

Air Toxics Hot Spots Program

Carbonyl Sulfide Reference Exposure Levels

Technical Support Document for the
Derivation of Noncancer Reference
Exposure Levels

Appendix D1

Final
February 2017



Air, Community, and Environmental Research Branch
Office of Environmental Health Hazard Assessment
California Environmental Protection Agency

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Final Report

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(carbon monoxide monosulfide, carbon oxide sulfide, carbonoxysulfide, oxycarbon sulfide)

CAS: 463-58-1

S=C=O

1. Summary

The Office of Environmental Health Hazard Assessment (OEHHA) is required to develop guidelines for conducting health risk assessments under the Air Toxics Hot Spots Program (Health and Safety Code Section 44360 (b) (2)). In response to this statutory requirement OEHHA developed a Technical Support Document (TSD) that describes acute, 8-hour, and chronic Reference Exposure Levels (RELs). The TSD presents methodology reflecting applicable scientific knowledge and approaches, and in particular explicitly includes consideration of possible differential effects on the health of infants, children, and other sensitive subpopulations, in accordance with the mandate of the Children's Environmental Health Protection Act (Senate Bill 25, Escutia, chapter 731, statutes of 1999, Health and Safety Code Sections 39669.5 *et seq.*). The TSD was adopted in December 2008 (OEHHA, 2008). These guidelines have been used to develop the acute, 8-hour and chronic RELs for carbonyl sulfide presented below. This document, which will be added to Appendix D of the TSD, describes the basis for these RELs.

Carbonyl sulfide (COS) is a chemical intermediate and a byproduct of oil refining, and could potentially be used as a grain fumigant. However, it is not currently registered in California as a fumigant. COS is classified as a California Toxic Air Contaminant (TAC) and a federal hazardous air pollutant (HAP). Inhalation of carbonyl sulfide results in adverse health effects mainly in the central nervous system (CNS). The RELs are based on CNS effects. The scientific literature published through February 2015 was considered in the derivation of these values.

1.1 Carbonyl Sulfide Acute REL

<i>Reference Exposure Level</i>	660 µg/m³ (270 ppb)
<i>Critical effect(s)</i>	CNS toxicity in male rats
<i>Hazard Index target(s)</i>	Nervous system

1.2 Carbonyl Sulfide 8-hour REL

<i>Reference Exposure Level</i>	10 µg/m³ (4 ppb)
<i>Critical effect(s)</i>	CNS toxicity in male and female rats
<i>Hazard Index target(s)</i>	Nervous system

1.3 Carbonyl Sulfide Chronic REL

<i>Reference Exposure Level</i>	10 µg/m³ (4 ppb)
<i>Critical effect(s)</i>	CNS toxicity in male and female rats
<i>Hazard Index target(s)</i>	Nervous system

2. Physical & Chemical Properties (HSDB, 2010)

<i>Description</i>	colorless, odorless gas
<i>Molecular formula</i>	COS
<i>Molecular weight</i>	60.075 g/mol
<i>Density/Specific gravity</i>	1.028 g/cm ³ @ 17°C/4°C
<i>Boiling point</i>	-50°C
<i>Melting point</i>	-138.8°C (sublimes appreciably at temperatures above melting point)
<i>Vapor pressure</i>	9,412 torr (mm Hg) @ 25°C (estimated)
<i>Vapor density</i>	2.1 (air = 1)
<i>Partition coefficient</i>	log Kow = -1.33 (estimated)
<i>Solubility</i>	1,220 mg/L in H ₂ O; sol. in ethanol and toluene; sol. in KOH and CS ₂
<i>Odor threshold</i>	no odor if pure (HSDB, 2010) 0.1 ppm (USEPA, 1992); sulfur odor
<i>Henry's law constant</i>	0.61 atm·m ³ /mol @ 25°C (estimated)
<i>Atmospheric half-life</i>	≥ 2 years
<i>Hydroxyl radical reaction rate constant</i>	2 x 10 ⁻¹⁵ cm ³ /molecule-sec at 25°C (estimated)
<i>Conversion factor</i>	2.46 µg/m ³ per ppb at 25°C

3. Occurrence and Major Uses

COS is naturally found in crude oil, salt marshes, soil, and volcanic gases (Haritos, 2000). COS has been used as a chemical starting material or intermediate, e.g., in the synthesis of thio-organic molecules, for some thiocarbamate pesticides and herbicides, and for the preparation of aliphatic polyureas (HSDB, 2010). COS has been identified as a product of captan decomposition by fungal spores (Somers et al., 1967). It is emitted from some oil refineries as an end product of sulfur combustion and is a product

of coal combustion. COS is a component of cigarette smoke and may occur at levels of 12 to 42 μg in the mainstream smoke from one unfiltered cigarette (National Research Council, 1986). It is present in side-stream smoke at 3-13% of the amount in mainstream smoke (CARB, 2005). COS is also a grain fumigant that has the potential to replace methyl bromide, which is being phased out due to its ozone-depleting ability, and to supplement phosphine gas, which is experiencing increased insect resistance (Bartholomaeus and Haritos, 2005).

In 2012, a total of 56 facilities subject to the California Air Toxics Hot Spots Act reported combined emissions of 15,528 pounds of carbonyl sulfide (CARB, 2012). The highest emission levels were from oil refineries in the South Coast Air Quality Management District (Table 1). Other facilities emitting reportable levels of COS include landfills, a paper company, and a tire company. Because of the quadrennial method of updating emission inventories in the Hot Spots program, the table should be considered a sample of high emitting facilities for the year indicated. Some high emitting facilities may be missing from the list for the specific year, even though they continuously emit COS.

Table 1. Major Hot Spots facilities which emitted COS in California in 2012.

<i>Facility type</i>	<i>Air District</i>	<i>Emissions (pounds)</i>
Oil Refinery 1	South Coast	7706.2
Oil Refinery 2	South Coast	2705.5
Oil Refinery 3	South Coast	2439.1
Oil Refinery 4	South Coast	1531.6
Oil Refinery 5	South Coast	708.6
Landfill 1	San Diego County	210.9
Landfill 2	San Diego County	57.5
Landfill 3	San Diego County	24.1
Landfill 4	San Diego County	23.3
Oil Refinery 6	South Coast	20.4
Military Camp	San Diego County	14.2
Landfill	Sacramento	11.6

Source: California Toxics Inventory (CTI) for 2012

URL = <http://www.arb.ca.gov/app/emsinv/facinfo/facinfo.php>

In the US Environmental Protection Agency (US EPA) Toxics Release Inventory (TRI) for 2012, 15 California facilities, mainly refineries, reported a total of 34,960 pounds COS in their on-site disposal and other releases (USEPA, 2015). The discrepancy between US EPA and ARB estimates is due to differences in reporting requirements including the quadrennial reporting in the Hot Spots program versus the annual reporting in TRI.

Data on ambient levels of COS are scarce. The ambient concentration of COS was reported to be 0.435 ± 0.028 ppb ($1.1 \mu\text{g}/\text{m}^3$) in Philadelphia, PA, 0.454 ± 0.031 ppb ($1.2 \mu\text{g}/\text{m}^3$) in Wallops Island, VA, and 0.511 ± 0.036 ppb ($1.3 \mu\text{g}/\text{m}^3$) in Lawton, OK (Maroulis et al., 1977; Aneja et al., 1982). The average world-wide atmospheric concentration of COS is considered relatively constant at 0.5 ppb ($1.2 \mu\text{g}/\text{m}^3$) (Sze and Ko, 1979; Melillo and Steudler, 1989).

Seasonal variations of atmospheric COS levels in Beijing, China (maximum average value of 0.85 ppb ($2.1 \mu\text{g}/\text{m}^3$) in winter and minimal value of 0.37 ppb ($0.9 \mu\text{g}/\text{m}^3$) in summer) were ascribed mainly to vegetation uptake and anthropogenic emissions. The dominant anthropogenic sources of COS in Beijing were identified as vehicle tire wear in summer and coal burning in winter (Cheng et al., 2015).

4. Metabolism

In vivo, carbonyl sulfide is primarily metabolized by carbonic anhydrase via mercaptoformic acid (HSCOOH) to hydrogen sulfide and CO_2 (Figure 1) (Chengelis and Neal, 1979; Sills et al., 2005).

A proposed secondary pathway (not shown in Figure 1) is metabolism via cytochrome P450 to CO_2 and a reactive S species which inactivates the P450 (Dalvi et al., 1975). However, Chengelis and Neal (1979) noted that the active metabolism of isolated rat hepatocytes as measured by COS disappearance was not inhibited by the cytochrome P450 inhibitors SKF 525-A, 4-methylpyrazole or metyrapone, or the P450 substrate carbon disulfide. COS is also a metabolite of carbon disulfide (CS_2) via cytochrome P450 mixed function oxidase (Dalvi et al., 1974), a pathway also used in the metabolism of Antabuse (disulfiram) by alcoholics (Johansson, 1989). COS is formed during the metabolism of the pesticide metam sodium (CDPR, 2004).

Pretreatment of rats with acetazolamide, an inhibitor of carbonic anhydrase, reduces the blood levels of hydrogen sulfide and decreases the lethality of COS (Chengelis and Neal, 1980; 1987). Sodium nitrite pretreatment, which converts a portion of hemoglobin to methemoglobin, also protects animals against a lethal dose of COS (Chengelis and Neal, 1980). Methemoglobin strongly binds sulfide (Smith and Gosselin, 1964).

