Cyclohexene oxide
286-20-4

NTP NOMINATION HISTORY AND REVIEW

A. Nomination History

1. Source: National Cancer Institute

2. Recommendations:
   - -Mechanistic carcinogencity studies
   - -Synergism studies or two-stage studies
   - - Test with benzene or other chemicals deemed appropriate by NTP

3. Rationale/Remarks:
   - -High annual production volume
   - -Potential for human exposure based on its use as a research and industrial chemical
   - -Environmental contaminant
   - -Representative cycloalkene monoepoxide; suspicion of carcinogenicity as a member of the epoxides chemical class

4. Priority: High

5. Date of Nomination: 4/93

B. Interagency Committee for Chemical Evaluation and Coordination Review

1. Date of Review:

2. Recommendations:

3. Priority:

4. NTP Chemical Selection Principles:

5. Rationale/Remarks:

SUMMARY OF DATA FOR CHEMICAL SELECTION

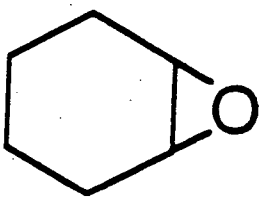
CHEMICAL IDENTIFICATION

CAS Registry Name: 286-20-4

Chemical Abstracts Name: 7-oxabicyclo[4.1.0]heptane(9CI,8CI)

Synonyms and Trade Names: Cyclohexene oxide; cyclohexene epoxide; 1,2-epoxycyclohexane; cyclohexane oxide tetramethyleneoxirane; CO; CHO

Structure, Molecular Formula and Molecular Weight:



$C_6H_{10}O$ Mol. wt.: 98.15

Chemical and Physical Properties

Description: Colorless liquid with strong (pungent, offensive) odor (Sax & Lewis, 1987; Uniroyal, 1990)

Boiling Point: 129-131°C; 266°F (Sax & Lewis, 1987; Uniroyal, 1990; Weast, 1989)

Melting Point: <-10°C (Weast, 1989)

Solubility: Very soluble in benzene; soluble in alcohol, ether, acetone, chloroform; low water solubility (~0.5 g/L) (Sax & Lewis, 1987; BASF, 1990; Weast, 1989)

Density: 0.967 g/cc @ 25°/4°C (Sax & Lewis, 1987; Uniroyal, 1990)

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Vapor Pressure:	12 mbar (BASF, 1990)
Flash Point:	27°C; 81°F (Sax & Lewis, 1989; Uniroyal, 1990)
Stability:	Stable at ambient temperatures and pressures. 1/2 life in Tris buffer (pH 7.4): 128 hours (Uniroyal, 1990; Turchi *et al.*, 1981)
Reactivity:	Corrosive, flammable liquid; highly reactive with acids, bases, and many organic compounds. A strong alkylating agent as measured by its reactivity with nucleophile 4-(p-nitrobenzyl) pyridine (NBP); substrate constant (K_1)=1.10, where K_1 is the *pseudo* 1st order rate constant of CO reacted with NBP and water (Turchi *et al.*, 1981).
Structural Analysis:	*cis*-configuration (Magdalou & Hammock, 1988)

BASIS OF NOMINATION TO THE CSWG

Cyclohexene oxide (CO) was one of the compounds considered in the Epoxides Class Study submitted to the CSPG by SRI on October 5, 1977. It was grouped with chemicals for which annual production of greater than 10^5g (>1,000 lbs) was reported; but it was not one of the five epoxides selected for nomination to the CSWG at that time. In May, 1991, a group of oxiranes including cyclohexene oxide, believed to be commercially available and important industrial chemical intermediates, were added to the ITC's Priority Testing List for a TSCA 4(e) voluntary reporting requirement with a recommendation only for ecological effects testing. While acknowledging that there are health concerns for some oxiranes, including possible carcinogenic, mutagenic, reproductive, development and neurological effects, the ITC focused on potential adverse ecological effects data needs and did not prioritized or make any recommendations for health effects testing at that time. The ITC based its 1991 recommendation regarding the oxiranes on "concerns and uncertainties related to production, use, persistence, potential exposures and releases from production, processing and use, and the potential for causing ecological effects."

CO is presented for consideration for chronic carcinogenicity testing as a representative cycloalkene monoepoxide which is commercially produced in substantial annual volumes and

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which, by virtue of a comparison of its properties with those of its analogs, can be anticipated to result in exposures. Furthermore, it represents a structural entity widely found in natural products, pharmaceuticals, and agricultural chemicals and for which cautious handling has been advocated. Voogd *et al.* (1981) advised that many epoxides are potent mutagens, some are known carcinogens, and some are neither carcinogenic nor mutagenic. Therefore, the potential risk both to human health and to the environment should be assessed for individual compounds in this class of chemicals.

