, 2000)</p> <hr/> <p><i>Genetic damage:</i> Mitotic recombination or gene conversion, <i>Saccharomyces cerevisiae</i> (+) (Genetox) DNA damage (Latour <i>et al.</i>, 1995; Epe <i>et al.</i>, 1990; Urios & Blanco, 1996)</p>	<p><i>Carcinogenicity:</i> Mice (20, ddh, f), skin, lifespan (-) (PHS-149)</p> <p><i>Tumor promotion:</i> Enhanced hepatocarcinogenesis in trout (Orner <i>et al.</i>, 1993)</p> <p>Enhanced skin tumors in initiated mice of SENCAR and SKH-1 strains (Athar <i>et al.</i>, 1989)</p>
<p>Hydrogen peroxide 7722-84-1</p> <p>HO - OH</p>	<p><i>Mutagenicity:</i> <i>S. typhimurium</i>, w/wo S-9, multiple strains, mixed response (CCRIS; Genetox) <i>E. coli</i> WP2, mixed response (CCRIS) <i>Neurospora crassa</i> (+) (Genetox), <i>B. subtilis</i> (+) & <i>Schizosaccharomyces pombe</i> (+) (Genetox)</p> <hr/> <p><i>Chromosome damage:</i> CA: <i>Vinca faba</i> (-) (Genetox); Chinese hamster V-79, mixed response (CCRIS) SCE: human & nonhuman cells (+) (Genetox)</p> <hr/> <p><i>Genetic damage:</i> UDS: human fibroblasts (+) (CCRIS; Genetox), DNA strand breaks: human breast epithelial cells (+) (HSDB) DNA repair: <i>E. coli</i> polA Rec-assay (+) (Genetox)</p>	<p><i>Carcinogenicity:</i> Mice (50/group, m, f), drinking water, 108 wk (+) (CCRIS) Mice (30, ddn, f) sc, lifespan (-) (PHS-149) Mice (18 C3H/HeN), oral, 6 months, duodenal tumors (PHS-149) Mice (20 Sencar, f) skin, ~51 weeks (PHS-149) Mice (99 C57BL, m,f) DW (0.4%), lifespan, 5% duodenal tumors (PHS-149)</p> <hr/> <p><i>Tumor Promotion:</i> Enhances trout hepatocarcinogenesis (CCRIS)</p>
<p>n-Butyl hydroperoxide 4813-50-7</p> $\begin{array}{c} \text{H}_3\text{C} \\ \\ \text{CH}_2 \\ \\ \text{CH}_2 \\ \\ \text{CH}_2 \\ \\ \text{O} \\ \\ \text{OH} \end{array}$	<p><i>Mutagenicity:</i> <i>S. typhimurium</i> TA100 (-) and TA102 (+) wo S-9 (CCRIS)</p>	<p>No information found in available literature</p>

Ethyl hydroperoxide 3031-74-1 $\begin{array}{c} \text{H}_3\text{C} \\ \\ \text{CH}_2 - \text{O} - \text{OH} \end{array}$	<i>Mutagenicity:</i> <i>S. typhimurium</i> TA 100 & TA102 w/o S-9 (+) (CCRIS)	No information found in available literature
Methylhydroperoxide 3031-73-0 CH_3OOH	<i>Mutagenicity:</i> <i>S. typhimurium</i> TA102 w/o S-9 (+); TA100 w/o S-9 (-) (CCRIS)	No information found in available literature
<i>N</i> -Propyl hydroperoxide 6068-96-8 $\begin{array}{c} \text{H}_3\text{C} - \text{H}_2\text{C} \\ \\ \text{CH}_2 - \text{O} - \text{OH} \end{array}$	<i>Mutagenicity:</i> <i>S. typhimurium</i> TA100 & TA102 w/o S-9 (+) (CCRIS)	No information found in available literature

(+) = positive; (-) = negative; w/w/o = with/without; CA = chromosomal aberration; SCE = sister chromatid exchange; UDS = unscheduled DNA synthesis

An evaluation of the carcinogenic potential of 21 organic peroxides was published by Lai and coworkers in 1996. According to these authors, hydroperoxides are positive in the *Salmonella* assay and have been shown to be genotoxic in various assay systems. *t*-BuOOH and cumene hydroperoxide induce significant elevations in revertant frequency in *Salmonella* strains TA98, TA100, and/or TA102. *t*-BuOOH, *n*-butyl hydroperoxide, cumene hydroperoxide, hydrogen peroxide, and bis(hydroxymethyl)peroxide are all positive in the *Neurospora* test. *t*-Butyl hydroperoxide, cumene hydroperoxide and bis(hydroxymethyl)peroxide are also positive in the *Drosophila* sex-linked recessive lethal test. Cumene hydroperoxide and hydrogen peroxide were also mutagenic in *E. coli*. Clastogenic effects of *t*-BuOOH, hydrogen peroxide, and 2-butanone peroxide have also been reported. Nine peroxides and one peroxide mixture induced malignant lymphomas in C57B1 mice after subcutaneous injection (note: *t*-butyl hydroperoxide was not tested). Of nine peroxides tested by skin painting in mice, three were considered positive. *t*-BuOOH failed to produce tumors in this protocol (Lai *et al.*, 1996).

The authors used OncoLogic, a knowledge rule-based expert system for predicting the carcinogenic potential of chemicals. OncoLogic predicted carcinogenicity concern levels for the 21 peroxides as follows: moderate, 7; low-moderate, 9; marginal, 4; low, 1. *t*-

BuOOH was predicted to have moderate concern for carcinogenic activity (Lai *et al.*, 1996).

A number of dialkylperoxides and hydroperoxides, including benzoyl peroxide, lauroyl peroxide, decanoyl peroxide, dicumyl peroxide, and cumene hydroperoxide are active as tumor promoters in the initiation-promotion model of the mouse epidermis. Experiments with benzoyl peroxide, cumene hydroperoxide, and butylated hydroxytoluene hydroperoxide have provided evidence that cell-mediated activation of these agents to free radicals is associated with their tumor-promoting activity. Once formed, primary radicals from these chemicals can undergo fragmentation, addition, abstraction, or substitution reactions resulting in the modification of cellular molecules. Reactive oxygen species have been shown to modify cellular macromolecules in a fashion relevant to the process of chemical carcinogenesis. For example, DNA damage can involve single or double strand breaks, rearrangements, or base modifications. Hydrogen peroxide in the presence of iron can oxidize pyrimidines and purines. Several of these oxidized bases can be demonstrated to be genotoxic (Trush & Kensler, 1991).

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