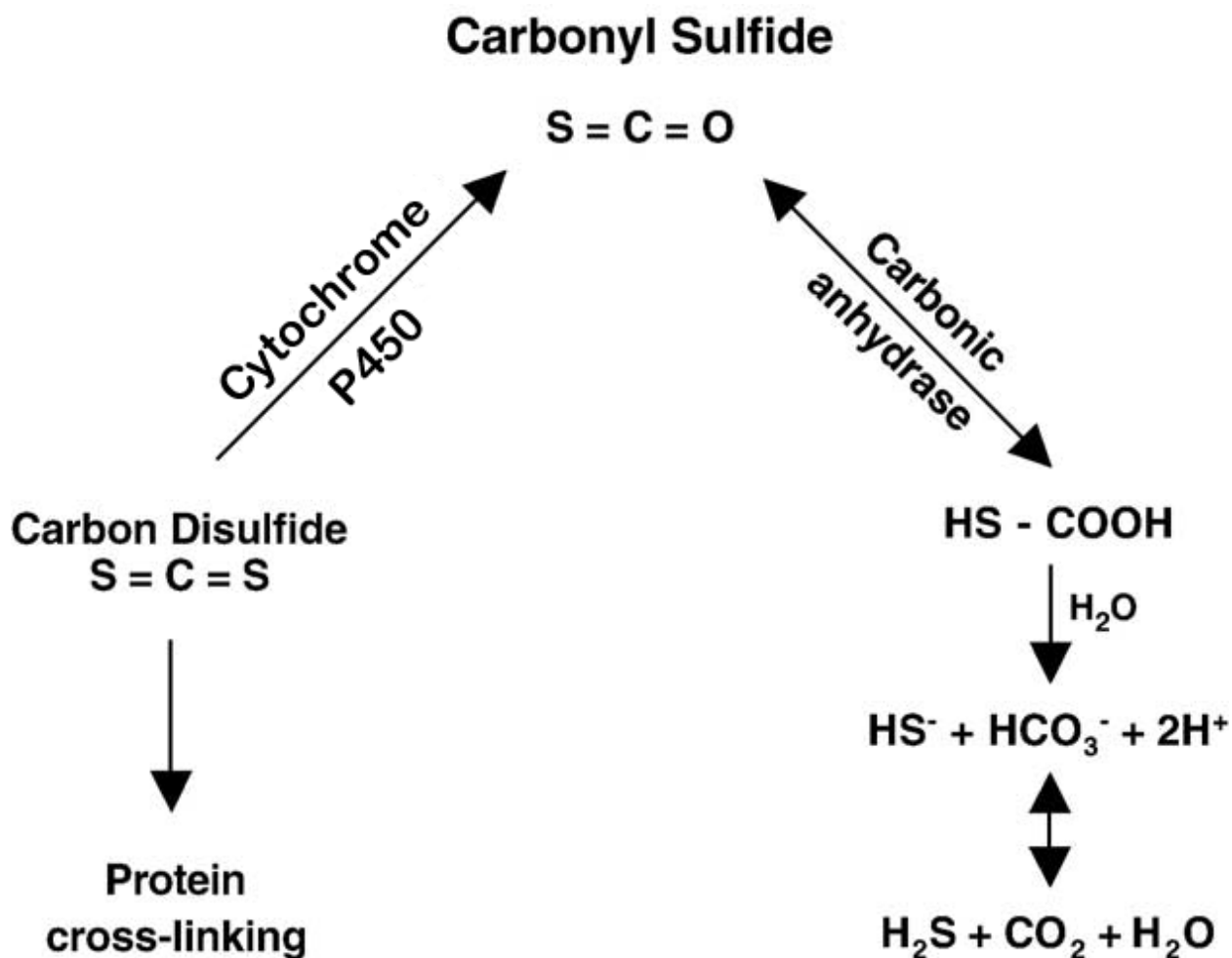


Figure 1. Metabolism of carbonyl sulfide in mammals (Sills et al., 2005)

COS is normally present in human breath; its source is uncertain, although one metabolic scheme implicates methionine as the sulfur source (Studer et al., 2001). The level in the breath of 109 volunteers with normal liver function was reported to be 3,778 ($\pm 7,660$) pmoles/L [mean (\pm SD); approximately 0.23 $\mu\text{g}/\text{m}^3$ or 0.09 ppb]. The ages and smoking status of the volunteers were not given. The breath level of COS was significantly increased (doubled) in 66 patients with either liver disease or hepatocellular injury and significantly decreased in 20 patients with bile duct injury (Sehnert et al., 2002). In a study of lung transplant recipients the level of COS was increased 6-fold in the breath of those acutely rejecting the transplant compared to stable recipients

(Studer et al., 2001). The investigators did not report if they had measured COS in the ambient air. Other investigators studying COS in the breath of cystic fibrosis sufferers have corrected for ambient air levels (Kambores et al., 2005). The levels of COS were significantly enhanced in the breath of most cystic fibrosis patients. The authors suggested that one source of COS could be the bacteria carried by the patients.

Carbonic anhydrase (CA) is a zinc metalloenzyme (Pastorekova et al., 2004) that is important in the respiration and transport of CO₂ and bicarbonate in many tissues (Imtaiyaz Hassan et al., 2013) and which also metabolizes COS. Fourteen isoforms (CA I through CA XIV) have been reported in mammals. At least five are cytosolic (I, II, III, VII, XIII), four are membrane bound (IV, IX, XII, XIV), one is mitochondrial (V), and one is secreted (VI). Three (VIII, X, XI) are inactive. Several, including carbonic anhydrase II, a high activity isozyme, are found in the nervous system. In the brain carbonic anhydrase II is found in oligodendrocytes and in the epithelium of the choroid plexus (Sly and Hu, 1995), and has been found in human fetal brain at mid-gestation (~17 weeks) (Kida et al., 2006). Carbonyl sulfide is metabolized to H₂S and HS⁻, both of which inhibit mitochondrial cytochrome oxidase. If hydrogen sulfide or HS⁻ is the agent of COS toxicity, then increased carbonic anhydrase activity should increase intoxication due to COS. Because of the limited data on COS exposure and subsequent toxicity in humans, it is uncertain as to what effect polymorphisms of carbonic anhydrase and other metabolic enzymes might have on COS-exposed humans.

The toxicity of COS may also involve protein cross-linking as has been shown for CS₂ (see Figure 1) (Graham et al., 1995). COS can react with an organic amine (e.g., a lysyl residue in protein) to form a monothiocarbamate which subsequently loses HS⁻ and forms an isocyanate (Graham et al., 1995). The isocyanate can then react with another amine. If the amine groups are on different proteins, the proteins become cross-linked and possibly dysfunctional.

5. Acute Toxicity of Carbonyl Sulfide

5.1 Acute Toxicity to Adult Humans

Little information on the acute toxicity of COS to humans could be found.

A review of COS toxicology (Bartholomaeus and Haritos, 2005) cites the experience of Klason who reported the effects of breathing pure COS in 1887. "The action of the gas on the nervous system is quite remarkable and similar to that of nitrous oxide. If pure carbonoxysulfide is inhaled, one notices not the slightest effect in the first 10 seconds. Suddenly, one becomes dizzy. One cannot stand upright without support. A peculiar feeling of oppression in the chest and a ringing in the ears occurs. If inhaling of the gas is discontinued, these symptoms remain for about 2 minutes and then suddenly disappear leaving no trace of a headache or other unpleasantness."

Thiess reported two cases of acute toxicity in workers, one of which was fatal, in what was considered to be exposure to COS (Thiess et al., 1968). Symptoms included dizziness and nausea, but there was no irritation of mucous membranes. Authors of the above review have questioned whether the gas was adequately identified as COS (Bartholomaeus and Haritos, 2005). Information in the Thiess paper, from the Institute for Judicial Medicine and Forensics in Mainz, implicated H₂S and CO as candidate acute toxicants. The absence of a reaction with lead paper ruled out H₂S. CO was likely ruled out by carboxyhemoglobin measurements which were below 1%. COS was implicated likely because it was being used or produced at the facility.

5.2 Acute Toxicity to Infants and Children

No information on acute toxicity of COS in infants and children was found.

5.3 Acute Toxicity to Experimental Animals

Carbonyl sulfide is acutely toxic to rats, with an LD₅₀ of 22.5 mg/kg, by intraperitoneal injection. The inhalation 4-hour LC₅₀ determined in male and female Sprague-Dawley rats exposed to COS by whole body inhalation was 1,082 ppm (2,700 mg/m³) (Monsanto, 1985). Other studies in rats from DuPont and Monsanto have determined similar 4-hour LC₅₀ values of 1,065 ppm (2,600 mg/m³) and 1,106 ppm (2,700 mg/m³) (Bartholomaeus and Haritos, 2005). In an abstract, the inhalation 4-hour LC₅₀ for F344 rats exposed to COS by nose-only was reported to be somewhat lower at 590 ppm (1,500 mg/m³) (Benson et al., 1995; Nutt et al., 1996).

The incidence of mortality (number died/total number exposed) varied among different species exposed to 1,000 ppm (2,500 mg/m³) COS for 90 minutes: cats (6/6) > rabbits (8/14) > rats (3/6) > guinea pigs (0/6) (Thiess et al., 1968). There were no deaths after 6 hours at 300 to 500 ppm (740 to 1,200 mg/m³) in cats, rabbits, or guinea pigs (n = 2/species).

In another study, Wistar rats were killed by 0.05% (500 ppm; 1,230 mg/m³) COS in 10 hours and by 0.2% (2,000 ppm; 4,900 mg/m³) COS in 30-60 minutes (Hayashi et al., 1971). Male ddy mice (n=5) showed no effects after exposure to 0.025% (250 ppm; 620 mg/m³) COS for 7 hours per day for 7 days. However, at 0.05% (500 ppm) the mice became sluggish and lost weight during the second 7-hour exposure and died during the third exposure. At 0.2% (2,000 ppm) mice died at approximately 30 minutes.