SELECTION STATUS

ACTION BY CSWG: 9/26/91

Studies Requested: Mechanistic studies

Priority: High

Comments: In May 1991, the ITC added oxiranes (including cyclohexene oxide) to their Priority Testing List for a TSCA 4(e) voluntary reporting requirement with a recommended only for ecological effects testing. Cyclohexene oxide is a compound that is present in natural products and has a wide range of uses including laboratory and production of other intermediates that have human exposure. It was suggested that it be tested in synergism studies or in two-stage studies. A strong suggestion was made that it be tested with benzene or some other chemicals NTP deems appropriate.

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EXPOSURE INFORMATION

Commercial Availability:

Production and Producers: Cyclohexene oxide can be manufactured by oxidation of cyclohexene with peroxybenzoic or peracetic acid (Madden, 1974). Typically, this chemical is supplied with a purity of 98 or 99%. Other variations in commerical preparations of CO involve oxidations of cyclohexene with hydrogen peroxide (H_2O_2). Some examples from patents granted to the Ugine Kuhlmann Company in the late 1970s include the following:

- H_2O_2oxidation catalyzed by B_2O_3
- H_2O_2oxidation catalyzed by $Mo(CO)_6$ with 89.7% yield, 87% selectivity
- With aq. H_2O_2,an Sb cmpd, MeCN, and $W(CO)_6$ with 89.7% yield
- C_6H_{10}(50 mmoles) + $Ph_4SbOP(O)(OH)_2$ + MeCN + aq. 70% H_2O_2 (32.5 mmoles) -> CO with 88% selectivity and 94% conversion of H_2O_2

In a search for new oxygen transfer agents, Rebek *et al.* (1979) investigated the reaction of N-benzoylperoxycarbamic acid and carbonylditriazole with cyclohexene in an anhydrous tetrahydrofuran solution of H_2O_2resulting in 91% yields of CO.

Recent annual production data on this compound were not found in the available literature. EPA's TSCAPP database lists only Uniroyal, Inc., as a manufacturer of this compound, but no production volume was reported. When contacted by telephone, a Uniroyal representative acknowledged that this company produces CO but declined to divulge any specific production information. BASF Corp. supplies their CO product, imported from Germany in bulk, according to a company source. Numerous other companies which manufacture, import, distribute or supply this compound were identified from various literature and database sources. They include Fluka Chemie A-G, Henkel Corp., Interchem Corp., Polysciences, Inc., Toray Industries, Ube Industries, and Union Carbide Corp. In addition, more than a dozen chemical supply houses list this compound in their catalogs. Companies which have shown an interest in this chemical, as evidenced by acquiring patents

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for its preparation, include Dow Chemical Co., Eastman Kodak Co., General Electric Co., Rhone-Poulenc Inc., Stauffer Chemical Co., and several pharmaceutical manufacturers.

The Hazardous Substances Data Bank (HSDB) reports the following annual production and importation volumes for CO for 1979: 10,000 lbs and 14,000 lbs, respectively.

Use Pattern: Cyclohexene oxide is primarily used as an industrial raw material in organic synthesis. It is a reactive epoxy compound used to synthesize various kinds of alicyclic chemical intermediates for pesticide, pharmaceutical, perfumery and dyestuff manufacture. A second major use is as a monomer in photoreactive polymerizations. Williams and Molaire (1982) reported CO as one of nine representative monomers which photopolymerize via an ionic mode of initiation not sensitive to oxygen or involving radical intermediates. Crivello and Lam (1979), working in polymerization development for GE, described CO as one of four typical monomers which undergo photoinitiated cationic polymerization with triaryl sulfonium salts. Some products derived from CO which are in use or have been developed for potential use, according to patent literature documentation, include the following:

- antiasthmatics (bronchodilators), platelet aggregation inhibitors, and veterinary analgesics (Upjohn)
- prostaglandin-type bronchodilators and antisecretory agents (Miles)
- antiasthmatics (Kuraray Co.)
- cardiovascular agents (Cassella A.-G.)
- polyolefin glassware coating compositions (Owens)
- photopolymerizable compositions for photoresists and other photomechanical devices (Eastman Kodak)
- additive/modifier for aminoalkyl siloxanes in dentrifices (Unilever)
- epoxide-extended polyol esters as non-digestible fat substitute of low caloric value
- stabilizer for polyoxymethylene and acrylic photopolymerizable compositions for dark storage without a decrease in cure rate (Stauffer Chemical Co.)
- epoxide stabilizer for isothiazolone microbiocides (bactericides, algicides, fungicides, fungistats) with potential for use in cosmetics, leather, paper, plastics, shampoos, shoes, and potable water supplies.

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A third use area for CO is that of laboratory reagent for research and analysis. CO has been used as a microbiological substrate material and biochemical reagent for pretreatment in numerous metabolism/enzyme activity studies, for example:

- Pelkonen *et al.* (1977) studied CO inhibitory effect on benzo[a]pyrene (BP) hydrolase activity and formation of phenol and dihydrodiol metabolites in human liver homogenates. Fahl, in the same year, reported using CO as a non-competitive inhibitor of arene oxide hydrase in metabolism studies of BP in rat liver microsomes.