Groups of 34 male and female F344/hsd rats inhaled 0, 450, 500, or 550 ppm (0, 1,100, 1,200 or 1,400 mg/m³) COS for 4 h and were observed for up to 14 d post exposure for signs of toxicity. Subgroups (5 males, 5 females) were euthanized at 1, 2, 4, 7, and 14 d. "Some" rats (incidence not stated) that survived 500 and 550 ppm COS exposure exhibited mild to severe behavioral changes (barrel rotations, ataxia, head tilt, body tilt). Partial to complete recovery occurred during the 14-d observation period. No behavioral changes were seen at 450 ppm COS. Thus OEHHA staff considers 450 ppm (1,100 mg/m³) an acute 4-hour NOAEL for COS in this report (Benson et al., 1995). OEHHA staff notes the closeness of the NOAEL to the 4 h LC₅₀ of 590 ppm (1,451 mg/m³) reported for this strain of rats by these investigators. The report indicates a steep increase in acute effects of COS between 450 and 500 ppm in rats. The effects were reversible after removal of the animals from COS exposure.

Investigators at NIEHS reported observations from single or repeated (up to 12 weeks) exposures to COS (Morgan et al., 2004). In the range-finding portion of their investigation into COS toxicity, groups of male F344 rats (5 per concentration) were exposed to 0, 75, 150, 300, or 600 ppm (0, 180, 370, 740 or 1,500 mg/m³) COS for 6 hours and then held for 2 weeks without exposure (Morgan et al., 2004). Thirty-six named regions of the brain were examined. At 600 ppm (1,500 mg/m³), some rats were moribund. The rats were lethargic after exposure and on day 2 were hypothermic and showed ataxia, lethargy, and head tilt. The head tilt lessened somewhat, but did not disappear during the two week holding period. Pathological lesions were noted only at 600 ppm (1,500 mg/m³) in the cerebellum and the fifth cranial nerve (Table 2). Thus OEHHA staff concludes that 300 ppm (740 mg/m³) was a NOAEL for this single-exposure experiment.

Table 2. Neuropathological lesion incidence in male rats 14 days after a single 6-hour inhalation exposure to 600 ppm COS (from Morgan, et al., 2004)

<i>CNS region</i>	<i>Neuropathological lesion</i>	<i>Control</i>	<i>Exposed</i>
Parietal cortex area 1	Cortical necrosis	0/5	0/5
Retrosplenial cortex	Cortical necrosis	0/5	0/5
Putamen	Necrosis	0/5	1/5
Internal capsule	Necrosis	0/5	2/5
Thalamus	Necrosis	0/5	2/5
Pyriform cortex	Necrosis	0/5	0/5
Red nucleus	Vacuolation of myelin	0/5	0/5
Anterior olivary nucleus	Vacuolation of myelin and/or necrosis, axonopathy	0/5	1/5
Posterior colliculus	Necrosis	0/5	0/5
Cerebellar cortex	Necrosis	0/5	1/4
Cerebellar roof nucleus	Vacuolation of myelin and/or hemorrhage	0/5	0/4
Cerebellar roof nucleus	Necrosis and cavitation	0/5	3/3*
Cerebellar medullary white	Vacuolation of myelin	0/5	5/5**
Fifth cranial nerve tract	Vacuolation of myelin	0/5	5/5**

Incidence = number of animals with the lesion/number of animals for which that area of the brain was examined.

*p < 0.05 compared to control (Fisher Exact Test)

**p < 0.001 compared to control (Fisher Exact Test)

Morgan also exposed F344 rats (5 males/concentration) to 0, 75, 150, 300, or 600 ppm (0, 180, 370, 740, or 1,500 mg/m³) COS 6 hours per day for 4 days (Morgan et al., 2004). The investigators reported no mortality, morbidity, or clinical toxicity in animals exposed up to 300 ppm COS for 4 days. No microscopic brain lesions were observed in these animals. In contrast, after 2 days rats exposed to 600 ppm were moribund and exhibited clinical signs of neurotoxicity including hypothermia, lethargy, ataxia, and impaired righting reflex. Among 5 male rats exposed to 600 ppm (1,500 mg/m³) for up to 4 days, necrosis, malacia (abnormal softening or loss of structural contiguity) and microgliosis (accumulation of microglia which usually occurs as a result of injury) were detected in several brain regions by microscopy.

These investigators also exposed male and female rats (10/sex/group) to 0, 300, 400, or 500 ppm (0, 740 980 or 1,200 mg/m³) COS for 6 hours/day on 12 days in a 2 week period. The investigators observed the animals for clinical signs of toxicity, and performed a functional observational battery (FOB) to evaluate neurobehavioral effects of COS exposure. The FOB includes general appearance, reactivity to handling, observations of posture, gait, arousal, activity level, a number of reflex tests, and fore limb and hind limb grip strength tests. Brain histopathology was subsequently

conducted. At 500 ppm (1,200 mg/m³) all 10 male rats and 4/10 female rats were moribund and were thus euthanized. These animals displayed extensive neurotoxicity including ataxia and poor motor control of the fore and hind limbs. Surviving females from the 500 ppm group showed hypotonia, decreased grip strength and slight gait abnormalities. At 400 ppm (980 mg/m³), slight gait abnormality was detected in half the rats (both sexes), and hypotonia was present in all rats. Decreases in motor activity and in fore- and hind-limb grip strength were also observed in the animals exposed to 400 ppm. Neuropathological lesions at 400 ppm included necrosis in the parietal cortex area 1 and in the putamen (Table 3). Several additional brain areas were affected at 500 ppm (1,200 mg/m³). (Results shown in Table 3 are for females; similar results were reported for males.)

Consistent with the above observations were changes in the amplitude of the brainstem auditory-evoked responses (BAER) for peaks N3, P4, N4, and N5 after exposure of a separate group of animals (10 males per group) for 2 weeks to 400 ppm (980 mg/m³). These represented changes in auditory transmission between the anterior olivary nucleus and the medial geniculate nucleus. OEHHA notes that 300 ppm (740 mg/m³) appears to be a NOAEL in this 2 week exposure study.

Table 3. Incidence of neuropathological lesions in female rats after inhalation exposure to COS for 2 weeks (from Morgan et al., 2004)

CNS region	Lesion	Control	300 ppm	400 ppm	500 ppm
Parietal cortex area 1	Necrosis	0/10	1/10	8/10**	10/10**
Retrosplenial cortex	Necrosis	0/10	0/10	0/10	7/10**
Hippocampus CA 1 and 3	Neuronal necrosis	0/10	0/10	1/10	3/10
Putamen	Necrosis	0/10	0/10	6/10**	8/9**
Thalamus	Necrosis or vacuolation	0/10	0/10	0/10	6/10**
Red nucleus	Necrosis	0/10	0/9	0/8	3/8
Posterior colliculus	Necrosis	0/8	0/0	3/9	8/10**
Anterior olivary nucleus	Necrosis	0/10	0/10	0/10	6/10**
Vestibular nucleus	Necrosis	0/10	0/10	0/10	1/10
Fifth cranial nerve tract	Vacuolation	0/10	0/10	0/10	0/10

Incidence = number of animals with the lesion/number of animals for which that area of the brain was examined.

**p < 0.001 compared to control (Fisher Exact Test)

Groups of 10 male and 10 female Sprague-Dawley rats were exposed to 0, 51, 151, 253, or 453 ppm (0, 120, 370, 620 or 1,100 mg/m³) COS on 11 days for 6 hours during a 2 week period (Monsanto, 1985b). Signs of CNS dysfunction (ataxia, head-tilting, circling, tremors, and convulsions) were observed at 453 ppm (1,100 mg/m³) in 3 males and 7 females after the first week. Of these, two males and 3 females were killed on

day 8 due to their extreme condition. Rats at 151 ppm (370 mg/m³) and above had dose-dependent increases in methemoglobin. No effects were seen at 51 ppm (120 mg/m³) COS, which is a NOAEL for this experiment.

6. Chronic Toxicity of Carbonyl Sulfide

6.1 Chronic Toxicity to Adult Humans

No reports of chronic toxicity to COS alone in adults were found.

Kilburn and Warshaw (1995) studied whether people exposed to sulfide gases as a result of working at or living downwind from the processing of a "sour" crude oil refinery in California had persistent neurobehavioral dysfunction. Controls (n = 32) were friends and relatives of the exposed subjects and had no exposure from the refinery. Thirteen former workers and 22 neighbors, who became plaintiffs in a class action lawsuit, reported headaches, nausea, vomiting, depression, personality changes, nosebleeds, and breathing difficulties.

The authors tested the exposed and control groups for differences in six brain function areas: neurophysiological, verbal recall, overlearned memory, cognitive function, perceptual motor speed, and affective status (using a profile of mood states (POMS) score). Neurophysiological function was evaluated using the following tests: simple reaction time, visual two-choice reaction time, body balance, blink reflex latency and color discrimination.

Decrements in exposed subject neurophysiological function were statistically significant compared to controls for the following tests: simple and visual two-choice reaction time, body balance, and color vision. Psychomotor speed in the exposed group was significantly reduced compared to controls as measured by two trail-making tests, but was not significantly reduced when measured by peg placement. No significant difference in verbal recall, overlearned memory, or cognitive function was noted between exposed subjects and controls. The mean exposed group POMS score (elevated anger, confusion, depression, tension-anxiety and fatigue scores) was significantly greater than that of the controls.

Outside the refinery desulfurization unit, the measured 24-hour average COS concentrations ranged from 2.6 to 51.1 ppm (6.4 to 125.7 mg/m³) over a 5-year period. In addition to COS, H₂S (0 to 8.8 ppm) and mercaptans (0.1 to 21.1 ppm) were also detected; these compounds can also affect the nervous system (Kilburn and Warshaw, 1995).