- Oesch *et al.* (1978) used CO as an enzyme inhibiting reagent in epoxide hydrolase (EH) activity studies on human and mammalian skin.

- deRaat (1978) used CO as a pretreating inhibitor of EH during studies of styrene and styrene oxide induction of SCEs in CHO cells with S9 activation. CO's inhibiting activity on the biotransformation of styrene oxide was demonstrated by the resulting enhancement of SCE induction by that compound.

- Gazzotte *et al.* (1980) used CO as experimental enzyme inhibitor in comparative studies of rat nuclear and microsomal monooxygenase activity on styrene.

- Dankovic and Billings (1985) added CO as a metabolic modifier in studies on bromobenzene toxicity and covalent binding in isolated perfused rat hepatocytes.

Guest and Dent (1980) recommended CO be preferentially used as a replacement for 1,1,1-trichloropropene-2,3-oxide (TCPO) for inhibition of EH activity in microbiological studies. Magdalou and Hammock (1988) measured inhibition of epoxide-metabolizing enzymes by six 1,2-epoxycycloalkanes ($C_{5-9}C_{12}$)and reported that CO substantially decreased the activity of microsomal EH, but not cytosolic EH, and that CO was the most powerful and selective inhibitor of the group with an $IC_{50}$of 4.0×10^{-6}M.

Human Exposure: According to the 1983 National Occupational Exposure Survey (NOES), 2,874 workers were potentially exposed to CO (NLM/RTECS on-line). The ITC reported that 2 out of 17 U.S. drinking water concentrates analyzed by Lucas (1984) contained CO (ITC, 1991). Because of its use by laboratory technicians and researchers, CO can be considered a potential human health hazard. Ringo *et al.* (1982) advised that other epoxy resin

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compounds - including the CO analog, vinylcyclohexene dioxide - used in microbiological laboratories be labeled and handled as suspected carcinogens.

With regard to CO, the BASF (1990) Material Safety Data Sheet stated that this chemical is a skin and eye irritant. It recommends that the following precautions be taken by handlers of CO. "Avoid contact with skin or eyes. Avoid breathing vapor or mists. Inhalation of vapors or mists may result in respiratory irritation. Wear NIOSH/OSHA approved organic vapor/mist respirator, chemical goggles and other protective apparel to minimize contact."

Primary routes of exposure include inhalation and dermal absorption (Uniroyal, 1990). Jung *et al.* (1981) advised that the 1,2-disubstituted oxirane structural unit of CO occurs in a wide variety of drugs and natural products.

Environmental Occurrence: Numerous epoxides, including CO, have been identified as environmental contaminants. Although CO is not reported in the EPA's Toxic Release Inventory (TRI) for 1987 and 1988, the ITC opined that the production and use of oxiranes as chemical intermediates are responsible for releases of these compounds to the environment. Pellizzari *et al.* (1976) collected CO as one of a number of volatile, hazardous, possibly carcinogenic vapors present in field samples used to develop analytical methods for the determination of organic vapor pollutants in ambient atmospheres. Agarwal *et al.* (1979) developed a method for detecting CO and other epoxides in liquid samples, with a detection limit for most samples of between 0.1 and 0.01 micromoles. Agarwal *et al.* (1980) based another method for detecting atmospheric epoxide contaminants, including CO, on their reaction with 4-nitrothiophenol (NTP). They found that the epoxides studied underwent a facile, nucleophilic ring-opening reaction yielding 1,2-adducts; in the case of CO, 2-(p-nitrophenoxy)cyclohexanol was formed. Breakthrough volumes for atmospheric pollutants, including CO, were determined by Krost *et al.*, (1982) in work to develop an analytical capability to collect, characterize, and quantify hazardous vapors. Colgan *et al.* (1985) developed a method for environmental monitoring and improved HPLC detection of alkyl halides and epoxides. The method, based on derivatization with the nucleophilic reagent, silver picrate, to form picryl ether derivatives of contaminant chemicals, can be performed

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on solid silica support or in solution. Of the chemicals studied, CO showed the most marked improvement in detection; it was not detectable without derivatization but could be detected in the parts per billion (ppb) range after derivatization. Joshi *et al.* (1989), in an evaluation of tests to monitor condition and quality of reclaimed industrial cleaning solvents, identified CO among various compounds in spent chlorinated hydrocarbon degreasing solvents. As cited above, the 28th ITC Report (1991) referenced a finding of CO in drinking water concentrates.

Several studies have identified CO as an intermediate or by-product in biological oxidations of cyclohexene. Leibman and Ortiz (1970) incubated hepatic microsomes from male New Zealand rabbits pretreated with sodium-phenobarbital with 20 mM cyclohexene for up to 20 minutes. They identified CO as the epoxide intermediate in the oxidation of the alkene to the corresponding glycol. This could have implications for the possible bioremediation of hazardous wastes containing cyclohexene, whereby CO might be formed as an intermediate/by-product under conditions of incomplete oxidation.

Environmental effects of CO have not been determined (Uniroyal, 1990). However, BASF (1990) recommends disposal in suitable containers in a landfill or other licensed facility. CO should not be discharged into waterways or sewer systems without authorization. According to Bellucci *et al.* (1984) the fundamental CO structural unit occurs in many natural, synthetic or metabolically formed products. Jung *et al.* (1981) identified this basic structural unit as common to many pharmaceutical and agricultural chemicals as well as natural products.

Regulatory Status: CO is listed in EPA's TSCA inventory. According to BASF (1990) this chemical is not regulated under RCRA. No exposure limits have been set, according to Uniroyal (1990). According to this source, the following OSHA classification applies to CO: flammable, mutagenic, toxic irritant; while the European Economic Community (EEC) classification states: flammable, mutagenic, harmful irritant. EPA has set the reportable quantity (RQ) for CO at 100 lbs. Disposal methods should be in accordance with EPA/RCRA regulations [40 CFR 261.21(a)(1)] according to Uniroyal (1990). A TSCA §4(e)

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voluntary reporting requirement cited in the 28th ITC report (1991) recommends ecological effects testing for this compound.

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EVIDENCE FOR POSSIBLE CARCINOGENIC ACTIVITY

Human Data: No epidemiological studies or case reports associating CO with a cancer risk in humans were found in the published literature [see Search Resource List]. According to Uniroyal Chemical Co. (1990) CO has not been assessed for carcinogenicity by NTP, IARC or OSHA.

Animal Data: Cyclohexene oxide is not currently on test or scheduled to be tested in a chronic carcinogenicity mammalian bioassay, according to a search of the available literature and databases. Two studies have been cited for this compound in PHS-149: Van Duuren *et al.* (1967b) and Kotin and Falk (1963).

Several early studies for evidence of carcinogenicity were carried out on CO by Van Duuren and coworkers. In Van Duuren *et al.* (1965) 10% CO in benzene solution was applied to 30 Swiss-Millerton female mice by dorsal application 3 times per week until all animals had succumbed (median survival time, 583 days). No papillomas and no carcinomas were reported. A reported skin irritancy of 3 indicated that moderate hair loss and crusting persisted throughout most of the experiment.

Van Duuren *et al.* (1967a) administered CO in a single 100 mg/0.1 ml tricaprylin dose by subcutaneous injection once a week to 20 female Sprague-Dawley rats, treating and observing the animals for their lifespan (median survival time, 531 days). No local subcutaneous sarcomas were reported during the 545 days of the test duration. Of all compounds tested, the CO test group had the highest incidence of mammary carcinomas (3); but this incidence was regarded as within the normal background incidence range.

Van Duuren, in a 1969 paper, summarized the above study results for CO, concluding that this compound did not demonstrate carcinogenicity.

Koten and Falk (1963), in a treatise on the implications of organic peroxides and epoxides in the induction of neoplasia by ionizing radiation, reported the results of bioassay

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experiments which they explained were, in some cases, still in progress and in which "data (were) obtained at the unsophisticated level of routine bioassay." Their observations include an experiment in which 30 C_3H mice received a dose of 20 μmoles of CO by inhalation, following which, after 7 months, 1 pulmonary adenoma was found among the 22 surviving mice (5%).

Uniroyal (1990) notes that, while CO has not been established as a carcinogen by NTP, IARC or OSHA, enhancement of the carcinogenicity of ethylene dibromide in rodents by CO has been reported by NIOSH.

CO has been reported in numerous sources to be a non-competitive inhibitor of the enzyme, epoxy hydrase (EH). Oesch *et al.* (1978) investigated EH activity in mammalian liver, kidney, lung, intestine and skin both *in vivo* and *in vitro*. They found that CO reduced hepatic glutathione (GSH) levels to 10% of controls and that in co-administration experiments CO significantly reduced the rate of metabolism of chlorobenzene and prevented the associated hepatic centrilobular necrosis in rats. These findings raised questions about the variety of factors, complexities, and discrepancies observed with multiple arene oxide-mediated hepatotoxic effects and their possible relationship to hepatocarcinogenicity. Perin-Roussel *et al.* (1978) reported that CO had an enhancing effect on the covalent binding of highly carcinogenic tritium-labeled dibenzo(a,e)fluoranthene (DBF) to DNA in mouse and rat liver microsomes. Nesnow *et al.* (1985), in a treatise on the role chemicals play in the etiology of cancer, reported the mechanistic effects of various chemicals as inhibitors or enhancers of oncogenic cell transformations in mouse embryo fibroblasts. CO was found to enhance cell transformation by inhibiting EH activity, allowing increased concentrations of arene oxides to accumulate in cells.