6.2 Chronic Toxicity to Infants and Children

No reports of chronic toxicity in infants and children were found.

6.3 Chronic Toxicity to Experimental Animals

There are no chronic or lifetime studies of COS toxicity by the inhalation route. Some investigators have fumigated the diet of rats with different levels of COS, then fed the treated diet to the animals for up to 2 years (Ruishu et al., 1999). No adverse effects were reported, but the study did not measure the kind and amount of residues in the diet (Bartholomaeus and Haritos, 2005).

In a subchronic study, Morgan and colleagues exposed F344 rats to 200, 300 or 400 ppm (490, 740, or 980 mg/m³) COS 6 hours per day, 5 days per week for 12 weeks (Morgan et al., 2004). The investigators observed the animals for clinical signs of toxicity, and evaluated neurobehavioral effects with an FOB at 6 and 12 weeks of exposure. Brain histopathology was performed after the final FOB. The investigators also exposed additional groups of rats to COS at various concentrations and durations up to 12 weeks to evaluate changes in electrophysiology, to image tissues by magnetic resonance microscopy, and to measure cytochrome oxidase in brain tissues. The authors reported no clinical signs of toxicity other than mild gait disturbances in a few animals. Results from the FOB were not consistent across time, dose, or gender. The authors note that there appear to be compensatory mechanisms operating in these animals as some of the symptoms were worse at 6 weeks than at 12 weeks. Unlike the 2 week exposures, hypotonia and decreased grip strength were not observed. After 12 weeks at 400 ppm (980 mg/m³), among groups of 10 male and 10 female rats the predominant lesions were necrosis in parietal cortex area 1 and neuronal loss, microgliosis, and hemorrhage in the posterior colliculus; occasional necrosis was seen in the putamen, thalamus, and anterior olivary nucleus (Table 4). Carbonyl sulfide targeted the auditory system including the olivary nucleus, nucleus of the lateral lemniscus, and posterior colliculus. OEHHA notes that for neuropathological lesions the LOAEL was 400 ppm and the NOAEL was 300 ppm (Table 4). Multiplying by 6/24 (hours/day) and 5/7 (days/week), the equivalent continuous exposures are 71 ppm (175 mg/m³) and 54 ppm (133 mg/m³), respectively.

Table 4. Incidence of neuropathology in rats after inhalation exposure to COS for 12 weeks (from Morgan et al, 2004)

<i>CNS region</i>	<i>Neuropathology</i>	<i>Sex</i>	<i>Control</i>	<i>300 ppm</i>	<i>400 ppm</i>
Parietal cortex area I	Necrosis or cavitation	M	0/10	0/10	5/10*
Parietal cortex area I	Necrosis or cavitation	F	0/10	0/10	4/10*
Posterior colliculus	Neuronal loss or microgliosis	M	0/9	0/9	7/9**
Posterior colliculus	Neuronal loss or microgliosis	F	0/9	0/9	5/9**
Posterior colliculus	Hemorrhage	M	0/9	0/9	2/9
Posterior colliculus	Hemorrhage	F	0/9	0/9	1/9
Thalamus	Necrosis	M	0/10	0/10	1/10
Thalamus	Necrosis	F	0/10	0/10	0/10

Incidence = number of animals with the lesion/number of animals for which that area of the brain was examined.

* $p < 0.05$ compared to control (Fisher Exact Test)

** $p < 0.001$ compared to control (Fisher Exact Test)

The lesions identified by traditional histological methods in the Morgan et al. study were confirmed and extended by magnetic resonance microscopy (Sills et al., 2004). The addition of magnetic resonance microscopy enabled the early identification of the most sensitive target in the CNS, the posterior colliculus.

Cytochrome oxidase catalyzes the oxidation of cytochrome c and is a rate-limiting enzyme in oxidative phosphorylation in the mitochondria. Inhibition of this enzyme decreases ATP production in the cell and would contribute to cell death. As noted earlier, both H_2S and HS^- are metabolites of COS and inhibitors of cytochrome oxidase.

A concentration-related decrease in mitochondrial cytochrome oxidase was detected in the posterior colliculus (data not shown) and in the parietal cortex of rats (10 per group) exposed to 0, 200, 300, or 400 ppm (0, 490, 740 or 980 mg/m^3) COS for 3 weeks (Morgan et al., 2004). Cytochrome oxidase activity in the posterior colliculus and parietal cortex was significantly decreased at all doses (Table 5). The decrease persisted through 7 and 12 weeks of exposure. After 12 weeks of exposure, only the rats exposed to 400 ppm (980 mg/m^3) showed detectable brain lesions (cortical necrosis, cavitation in the parietal cortex, and bilateral symmetrical neuronal loss in the posterior colliculus). Thus the results suggest that the inhibition of the mitochondrial respiratory chain may precede brain lesions. OEHHA staff concludes that the LOAEL was 200 ppm (490 mg/m^3), the lowest concentration tested, for decreased cytochrome oxidase activity in male and female rat brain (Table 5).

Table 5. Levels of cytochrome oxidase in the parietal cortex of rats after repeated inhalation exposure to COS (from Morgan et al., 2004)

COS (ppm)	Female		Male	
	Cytochrome oxidase ^a	% control	Cytochrome oxidase ^a	% control
<i>Day 24 (3 weeks)</i>				
0	1,829 ± 163 ^b	100	1,841 ± 170	100
200	1,642 ± 88 ^{**}	90	1,580 ± 204 [*]	86
300	1,129 ± 127 ^{**}	62	1,258 ± 190 ^{**}	68
400	1,182 ± 104 ^{**}	65	1,066 ± 234 ^{**}	58
<i>Day 52 (7 weeks)</i>				
0	2,131 ± 257	100	1,898 ± 334	100
200	1,629 ± 209 ^{**}	76	1,755 ± 139	80
300	1,171 ± 232 ^{**}	55	1,277 ± 108 ^{**}	67
400	1,227 ± 139 ^{**}	58	1,227 ± 94 ^{**}	65
<i>Day 86 (12 weeks)</i>				
0	1,711 ± 125	100	1,687 ± 214	100
200	1,268 ± 232 ^{**}	74	1,349 ± 111 ^{**}	80
300	928 ± 175 ^{**}	54	816 ± 129 ^{**}	48
400	857 ± 72 ^{**}	50	935 ± 185 ^{**}	55

^a μmol cytochrome c/min/mg protein

^b mean ± SD (n=10)

* p < 0.05 compared to control (Dunnett's Test)

** p < 0.001 compared to control (Dunnett's Test)

Herr and co-workers reported the results of expanded neurophysiological examinations (Herr et al., 2007) that were a part of the above study (Morgan et al., 2004). Fischer rats were exposed using whole-body inhalation chambers to 0, 200, 300, or 400 ppm (0, 490, 740, or 980 mg/m³) COS for 6 hours/day, 5 days/week for 12 weeks or to 0, 300, or 400 ppm for 2 weeks. After treatment, the animals were subjected to neurophysiological tests to examine: (1) peripheral nerve function, (2) somatosensory-evoked potentials (SEPs) (tail/hind limb and facial cortical regions), (3) brainstem auditory-evoked responses (BAERs; a measure of auditory neural function), and (4) visual flash-evoked potentials (2-week study). In addition, rats exposed for 2 weeks were examined using an FOB and response modification audiometry (RMA). Peripheral nerve function was not altered for any exposure scenario. Amplitudes of somatosensory-evoked potentials from the cerebellum were not altered by COS exposure. However, after 12 weeks of exposure to 400 ppm (980 mg/m³), amplitudes and latencies of somatosensory-evoked potentials from cortical areas were altered. These effects were not seen at 300 ppm (740 mg/m³).

In the 2-week study the somatosensory-evoked potential waveforms were changed to a greater extent after forelimb stimulation than after tail stimulation. The most consistent

findings were decreased amplitudes of BAER peaks associated with brainstem regions after exposure to 400 ppm (980 mg/m³) COS.

Additional BAER peaks were affected after 12 weeks, compared to 2 weeks of treatment, indicating that additional regions of the brainstem were damaged with longer exposures to COS (Table 6). The changes in BAERs were observed in the absence of altered auditory responsiveness in FOB or response modification audiometry. The authors concluded that COS produces changes in brainstem auditory and cortical somatosensory neurophysiological responses that correlate with previously described histopathological damage. For the 12 week exposure, OEHHA considers that the LOAEL for changes in BAER was 400 ppm (980 mg/m³) and the NOAEL was 300 ppm (740 mg/m³) COS.

Table 6. Brainstem auditory-evoked responses (BAERs) in rats after inhalation exposure to COS for 12 weeks (Herr et al., 2007)

BAER Peak	Region where generated	Altered by COS
P1	Auditory nerve	No
P2	Cochlear nucleus	No
P3	Olivary complex	Yes
P4	Lateral lemniscus	Yes
P5	Brainstem and posterior colliculus	Yes
P6	Brainstem and medial geniculate nucleus	Yes

Kamstrup and Hugod (1979) exposed 18 White Danish country female rabbits continuously to 54 ± 13 ppm (130 ± 32 mg/m³) COS for 7 weeks; 17 female rabbits served as controls. Three of the 18 rabbits (17%) died after 5 days of exposure and two others had serious symptoms of CNS intoxication. The other 13 exposed rabbits were reported by the authors to be “clinically unaffected” during the exposure. The authors suggested possible inter-individual differences in sensitivity to the neurotoxicity of COS. Exposure slightly elevated mean serum cholesterol. The mean serum triglyceride level was largely unaffected by exposure, although an increasing trend with time of exposure was observed. A small increase in free cholesterol in serum from the aorta was likely caused by the difference in serum cholesterol concentrations between experimental and control animals. No significant difference in arterial uptake of labeled cholesterol between exposed animals and controls could be demonstrated. By light microscopic investigation, no histopathological changes in lungs or atherosclerosis-like changes in the intima of the coronary arteries, aorta, or pulmonary arteries were found (Kamstrup and Hugod, 1979). OEHHA considers the strong dichotomy in effect, clinically unaffected vs. serious CNS intoxication or death, reported in this study an unusual finding. The very large variability in rabbits in the activity and tissue distribution of isoenzymes of carbonic anhydrase (which metabolizes COS) may be involved in the dichotomy (McIntosh, 1970).

The three chemicals, COS, CS₂, and H₂S, are interconnected by metabolism (Figure 1) and all have as a principal target of toxicity the nervous system. The most sensitive adverse effect detected for COS was depression of cytochrome c oxidase activity in brain (Table 5). The mechanism of H₂S toxicity is cellular hypoxia caused by inhibition of cytochrome oxidase (Nicholls, 1975). The specific target of CS₂ is less certain although it can bind to the cytochrome in P450 enzymes (Obrebska et al., 1980) in addition to cross-linking proteins. OEHHA has reviewed the nervous system toxicity of CS₂ and H₂S in its acute and chronic REL summaries for these chemicals (OEHHA, 2014).

H₂S has also been described as having a physiological role as a gasotransmitter. Gasotransmitters are low molecular weight gases that are endogenously generated, exhibit enzymatic regulation of their production and metabolism, and exert several physiological functions that do not depend on specific membrane receptors. Some other examples of gasotransmitters are nitric oxide (NO) and carbon monoxide (CO) (Salloum, 2015).

H₂S has been proposed as a regulator of homeostasis in several organ systems, including the cardiovascular, neuronal, gastrointestinal, respiratory, renal, liver and reproductive systems (Wallace and Wang, 2015).

However, the effective physiological plasma or tissue concentrations of H₂S are several orders of magnitude below the corresponding concentrations believed to induce toxicity (15 nM and >250 μM, respectively)(Sulaieva and Wallace, 2015). Thus, it would be expected that the toxic effects of H₂S resulting from COS metabolism would be distinct from any effects exhibited by H₂S in its gasotransmitter role.

7. Developmental and Reproductive Toxicity of COS

7.1. Teratology

In a teratology study conducted at Monsanto Agricultural Company (available in an abstract (Monsanto, undated)), mated female Sprague-Dawley rats were exposed to 0, 50, 200, or 400 ppm (0, 120, 490, or 980 mg/m³) COS by inhalation 6 hours/day on gestation days (gd) 6 through 15 and terminated at day 21. Maternal toxicity (death, reductions in weight gain and food consumption) was seen at 400 ppm (980 mg/m³), but not 200 ppm (490 mg/m³) (a NOAEL for maternal toxicity). The authors found no evidence for embryotoxicity, fetotoxicity, or treatment-related increase in variations or malformations.

7.2. Reproductive Toxicity

In an unpublished, non-peer reviewed one generation reproduction study also done at Monsanto, groups of 24 male Sprague-Dawley rats were exposed to 0, 10, 60, or 180 ppm (0, 25, 150, or 450 mg/m³) COS by inhalation 6 hours/day, 5 days per week for approximately 13 weeks, then mated with unexposed females (Reyna and Ribelin, 1987). Although not peer-reviewed, this study was GLP compliant and appeared to be well-conducted. Measured endpoints included parental body weight, pregnancy rate, number and sex of live and dead pups, and pup weight. The reproductive effect noted in the experiment was a significant decrease in the pregnancy rate of unexposed females mated with males exposed to 180 ppm (450 mg/m³) COS (Table 7). Additional experimentation showed that, when males were allowed a recovery period of 10 weeks after exposure and prior to mating, female pregnancy rate was unaffected. Thus the effect on male fertility appears reversible. OEHHA staff concludes that in this study, 60 ppm (150 mg/m³) is a NOAEL and 180 ppm (450 mg/m³) is a LOAEL for reproductive toxicity in male rats. In another experiment, when females were exposed to 0, 10, 60, or 180 ppm COS for 6 hours/day, 5 days per week for 13 weeks prior to mating with unexposed males, the NOAEL for reproductive toxicity was 180 ppm (450 mg/m³) in female rats based on lack of effect on the pregnancy rate.

Table 7. Pregnancy rate in female rats mated with male rats exposed to COS by inhalation for 13 weeks

Male pre-exposure	None	10 ppm	60 ppm	180 ppm
Paired females (unexposed)	24	24	24	24
Pregnant females	20	20	20	12
Pregnancy rate (%)	83.3	83.3	83.3	50*

* p < 0.05 compared to mating with male rats with no COS pre-exposure (Fisher Exact Test)

7.3. Genotoxicity

There are few genotoxicity studies using COS; reported results were generally negative. Tests included gene reversion in four *Salmonella his⁻* tester strains (TA97, TA98, TA100, and TA102) ± S9 induced liver extract at 1, 5, 10, and 50 g/m³ (400, 2,000, 4,000, and 20,000 ppm) COS and in five *E. coli* reverse mutation tester strains ± S9 at 50, 100, 500, and 1,000 mg/m³ (20, 40, 200, and 400 ppm) COS for 2 hours in vitro. The authors used previously published exposure methods (Maron and Ames, 1983). Mammalian tests included a marrow red cell micronucleus test in mice exposed by inhalation to 0.1, 0.5, 1.0, and 2.0 g/m³ (40, 200, 400, and 800 ppm) COS for 2 hours and in a chromosomal alteration test in mice exposed to 0.25, 0.5, and 1.0 g/m³ (100, 200, 400, and 800 ppm) COS by inhalation for 2 hours (Ruishu et al., 1999). The authors used positive and negative controls in the various test systems.

The National Toxicology Program (NTP) studied 4 strains of mutant *Salmonella* (TA97, TA98, TA100, and TA1535) in the Ames test and used from 0.58 to 2.89 µg COS per

test plate with and without induced liver extract from rats or hamsters. They reported a weakly positive response based on positive results in one strain (TA97) (NTP, 2010).

7.4. Gene Expression

In order to study the time course for the development of the neurotoxicological lesions and the gene expression changes occurring in the posterior colliculus, Fischer 344 rats were exposed to 0 or 500 ppm (1,200 mg/m³) COS 6 hours per day for 1, 2, 3, 4, 5, 8, or 10 days (Morrison et al., 2009). Gene expression was determined by both microarray analysis of the expressed genome and by Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) of specific genes. No morphological changes were detected on day 1 or 2. On day 3, 10/10 (100%) rats had necrosis in the posterior colliculi. On day 4 and following, numerous areas of the brain were necrotic. Gene expression changes in the posterior colliculi from one or two days of COS exposure were predictive of the subsequent neuropathology. Collicular changes included up-regulation of genes associated with DNA damage and G1/S cell-cycle checkpoint regulation (KLF4, BTG2, GADD45g), apoptosis (TGM2, GADD45g, RIPK3), and vascular mediators (ADAMTS, CTGF, CYR61, VEGFC). The pro-inflammatory mediators CCL2 and CEBPD were up-regulated prior to increases in the astrocyte marker GFAP and the macrophage marker CSF2rb1 (Table 8).

Table 8. Genes, mediators and markers up-regulated in rats after inhalation to 500 ppm COS for 2 days

Factor	Description of rat brain factor	Fold increase after COS	
		Microarray	RT-PCR*
KLF4	Kruppel-like transcription factor 4 (gut), an upstream regulator of p53	2.19	2.02
GADD45g	growth arrest and DNA-damage-inducible, gamma	1.91	2.64
TGM2	transglutaminase 2, an effector of apoptosis; crosslinks proteins at glutamine residues	2.35	2.44
ADAMTS1	a disintegrin and metalloproteinase with thrombospondin type 1 motif	2.03	4.82
CYR61	cysteine-rich, angiogenic inducer, 61; stimulates endothelial cell growth	2.61	13.05
CEBPD	CCAAT/enhancer binding protein (C/EBP), delta; a modulator of inflammation (CCAAT is a specific base sequence recognized in DNA)	2.48	3.59
GFAP	glial fibrillary acidic protein	1.81	2.06
CSF2rb1	colony stimulating factor 2 receptor, beta, low-affinity (granulocyte-macrophage)	3.43	17.37
HSPA1A	heat shock 70 kDa protein	2.45	3.16

* Reverse Transcriptase-Polymerase Chain Reaction

8. Derivation of Reference Exposure Levels

8.1 Carbonyl Sulfide Acute Reference Exposure Level

<i>Study</i>	(Morgan et al., 2004)
<i>Study population</i>	Groups of 5 F344 male rats, 8-9 weeks old
<i>Exposure method</i>	Inhalation of 0, 75, 150, 300, or 600 ppm COS
<i>Exposure continuity</i>	Single exposure
<i>Exposure duration</i>	6 hours (plus 14 days observation)
<i>Critical effects</i>	CNS effects (ataxia, head tilt, necrotic lesions, and vacuolation of myelin)
<i>LOAEL</i>	600 ppm (1,500 mg/m ³)
<i>NOAEL</i>	300 ppm (740 mg/m ³)
<i>Benchmark concentration</i>	Not derived (effect only at highest dose, with 100% incidence) (Table 2)
<i>Time-adjusted exposure (one-hour)</i>	540 ppm [(300 ppm) ³ x 6 hours = (x ppm) ³ x 1 hour]
<i>Human Equivalent Concentration</i>	540 ppm (1,300 mg/m ³) (RGDR* = 1)(systemic effect)
<i>LOAEL uncertainty factor (UF_L)</i>	1 (NOAEL determined)
<i>Interspecies uncertainty factor</i>	
<i>Toxicokinetic (UF_{A-k})</i>	2 (default; no PBPK model)
<i>Toxicodynamic (UF_{A-d})</i>	√10 (default: no interspecies toxicodynamic data)
<i>Intraspecies uncertainty factor</i>	
<i>Toxicokinetic (UF_{H-k})</i>	10 (default)
<i>Toxicodynamic (UF_{H-d})</i>	10 (potential for increased sensitivity of infants and children to neurotoxicants)
<i>Database uncertainty factor (UF_D)</i>	√10 (limited database)
<i>Cumulative uncertainty factor</i>	2,000
<i>Acute Reference Exposure Level</i>	660 µg/m ³ (270 ppb)

*The RGDR (Regional Gas Dose Ratio) is the ratio of the regional gas dose calculated for a given exposure for the respiratory region affected by a toxicant in the animal species to the regional gas dose of the same exposure in humans. For a systemic effect such as CNS toxicity the default value is 1.