The following results of acute toxicity tests are summarized in Uniroyal's MSDS:

Oral toxicity:	LD_{50}(rats) - 1.05 g/kg
Dermal toxicity:	LD_{50}(rabbits) - 0.6 g/kg
Inhalation toxicity:	LC_{50}(rats, 4 hr.) - 2649 ppm
Irritation:	eye (rabbits) - severe

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In addition, this source states the following with regard to the chronic toxicity of CO. "The feeding to mice of up to 2,200 ppm and rats of up to 600 ppm for two years did not produce an increased tumor incidence."

Short-Term Tests: Guest and Dent (1980) tested CO for mutagenicity in the Ames histidine-reversion assay with *S. typhimurium* strains TA98, TA100, TA 1535, TA1537 and TA1538, reporting a slight but significant effect in TA100 both with and without S9 and in TA1535 only in the absence of S9. In this assay, 10^{-3}M CO showed weak mutagenic activity in the TA100 strain with an increase in the number of revertant from 90 to 130, in reasonably close agreement with results reported by Wade *et al.* (1978) who observed that 1,000 μg of CO (~ 4.5×10^{-3}M)increased the number of revertants from a level of 180 (spontaneous) to 280. Guest and Dent (1980) also reported a non-mutagenic but somewhat toxic response using a forward mutation assay using 8-azaguanine resistance in TM677 cells as the genetic marker.

Jung *et al.* (1981) tested CO for mutagenicity as one compound in a series of 17 structurally related oxiranes. The test system was a standard Ames/*Salmonella* assay using strains TA98, TA100, TA1535 and TA1537. The procedure was modified slightly for CO and three of its close analogs because of volatility under the test conditions; prepared petri dishes were placed in 20 L desiccators and exposed to the volatile compounds in varying amounts overnight. CO was reported to be weakly mutagenic in TA100 and moderately mutagenic in TA1535 (both base-pair substitution mutant strains) by comparison with positive controls, benzo[a]pyrene-4,5-oxide and N-methyl-N'-nitro-N-nitrosoguanidine. CO did not cause reversions in the frameshift mutant strains TA98 and TA1537. A summary of the test results for CO was reported as His^+ revertants per μmole of compound (values taken from the linear part of dose-response curves) and maximal numbers of revertant colonies per dose, as follows:

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Mutagenicity of epoxides: CO. His^+ revertant colonies in strain (from Jung *et al.*, 1981)

TA98		TA100		TA1535		TA1537	
per μmole	per dose (col/μg)	per μmole	per dose (col/μg)	per μmole	per dose (col/μg)	per μmole	per dose (col/μg)
<0.6	27/3300	41	980/3300	39	925/3300	<0.3	6/3300

N.B. The following numbers of colonies per μmole were considered to be significant: >20 (TA98), >40 (TA100), >20 (TA1535), >10 (TA1537).

Frantz and Sinsheimer (1981) studied the mutagenicity and toxicity of 12 cycloaliphatic epoxides in a modified Ames *Salmonella* assay in strains TA92, TA100, TA1535, TA1950, TA1975 and TA2410 without S9. They reported a positive mutagenic response at high doses for CO and 7 analogs and observed decreasing mutagenicity and increasing bacterial growth inhibition with increasing ring size.

Turchi *et al.* (1981) tested CO for mutagenicity, reporting a positive result in an Ames assay with *S. typhimurium* strain TA100 (364.4 His^+ revertants per plate induced by a 1 mM concentration). These researchers also tested CO in V79 Chinese hamster cells, measuring point mutations (6-thioguanine resistance) and chromosomal damage (anaphase bridges and micronuclei). CO induced significant dose-dependent increases in mutant frequency and induced micronuclei and, based on the observed chromosomal damage, was determined to be mutagenic.

Turchi *et al.* (1981) reported that CO showed strong clastogenic activity when tested in a cultured Chinese hamster cell assay.

Voogd *et al.* (1981) reported CO to be mutagenic in a Luria and Delbrück's fluctuation test with *Klebsiella pneumonia*, producing increases in the mutation rate of 1.9, 2.4 and 4.6 at 5, 10 and 20 mM respectively.

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Bellucci *et al.* (1984) tested CO in a mutagenicity assay for induction of 6-thioguanine resistant mutants in V79 Chinese hamster cells, reporting this compound to be positive with a mutagenic rate of 3.0 mutants/10^6 cells/mmole in a linear dose-response fashion. These researchers also reported CO to be positive in a determination of alkylating ability by measuring the rate of alkylation of nicotinamide indicated by increased fluorescence.

von der Hude *et al.* (1989) investigated the genotoxicity of CO in an SCE assay with V79 cells in the absence of external metabolizing system. While most of the cycloepoxides tested gave negative results, CO was positive.

von der Hude *et al.* (1990) reported CO and other cycloaliphatic epoxides to be negative for increased *beta*-galactosidase activity and SOS induction factor in an SOS-Chromatest assay to measure SOS repair in *Escherichia coli* PQ37 strain with and without S9. They also performed an Ames assay and reported this compound to be positive in strain TA100.