Reference Exposure Levels are based on the most sensitive and relevant health effects reported in the medical and toxicological literature. Acute Reference Exposure Levels are levels at which infrequent one-hour exposures are not expected to result in adverse health effects (see Section 5 of the Technical Support Document (OEHHA, 2008)).

In the key study (Morgan et al., 2004) rats exposed to 600 ppm (1,500 mg/m³) COS for 6 hours showed ataxia and head tilt, as well as neuropathological lesions in the brain (Table 2), while those at 300 ppm (740 mg/m³) did not exhibit these nervous system effects. There were only five animals (all males) per exposure level.

Time extrapolation from the 6 hour exposure to the 1 hour period of the acute REL used OEHHA's default modification of Haber's relationship where concentration cubed multiplied by time equals a constant (OEHHA, 2008).

The default interspecies UF_{A-k} of 2 was used for residual pharmacokinetic differences not accounted for by the Human Equivalent Concentration (HEC) adjustment, and because there were no pharmacokinetic modeling data available to estimate the internal dose in humans, when the point of departure (NOAEL) is based on experimental animal studies. The default interspecies UF_{A-d} of $\sqrt{10}$ was applied to account for the absence of data on potential pharmacodynamic differences for COS between rats and humans (OEHHA, 2008).

The default intraspecies UF_{H-k} of 10 was used because there were no pharmacokinetic modeling data available for COS to account for variability among the human population. In infants and newborns, the incompletely formed blood brain barrier might allow more access of carbonyl sulfide to the CNS. An intraspecies UF_{H-d} (toxicodynamics) of 10 was used because of the increased susceptibility of infants and children to neurotoxicants and the steepness of the acute dose-response curve for COS effects, which are severe.

A database uncertainty factor of $\sqrt{10}$ was used because of the limited database for acute toxicity, including a handful of lethality studies, and no neurodevelopmental data for COS. Some possibly relevant data have only been reported in abstracts.

As a comparison, the data of Benson and coworkers yielded a NOAEL of 450 ppm, which leads to an acute REL of 410 ppb (1,000 µg/m³) using the same uncertainty factors listed above (Benson et al., 1995).

8.2 Carbonyl Sulfide 8 hour Reference Exposure Level

The 8-hour Reference Exposure Level is a concentration at or below which adverse non-cancer health effects would not be anticipated for repeated 8-hour exposures (see Section 6 in the Technical Support Document (OEHHA, 2008)). Because chemicals that have the endpoint of neurotoxicity often have cumulative and sometimes irreversible effects, the 8 hour REL is the same as the chronic REL that is derived below.

8.3 Carbonyl Sulfide Chronic Reference Exposure Level

<i>Study</i>	(Morgan et al., 2004)
<i>Study population</i>	F344/N rats (10/sex/exposure level)
<i>Exposure method</i>	Discontinuous whole-body inhalation exposures to 0, 200, 300, or 400 ppm COS
<i>Exposure continuity</i>	6 hours/day for 5 days/week
<i>Exposure duration</i>	12 weeks
<i>Critical effects</i>	Low cytochrome oxidase levels in exposed females (Table 5)
<i>LOAEL</i>	200 ppm (980 mg/m ³)
<i>NOAEL</i>	not found
<i>Benchmark concentration</i>	44 ppm (BMCL _{1SD} ; Exponential Model 2)
<i>Time-adjusted exposure</i>	7.9 ppm (44 ppm x 6 hours/24 hours x 5 days/7 days)
<i>Human Equivalent Concentration</i>	7.9 ppm (19 mg/m ³) (RGDR = 1)(systemic effect)
<i>LOAEL uncertainty factor (UF_L)</i>	Not relevant since BMCL used
<i>Subchronic uncertainty factor (UF_s)</i>	√10 (12 week study)
<i>Interspecies uncertainty factor</i>	
<i>Toxicokinetic (UF_{A-k})</i>	2 (no PBPK model)
<i>Toxicodynamic (UF_{A-d})</i>	√10 (default: no interspecies toxicodynamic data)
<i>Intraspecies uncertainty factor</i>	
<i>Toxicokinetic (UF_{H-k})</i>	10 (default)
<i>Toxicodynamic (UF_{H-d})</i>	√10 (default)
<i>Database uncertainty factor (UF_D)</i>	√10 (limited database)
<i>Cumulative uncertainty factor</i>	2,000
<i>Chronic Reference Exposure Level</i>	10 µg/m ³ (4 ppb)

The chronic Reference Exposure Level is a concentration at which adverse noncancer health effects would not be expected from continuous chronic exposures (see Section 7 in the Technical Support Document (OEHHA, 2008)).

OEHHA staff participated in a workshop that explored the use in health risk assessment of “upstream” effects that are not deleterious by themselves but usually are precursors of adverse effects (Woodruff et al., 2008). The workshop concluded: “For certain classes of early perturbations, sufficient information on the disease process is known, so hazard and quantitative risk assessment can proceed using information on upstream biological perturbations.” For carbonyl sulfide such an upstream effect may be a decrease in cytochrome oxidase levels in certain areas of the brain. As discussed above, concentration-related, statistically significant decreases in (mitochondrial)

cytochrome oxidase activity were found in the posterior colliculus and parietal cortex of the brains of male and female rats exposed to 200, 300, and 400 ppm (490, 740, and 980 mg/m³) COS for 3, 6, and 12 weeks (Table 5). These decreases in activity were present in the brains of rats exposed to 200 and 300 ppm COS where no histopathological findings were present.

Models in US EPA's Benchmark Dose (BMDS) software versions 2.2 and 2.5 for continuous data were fit to the cytochrome oxidase data for female rats at 12 weeks. Results are shown in Table 9.

Table 9. BMDS models fit to cytochrome oxidase data in female rats after inhalation exposure to COS for 12 weeks

Model ¹	Deviation	BMC	BMCL	p for fit	AIC(fitted) ²
Hill	1 SD	148	85	NA ³	452.90
Hill	0.5 SD	127	56	NA	452.90
Hill	0.05 relative	130	59	NA	452.90
Power	1 SD	73	59	0.075	454.07
Linear	1 SD	73	59	0.075	454.07
Polynomial(n=2) ⁴	1SD	58	43	0.046	454.88
Exponential Model 2	1 SD (normal dist.)	55	44	0.120	453.15
Exponential Model 3	1 SD	69	44	0.051	454.70
Exponential Model 4	1 SD	55	40	0.120	453.16
Exponential Model 5	1 SD	130	78	NA	452.90

¹ The results for all models are from BMDS 2.2, 2.4 and 2.5 runs with constant variance. Runs using non-constant variance yielded p values for fit < 0.1. A deviation of 1 standard deviation (SD) from the mean, [using an assumption that the data are normally distributed](#), was selected as a defensible point of departure (POD). It is also recommended by the US EPA to always be shown as a standardized basis of comparison (USEPA, 2012).

² AIC = Akaike Information Criterion

³ NA = not applicable since the degrees of freedom for chi-square ≤ 0.

⁴ Since the adverse effect was in the down direction with increasing dose, the beta coefficients were constrained to be non-positive (Davis et al., 2011). In this case the polynomial result was similar to the linear and power models.

The complete BMDS file for the exponential models is presented in Section 9 below.