Metabolism: The metabolism of CO, as expected, follows the general pathway outlined for epoxides by Klaassen (1986): enzymatic hydration catalyzed by EH to the corresponding *trans*-1,2-dihydrodiol. It is thought to involve EH-catalyzed hydration of the oxide moiety by activation of water to a nucleophilic species which precipitates ring opening to the diol in the *trans* configuration. EH is widely distributed in a variety of tissues, including liver, lung, kidney, spleen, adrenal gland, intestine, colon, ovary, testis, thymus, brain, heart and skin. Certain chemicals (including some alcohols, ketones, and imidiazoles) stimulate microsomal EH activity *in vitro*, and the enzyme's activity in the liver of rats has been found to be twice as great in males as in females. CO is a widely known and used inhibitor of this enzyme. For example CO was found to inhibit metabolism of the triepoxide antitumorigenic investigative drug, teroxirone, in rat liver and lung microsomal incubations (Ames *et al.*, 1984).

Sakurai (1988) reported that the oxidative metabolism of cyclohexene by various cytochrome P-450 model systems gave rise to CO as one of several oxidation products with varying yields depending on the components of the system.

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Another metabolic pathway followed by CO involves biocatalysis by glutathione transferases. The conjugation of CO with glutathione gives rise to mercapturic acid derivatives which are excreted in the urine. van Bladeren *et al.* (1981) studied the stereospecificity of this CO metabolism by measuring urinary excretion of metabolites in rats following intraperitoneal and intravenous administration. They found that rats excreted only the two *trans* forms of the four possible diastereioisomers of N-acetyl-S-2-hydroxycyclohexyl-L-cysteines. The portion of the administered dose excreted as mercapturic acids was found to be 21±4% up to a dose of 0.5 mmole of CO per rat; with higher doses, the amount excreted remained constant indicating that glutathione synthesis pathways were saturated.

CO has been investigated by several research groups for its inhibitory effect on the biliary excretion of metal toxins. Refsvik (1978) postulated that high levels of liver GSH were required for the normal transport of methyl mercury from liver to bile in the rat via a GSH complex. When CO was administered intraperitoneal to rats, biliary methyl mercury excretion declined as well as the bile sulfhydryl and sulfide content, suggesting the complexation of GSH by CO. Norseth *et al.* (1982) investigated the importance of GSH in rat liver metabolism of various chromium compounds. After intraperitoneal instillation of CO as a GSH-depleting agent, only hexavalent forms of chromium were found in the bile. Refsvik reported that CO by its action as a GSH depletor depressed the rate of excretion of mercury in the bile of rats treated with methyl mercuric chloride. Alexander *et al.* (1986) made similar observations about the hepatic detoxification of lead administered as lead nitrate to rats based on injection of CO which was followed by a rapid decline in the concentration of lead and GSH in the bile.

Direct enhancement of cellular resistance such as elevation of intracellular glutathione is one of the mechanisms cited by Szabo (1991) for protection against injury to the gastrointestinal mucosa by such factors as ischemia (hypoxia), exogenous (drug) and endogenous (chemical) mediators, and biologic agents. GSH-depleting agents such as CO may interfere with this protective mechanism.

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Structure/Activity Relationships: IARC has evaluated the following epoxy alkane analogs of CO with results and conclusions as follows (IARC, 1987):

	Degree of evidence for toxicity[a]		Overall evaluation[a]
	Human	Animal	
ethylene oxide (based on other relevant data)	L	S	2A
propylene oxide[e] (based on other relevant data)	I	S	2A
diepoxybutane[b] (Vol. 11, 1976)	ND	S	2B
1-epoxyethyl-3,4-epoxycyclohexane[d] (Vol. 11, 1976)	ND	L	3
3,4-epoxy-6-methylcyclohexane carboxylate[d] (Vol. 11, 1976)	ND	L	3

[a] ND, no adequate data; I, inadequate evidence; L, limited evidence; S, sufficient evidence; Group 2A - the agent is probably carcinogenic to humans; Group 2B - the agent is possibly carcinogenic to humans; Group 3 - the agent is not classifiable as to its carcinogenicity to humans
[b] Overall evaluation based only on evidence of carcinogenicity in monograph [volume, year]
[c] Degree of evidence in animals revised on the basis of data that appeared after the most recent monograph and/or on the basis of present criteria
[d] Degree of evidence not previously categorized; evaluation made according to present criteria on the basis of data in monograph [volume, year]
[e] Other relevant data, as given in the summaries here or in monograph [volume, year], influenced the making of the overall evaluation

The NTP has tested 4-vinyl-1-cyclohexene diepoxide (VCHD) [evaluated by IARC under the synonym, 1-epoxyethyl-3,4-epoxycyclohexane] for carcinogenicity in a 2-year bioassay in rats and mice by the dermal route of application. VCHD was clearly carcinogenic to the skin of both male and female rats and mice; squamous cell carcinomas were the predominant tumor type found. Other types of tumors such as basal cell adenomas and carcinomas were more frequent in rats than in mice (Chhabra *et al.*, 1990). This compound also gave positive genetic toxicity results in the Ames *Salmonella*, mouse lymphoma and cytogenics (CA/SCE) *in vitro* test systems, and a finding of positive in an immunotoxicity study (NTP, 1991). Voogd *et al.* (1981) reported this diepoxide analog to be more mutagenic than CO. Other epoxide compounds for which the NTP has reported test results include epoxyalkanes and epoxyalkane derivatives. 1,2-epoxybutane was tested in a 2-year bioassay by inhalation in rats and mice with the following results: clear evidence of carcinogenicity in male rats,

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equivocal evidence in female rats, and no evidence in male or female mice. This compound was reported positive for genetic toxicity in the following test systems: *Salmonella*, mouse lymphoma, *Drosophila* (SLRL/RT) and *in vitro* cytogenetics (CA/SCE). 2,3-Epoxybutane and 1,2-epoxy-3-butene tested positive for mutagenicity in the *Salmonella* bioassay and 1,2-epoxydecane and 1,2-epoxydodecane gave negative results in the same systems, the only test result reported for these compounds. Other alkyl epoxides tested for genetic toxicity, with varying results, were halogenated derivatives.

Frantz and Sinsheimer (1981) studied mutagenicity of 12 cycloaliphatic epoxides in the Ames *Salmonella* assay without metabolic activation in strains TA98, TA100, TA1535, TA1537 and TA1538. Based on their results of mutagenic response at high doses for 7 of the 12 epoxides, they postulated that *cis*-1,2-disubstituted oxiranes are mutagenic. Mutagenic activity occurred only in the base-pair indicator strains TA100 and TA1535. Six compounds were mutagenic in both strains and a 7th, oxaspirooctane, was weakly mutagenic in TA100 only. The mutagenic response decreased for the compounds reported positive in the following order:

- in TA100: vinylcyclohexane dioxide > cyclopentane oxide > 4,5-epoxycyclohexene ≈ cyclohexane oxide > norbornane oxide > 3,4-epoxycyclohexene > oxaspirooctane.
- in TA1535: 4,5-epoxycyclohexene > cyclopentane oxide > cyclohexane oxide ≈ norbornane oxide > vinylcyclohexane oxide > 3,4-epoxycyclohexene.

Microbial cytotoxicity prevented correlations of other structural features with mutagenic activity. For four compounds (cycloheptane oxide, cyclooctane oxide, cyclododecane oxide and 3,4-epoxycyclopentane) toxic inhibition was very pronounced to the extent that these compounds did not show any mutagenic dose-response effect. For CO and its 1,2-cycloalkene epoxide analogs in this series of C_{5-8} and C_{12} saturated ring epoxides, toxicity increased with enlarging ring size, but mutagenicity was shown only for compounds with 5- or 6-membered rings.

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Comparative Mutagenicity for the Expanding Ring Cycloalkane Epoxides (From Frantz and Sinsheimer, 1981)

No.	Compound	Dose[a] (μmole/plate)	Revertants per plate[b] TA153		TA100
1	Cyclopentane oxide	12	678 ± 101	$p < 0.05$[c]	234 ± 24
2	Cyclohexane oxide	12	534 ± 74		187 ± 47
3	Cycloheptane oxide	12[d]	29 ± 7	$p < 0.05$[e]	67 ± 17
4	Cyclooctane oxide	12	22 ± 5		53 ± 12
Spontaneous reversion counts[f]			20 ± 4		77 ± 9

[a] Test compound is dissolved in 0.1 ml dimethylsulfoxide per plate.
[b] Mutagenicity for 6 replicates per compound is reported as mean counts ± standard deviation. Background counts are not subtracted.
[c] Statistical comparison of results for compounds 1 and 2 in TA 1535 using a one-sided Student's t-test.
[d] This compound was only partially soluble in dimethyl sulfoxide and was delivered to the plates as a suspension rather than a solution.
[e] Statistical comparison of results for compound 3 with spontaneous reversion in TA1535 using a one-sided Student's t-test.
[f] Background counts are the results of adding 0.1 ml dimethylsulfoxide per plate to determine the spontaneous reversion rate. The counts have not been subtracted from the data for mutagenicity counts.

Sixteen oxiranes structurally related to CO were evaluated for mutagenicity in an Ames *Salmonella* assay by Jung *et al.* (1981), and the results were evaluated for structure activity relationships. They reported the following observations.