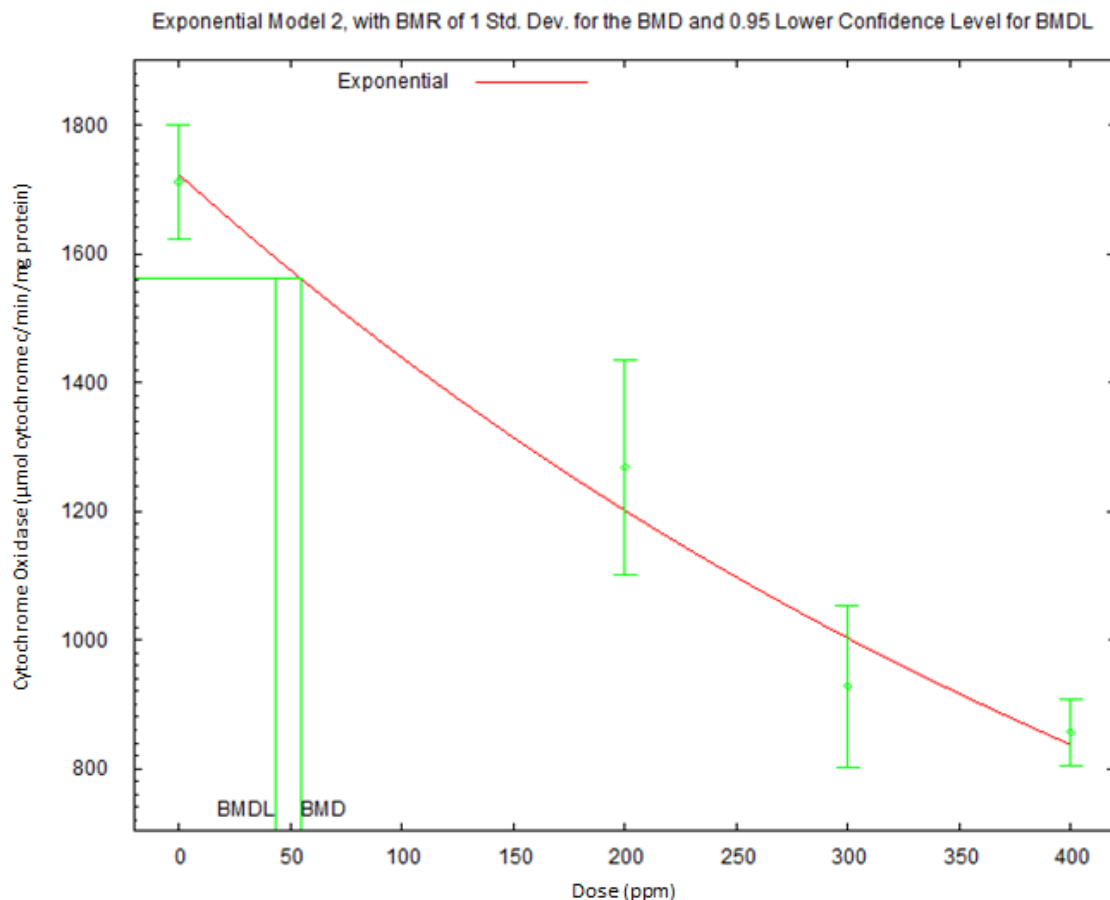


Figure 2. Exponential Model 2 fit to female rat cytochrome oxidase data at 12 weeks

Use of these data (the $BMCL_{1SD}$ of 44 ppm (110 mg/m³) from Exponential Model 2) (Figure 2) for cytochrome oxidase decrease as the point of departure, compared to the NOAEL of 300 ppm (740 mg/m³) for brain lesions, results in a lower, more health protective chronic REL. (The BMDS modelling output for the four exponential models are appended to this document.) OEHHA has previously used “upstream” effects such as the inhibition of the symporter for iodine as the critical effect in its Public Health Goal (PHG) for perchlorate in drinking water, and increased serum activities of alkaline phosphatase, gamma-glutamyl transferase and glutamic-pyruvic transaminase in the chronic REL for dioxins and dibenzofurans. The Department of Pesticide Regulation considers inhibition of acetylcholinesterase to be a key adverse effect of pesticides. In the case of COS, the demonstration of dose-dependent lower levels of a key mitochondrial enzyme in the brain by a neurotoxicant indicates that this is likely a precursor effect to the neuropathology. It also argues against lowering the default uncertainty factors for the RELs derived for COS based on neurological effects, since metabolic effects that may lead to adverse health effects are present in the central nervous system at COS concentrations as low as 200 ppm (490 mg/m³).

A subchronic uncertainty factor, UF_s , of $\sqrt{10}$ was used since a 12 week rodent exposure is considered a subchronic exposure by OEHHA, but it is not so short as to require a factor of 10.

The default interspecies UF_{A-k} of 2 for residual pharmacokinetic differences was used because (1) these differences were not accounted for by the HEC adjustment, and (2) there were no pharmacokinetic modeling data available to estimate the internal dose in humans, when the point of departure is based on experimental animal studies. The default interspecies UF_{A-d} of $\sqrt{10}$ was applied to account for the absence of data on potential pharmacodynamic differences for COS between rats and humans (OEHHA, 2008).

The default intraspecies UF_{H-k} of 10 was used because (1) there were no pharmacokinetic modeling data available for COS to account for variability among the human population in infants and newborns and (2) the incompletely formed blood-brain barrier in humans might allow more access of COS to the CNS. In addition, levels of carbonic anhydrase II in brain regions were lower in neonates than in children and adults (Kida et al., 2006).

An intraspecies UF_{H-d} (toxicodynamics) of $\sqrt{10}$ (default) was used. Although OEHHA staff sometimes uses a UF of 10 to address the usually increased sensitivity of infants and children to neurotoxicants, in this case it is not necessary because an upstream precursor effect is the endpoint for the REL, the pathological findings occurred at higher dose levels, and the dose-response is not steep (Table 5). There is no specific information on potential pharmacodynamic differences for COS among the human population.

A database uncertainty factor of $\sqrt{10}$ was used because of the limited database available on COS, including on the neurodevelopmental toxicity of COS.

For a comparison chronic REL, OEHHA staff used the less sensitive but more traditional endpoint of CNS pathology (Table 4).

Comparison Carbonyl Sulfide Chronic Reference Exposure Level

<i>Study</i>	(Morgan et al., 2004)
<i>Study population</i>	F344/N rats (10/sex/exposure level)
<i>Exposure method</i>	Discontinuous whole-body inhalation exposures to 0, 200, 300, or 400 ppm COS
<i>Exposure continuity</i>	6 hours/day for 5 days/week
<i>Exposure duration</i>	12 weeks
<i>Critical effects</i>	CNS pathology (Table 4)
<i>LOAEL</i>	400 ppm (980 mg/m ³)
<i>NOAEL</i>	300 ppm (740 mg/m ³)
<i>Benchmark concentration</i>	Not derived
<i>Time-adjusted exposure</i>	54 ppm (300 ppm x 6 hours/24 hours x 5 days/7 days)
<i>Human Equivalent Concentration</i>	54 ppm (130 mg/m ³) (RGDR = 1)(systemic effect)
<i>LOAEL uncertainty factor (UF_L)</i>	1 (NOAEL observed)
<i>Subchronic uncertainty factor (UF_s)</i>	√10 (12 week study)
<i>Interspecies uncertainty factor</i>	
<i>Toxicokinetic (UF_{A-k})</i>	2 (RGDR but no PBPK model)
<i>Toxicodynamic (UF_{A-d})</i>	√10 (default: no interspecies toxicodynamic data)
<i>Intraspecies uncertainty factor</i>	
<i>Toxicokinetic (UF_{H-k})</i>	10 (default)
<i>Toxicodynamic (UF_{H-d})</i>	10 (increased sensitivity of infants and children to neurotoxicants)
<i>Database uncertainty factor (UF_D)</i>	√10 (limited database)
<i>Cumulative uncertainty factor</i>	6,000
<i>Chronic Reference Exposure Level</i>	22 µg/m ³ (9 ppb)

In the comparison study, there was a high incidence of necrosis or cavitation of the parietal cortex (area 1) or lesions in the posterior colliculus in male and female rats exposed to 400 ppm (980 mg/m³) COS 6 hours/day, 5 days/week for 12 weeks, while no animals exposed to 300 ppm had such lesions. This indicates a very steep dose-response curve for a severe effect. The calculated value is somewhat higher than the chronic REL.

A comparison chronic REL can also be derived from the reproductive/developmental toxicity study by Reyna and Ribelin (unpublished), although this report has not been published in the peer-reviewed literature. Measured endpoints included parental body weight, pregnancy rate, number and sex of live and dead pups, and pup weight. The

effect noted in the experiment was a significant decrease in the pregnancy rate of unexposed females mated with males exposed to 180 ppm COS (440 mg/m³) (Table 7). The study found a LOAEL of 180 ppm (440 mg/m³) and a NOAEL of 60 ppm (150 mg/m³). The NOAEL corresponds to a continuous exposure of 10.7 ppm (26 mg/m³). A subchronic uncertainty factor of $\sqrt{10}$ was applied since the study duration was 13 weeks. To accommodate possible differences between rats and humans, OEHHA staff applied the default interspecies toxicokinetic and toxicodynamic UFs of 2 and $\sqrt{10}$, respectively. We applied the default intraspecies toxicokinetic and toxicodynamic UFs of 10 and $\sqrt{10}$, respectively, to account for intra-individual variation when using a “sensitive animal model. A database UF of $\sqrt{10}$ was included due to the limited database for COS, including a lack of neurodevelopmental data. The cumulative UF equals 2,000 resulting in a comparison chronic REL of 5 ppb (13 µg/m³).

COS has a relatively steep dose-response curve for acute effects; the acute REL is substantially greater (66-fold) than the chronic REL (Table 10). Since the toxicity of COS may result from conversion to H₂S (Figure 1), it is unexpected that the chronic RELs for both COS and H₂S are 10 µg/m³, but the acute RELs vary by a factor of 16 (Table 10). The data available and the methods used to calculate these RELs account for the differences seen. Brown and Strickland (2003) published a dose-duration meta-analysis of H₂S acute toxicity; steep curves for acute lethality by inhalation in rats were observed at eight time points ranging from 5 minutes to 16 hours. The amount of acute exposure data for COS (one 4 hour and one 6 hour study) is insufficient for a similar meta-analysis. The acute REL for H₂S is based on a human study where the adverse effect is a LOAEL for odor perception, while the chronic REL is based on inflammation of nasal mucosa in mice. The acute REL for COS is based on an animal study NOAEL for more severe adverse CNS effects (ataxia, head tilt, necrotic lesions, and vacuolation of myelin), while the COS chronic REL is based on a BMCL₀₅ for cytochrome oxidase inhibition, an up- stream event to a pathological endpoint. OEHHA staff notes that hydrogen cyanide (HCN) also inhibits cytochrome oxidase and has a steep dose-response curve; the acute REL for HCN is 38 times the chronic REL (Table 10).