- CO and 3 analogs which are also volatile and exhibit similar physicochemical properties and dispositions, gave results as follows:

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Compound	TA98 per mole	TA98 per dose (col/ g)	TA100 per mole	TA100 per dose (col/ g)	TA1535 per mole	TA1535 per dose (col/ g)	TA1537 per mole	TA1537 per dose (col/ g)
cyclohexene oxide [A]	<0.6	27/3300	41	980/3300	39	925/3300	<0.3	6/3300
cyclohexa-1,4-diene oxide [B]	<0.6	27/3400	43	1420/3400	48	1325/3400	<0.3	12/3400
cyclohexa-1,3-diene oxide [C]	<0.6	20/3350	115	1300/3350	58	730/3350	<0.3	10/3350
benzene oxide [D]	<0.6	13/2000	<2	78/2000	<0.9	22/2000	<0.5	10/2000

- In a comparison of the mutagenic activities relative to structure, the authors observed that compound [C], which contains a double bond in conjugation to the epoxide moity, exhibited stronger mutagenicity than the analogous fully saturated CO. The increase in mutagenicity was less when the structure contained a double bond not vicinal to the epoxide ring, as in compound [B].

- A similar effect was observed when the saturated cyclohexane diepoxides were compared with their mono-unsaturated analogs (benzene dioxide isomers). The arene oxide analog of CO, benzene oxide (compound [D]), which might be expected to have greater mutagenicity resulting from increased allylic unsaturation, was non-mutagenic, possibly because of instability in aqueous media (1/2 life of <2 min. in water @ pH7, 1M KCl, 30°C).

- A further comparison of mutagenic activity of CO with several of its bromine and/or hydroxyl substituted derivatives was also made by these authors. They reported that the introduction of a bromine atom on the carbon adjacent to the epoxide ring resulted in increased mutagenicity. On the other hand, analogs in this cyclohexane epoxide series in which monoepoxidation was changed to diepoxidation showed mutagenicities of the

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same order of magnitude. The authors concluded that increasing the number of oxirane rings is not accompanied by an enhancement of mutagenic activity.

Voogd *et al.* (1981) tested 45 epoxides for mutagenicity in Luria and Delbrück's fluctuation test with *Klebsiella pneumoniae*. Mutagenic activity was observed for 36 of the 45 epoxides including CO. Of the four 1,2-epoxycycloalkanes among the test compounds, the other three (4-vinylcyclohexenedioxide, cyclopenteneoxide, *exo*-2,3-epoxynorbornane) were all stronger mutagens than CO at a concentration of 5 mM. For comparison, 1,2,5,6-diepoxycyclooctane showed little mutagenic activity [see table below].

Increase of the Mutation Rate of *Klebsiella pneumoniae* by Some Cycloaliphatic Epoxides (from Voogd *et al.*, 1981)

Compound	Concentration (mmoles/l) 100	50	20	10	5	2	1	0.5	0.2
4-Vinylcyclohexenedioxide					4.2	2.9	1.8	-	-
Cyclohexeneoxide			4.6	2.4	1.9				
Cyclopenteneoxide					2.1	1.7			
exo-2,3-Epoxynorbornane				5.8	3.2	1.6			
1,2,5,6-Diepoxycyclooctane				1.7	-		-		

- no mutagenic action
Average of triplicate experiments, coefficient of variation ±18%

Bellucci *et al.* (1978) demonstrated that considerable species differences occurred in the velocity of enzymatic hydration between the 4 stereoisomers of the optically active analog, 4-*tert*-butylcyclohexene oxide. van Bladeren *et al.* (1981), based on results with CO as a model compound, speculated that similar selectivity of activity might be shown by glutathione S-transferases toward optically active epoxides. Differences in the mutagenic

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and carcinogenic action of different optical isomers is likely to result from differences in the affinity of the detoxifying enzymes.

Bellucci *et al.* (1984) examined the effect on mutagenicity of bromination at the carbon adjacent to the oxirane ring of CO. No increase in mutagenicity was found. However, *trans*-3-bromo-1,2-epoxycyclohexane exhibited mutagenic activity comparable to the parent, CO, while its *cis*-isomer was 2 1/2 times less active, suggesting a stereo-specific enzymatic detoxifying mechanism. Furthermore, all three compounds underwent a nucleophilic ring opening alkylating reaction of very similar rate toward nicotinamide in V79 Chinese hamster cell microsomal fraction, supporting a conclusion that alkylation takes place exclusively at the oxirane carbons for all three.

Various biologically active CO derivatives are found in numerous plants of different families, including crotepoxide, senepoxide and pipoxide. Crotepoxide has been described as an antitumorigenic agent that exhibits significant inhibitory activity against Lewis lung carcinoma in mice. (Thebtaranonth and Thebtaranonth, 1986)

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