Table 10. Comparison of acute and chronic RELs of related sulfur-containing chemicals and hydrogen cyanide

Chemical	Acute REL (µg/m ³)	Chronic REL (µg/m ³)	Acute/chronic ratio
COS	660	10	66
HCN	340	9	38
CS ₂	6200	800	8
H ₂ S	42	10	4

8.4 Carbonyl Sulfide as a Toxic Air Contaminant Especially Affecting Infants and Children

In the Clean Air Act Amendments of 1989, US EPA listed carbonyl sulfide as a Hazardous Air Pollutant (HAP). As a result of this listing, in 1993 the California Air Resources Board identified carbonyl sulfide as a Toxic Air Contaminant (TAC).

Under Health and Safety Code Section 39669.5, OEHHA establishes and maintains a list of Toxic Air Contaminants (TACs) that may disproportionately impact infants and children. OEHHA evaluates TACs for addition to this list as we develop Reference Exposure Levels for TACs.

As described in OEHHA's "Prioritization of Toxic Air Contaminants Under the Children's Environmental Protection Act" (OEHHA, 2001), the initial peer-reviewed document produced to prioritize TACs that may disproportionately impact children, neurotoxicity is a "red flag" toxicological endpoint for concern about higher impacts from early life-stage exposures:

"Certain systems have critical periods during their development when they are particularly vulnerable to lasting injury by xenobiotic chemicals or other agents. Among these critical systems are the three information processing systems of the body: the central nervous, the endocrine, and the immune systems. Each of these complex systems is programmed as it develops, and xenobiotic chemicals can interfere with programming of these critical systems. The central nervous system is programmed to recognize and respond to internal and external stimuli in an adaptive manner that supports the survival of the organism. ... Effects on one of these systems can be expected to have collateral effects on the others..."

"Because these organ systems have long developmental periods and are known to be irreversibly impacted by specific toxicants, any toxicological or epidemiological information indicating impacts on these systems was considered a red flag."

Since COS is a neurotoxicant, concern arises for early life-stage exposures. In the animal studies used as a basis for our Reference Exposure Levels, COS caused inhibition of brain cytochrome oxidase activity, neurotoxicity and frank histopathological lesions in the brain of adult rats. COS may differentially affect infants and children since their nervous systems are undergoing development. Thus, OEHHA recommends adding COS to the list of Toxic Air Contaminants that may disproportionately impact children under Health and Safety Code Section 39669.5.

9. BMDS Output File for Exponential Model

Female rat cytochrome oxidase in parietal cortex after 12 weeks of COS

```
=====
Exponential Model. (Version: 1.10; Date: 01/12/2015)
Input Data File:
C:/Users/jcollins/Downloads/BMDS260_20150223/BMDS260/Data/exp_Dax_Setting.(d)
Gnuplot Plotting File:
```

Tue Oct 27 12:10:39 2015

BMDS Model Run

The form of the response function by Model:

```
Model 2:    Y[dose] = a * exp{sign * b * dose}
Model 3:    Y[dose] = a * exp{sign * (b * dose)^d}
Model 4:    Y[dose] = a * [c-(c-1) * exp{-b * dose}]
Model 5:    Y[dose] = a * [c-(c-1) * exp{-(b * dose)^d}]
```

Note: Y[dose] is the median response for exposure = dose;
 sign = +1 for increasing trend in data;
 sign = -1 for decreasing trend.

Model 2 is nested within Models 3 and 4.
 Model 3 is nested within Model 5.
 Model 4 is nested within Model 5.

Dependent variable = Mean
 Independent variable = Dose
 Data are assumed to be distributed: normally
 Variance Model: $\exp(\ln\alpha + \rho * \ln(Y[dose]))$
 rho is set to 0.
 A constant variance model is fit.

Total number of dose groups = 4
 Total number of records with missing values = 0
 Maximum number of iterations = 500
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008

MLE solution provided: Exact

Initial Parameter Values

Variable	Model 2	Model 3	Model 4	Model 5
lnalpha	10.0725	10.0725	10.0725	10.0725
rho	0 *	0 *	0 *	0 *
a	760.723	760.723	1796.55	1796.55
b	0.00182158	0.00182158	0.00194593	0.00194593
c	0 *	0 *	0.000477025	0.000477025
d	1 *	1	1 *	1

* Indicates that this parameter has been specified

Parameter Estimates by Model

Variable	Model 2	Model 3	Model 4	Model 5
lnalpha	10.1786	10.1674	10.1786	10.0725
rho	0 *	0 *	0 *	0 *
a	1723.32	1714.69	1723.32	1711
b	0.00180415	0.00192534	0.00180415	0.0044978
c	--	--	0	0.499383
d	--	1.14436	--	2.99744

-- Indicates that this parameter does not appear in model

* Indicates that this parameter has been specified

Std. Err. Estimates by Model

Variable	Model 2	Model 3	Model 4	Model 5
lnalpha	NA	NA	5888.65	5295.69
rho	NA	NA	NA	NA
a	NA	NA	49.2942	48.6652
b	NA	NA	0.000138402	0.000319749
c	NA	NA	NA	0.0354948
d	NA	NA	NA	0.96265

NA - Indicates that this parameter was specified (by the user or because of the model form) or has hit a bound implied by some inequality constraint and thus has no standard error.

Table of Stats From Input Data

Dose	N	Obs Mean	Obs Std Dev
0	10	1711	125
200	10	1268	232
300	10	928	175
400	10	857	72

Estimated Values of Interest

Model	Dose	Est Mean	Est Std	Scaled Residual
2	0	1723	162.3	-0.2402
	200	1201	162.3	1.299
	300	1003	162.3	-1.462
	400	837.4	162.3	0.3812
3	0	1715	161.4	-0.07225
	200	1226	161.4	0.8239
	300	1006	161.4	-1.522
	400	816.8	161.4	0.7885
4	0	1723	162.3	-0.2402

	200	1201	162.3	1.299
	300	1003	162.3	-1.462
	400	837.4	162.3	0.3812
5	0	1711	153.9	1.058e-007
	200	1268	153.9	5.334e-007
	300	928	153.9	3.353e-007
	400	857	153.9	3.472e-007

Other models for which likelihoods are calculated:

Model A1: $Y_{ij} = \mu(i) + e(ij)$
 $\text{Var}\{e(ij)\} = \sigma^2$

Model A2: $Y_{ij} = \mu(i) + e(ij)$
 $\text{Var}\{e(ij)\} = \sigma(i)^2$

Model A3: $Y_{ij} = \mu(i) + e(ij)$
 $\text{Var}\{e(ij)\} = \exp(\alpha + \log(\mu(i))) * \rho$

Model R: $Y_{ij} = \mu + e(i)$
 $\text{Var}\{e(ij)\} = \sigma^2$

Likelihoods of Interest			
Model	Log(likelihood)	DF	AIC
A1	-221.4503	5	452.9006
A2	-215.0578	8	446.1156
A3	-221.4503	5	452.9006
R	-256.6924	2	517.3849
2	-223.573	3	453.1459
3	-223.348	4	454.6961
4	-223.573	3	453.1459
5	-221.4503	5	452.9006

Additive constant for all log-likelihoods = -36.76. This constant added to the above values gives the log-likelihood including the term that does not depend on the model parameters.

Explanation of Tests

- Test 1: Does response and/or variances differ among Dose levels? (A2 vs. R)
 Test 2: Are Variances Homogeneous? (A2 vs. A1)
 Test 3: Are variances adequately modeled? (A2 vs. A3)
 Test 4: Does Model 2 fit the data? (A3 vs. 2)
- Test 5a: Does Model 3 fit the data? (A3 vs 3)
 Test 5b: Is Model 3 better than Model 2? (3 vs. 2)
- Test 6a: Does Model 4 fit the data? (A3 vs 4)
 Test 6b: Is Model 4 better than Model 2? (4 vs. 2)
- Test 7a: Does Model 5 fit the data? (A3 vs 5)
 Test 7b: Is Model 5 better than Model 3? (5 vs. 3)
 Test 7c: Is Model 5 better than Model 4? (5 vs. 4)

Tests of Interest

Test	-2*log(Likelihood Ratio)	D. F.	p-value
Test 1	83.27	6	< 0.0001
Test 2	12.78	3	0.005126
Test 3	12.78	3	0.005126
Test 4	4.245	2	0.1197
Test 5a	3.795	1	0.05139
Test 5b	0.4499	1	0.5024
Test 6a	4.245	2	0.1197
Test 6b	2.842e-013	0	N/A
Test 7a	5.684e-013	0	N/A
Test 7b	3.795	1	0.05139
Test 7c	4.245	2	0.1197

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels, it seems appropriate to model the data.

The p-value for Test 2 is less than .1. Consider running a non-homogeneous variance model.

The p-value for Test 3 is less than .1. You may want to consider a different variance model.

The p-value for Test 4 is greater than .1. Model 2 seems to adequately describe the data.

The p-value for Test 5a is less than .1. Model 3 may not adequately describe the data; you may want to consider another model.

The p-value for Test 5b is greater than .05. Model 3 does not seem to fit the data better than Model 2.

The p-value for Test 6a is greater than .1. Model 4 seems to adequately describe the data.

Degrees of freedom for Test 6b are less than or equal to 0. The Chi-Square test for fit is not valid.

Degrees of freedom for Test 7a are less than or equal to 0. The Chi-Square test for fit is not valid.

The p-value for Test 7b is greater than .05. Model 5 does not seem to fit the data better than Model 3.

The p-value for Test 7c is greater than .05. Model 5 does not seem to fit the data better than Model 4.

Benchmark Dose Computations:

Specified Effect = 1.000000

Risk Type = Estimated standard deviations from control

Confidence Level = 0.950000

BMD and BMDL by Model

Model	BMD	BMDL
2	54.8181	43.5318
3	68.74	44.3399
4	54.8181	40.2654
5	129.535	78.2588

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