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Review Article

A Review of Developmental and Reproductive Toxicity of CS_2 and H_2S Generated by the Pesticide Sodium Tetrathiocarbonate

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BACKGROUND: Sodium tetrathiocarbonate (STTC) is an example of a pesticide that when prepared for use in aqueous solution releases two toxic products carbon disulfide (CS_2) (active ingredient) and hydrogen sulfide (H_2S) in ambient air in equimolar concentrations resulting in potential exposure to workers and bystanders. CS_2 and H_2S are pollutants that are generated from several pesticides as well as in industrial settings. **METHODS:** Registrant submitted reports and open literature studies for STTC, CS_2 and H_2S were reviewed. Previous reports suggest that CS_2 was a concern as a developmental and reproductive toxicant. H_2S was also examined since it is a neurotoxicant and potentially harmful to developing fetuses. **RESULTS:** STTC did not induce developmental or reproductive effects in animal studies. CS_2 was a developmental neurobehavioral toxin in rat pups (inhalation no observed effect level [NOEL] = 0.01 ppm). Reproductive effects occurred in male and female factory workers after CS_2 exposure (NOEL = 1 ppm). H_2S had developmental effects in rats at doses at or above those observed for nasal pathology (NOEL = 10 ppm) but was not a reproductive or developmental toxin in humans. **CONCLUSIONS:** The database for CS_2 indicates a strong potential for developmental neurotoxicity in animals at low doses but it is lacking in acceptable, well-performed studies. There is also a lack of studies performed with CS_2 and H_2S as a mixture. *Birth Defects Res (Part B)* 98:119–138, 2013. © 2013 Wiley Periodicals, Inc.

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INTRODUCTION

The pesticidal fumigant sodium tetrathiocarbonate (STTC; Na_2CS_4) was prioritized for risk assessment by the Department of Pesticide Regulation (DPR) in the California Environmental Protection Agency (Cal/EPA) due to previous high agricultural use of the product ENZONE. Dissociation of STTC by hydrolysis generates equimolar concentrations of carbon disulfide (CS_2 pesticidal active ingredient; a.i.) and hydrogen sulfide (H_2S; Young and Green, 1988; Pino et al., 2010).

$$Na_2CS_4 + 2H_2O \rightarrow CS_2 + H_2S + 2NaOH + S$$

Occupational use of STTC resulted in CS_2 and H_2S release in air. Exposure to STTC, CS_2, or H_2S may have occurred to agricultural workers during preparation and use of STTC (CARB, 2006, 2007; DPR, 2010; Reeve, 2012). Only one STTC study (chemigation mixers/loaders and applicators) was available to estimate STTC, CS_2, and H_2S worker exposure (detailed explanation in Reeve, 2012). No other studies were found for handler exposure to STTC or the degradation products, CS_2 and H_2S. Despite this, in California CS_2 is listed under California's Proposition 65 (California Proposition 65, 1986) as a chemical known to the state to cause developmental toxicity. In addition, CS_2 air exposure levels for infants and children were evaluated under California's Senate Bill 25 (Prioritization of Toxic Air Contaminants—Children's Environmental Health Protection Act, October, 2001; OEHHA, 2001). CS_2 is currently listed as a Toxic Air Contaminant in California (CCR, 2009). Studies performed on workers exposed to CS_2 in the viscose rayon industry worldwide have reported that H_2S is also a byproduct of manufacturing (Van Doorn et al., 1981; Vanhoorne et al., 1993).

In 2011, STTC and products containing it were voluntarily canceled nationally by the registrant (Federal Register, 2011). However, there are a multitude of potential sources generating CS_2 and H_2S as byproducts including the widely used pesticides (metam sodium, metam potassium, dazomet), coal blast furnaces, household cleaning products, oil refinieries, and artificial fabric manufacturing (ATSDR, 1996, 2006; OEHHA, 1999, 2001; Gelbke,

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2009). They remain a concern for potential reproductive and developmental effects in humans after inhalation exposure where data are often lacking.

The purpose of this article is to present animal and human data from developmental and reproductive studies after exposure to the STTC, CS_2, and H_2S, focusing on the latter two. The information, based on a risk assessment for STTC, is currently relevant to human exposure scenarios in industrial, agricultural, and other settings.

METHODS

STTC (ENZONE, sodium perthiocarbonate) was last registered in California by Arysta LifeScience Corporation. For registration, Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Guideline studies (required for Federal pesticide registration; USEPA, 1997a, 1997b) were submitted for the parent compound STTC, and the a.i. (CS_2) as required under the California Birth Defects Prevention Act (1984), and the California Assembly Bill 2161 (Bronzan and Jones, 1989) which entails a dietary risk determination for all pesticides with food crop uses. The risk determination in California focused on CS_2, due to its pesticidal activity and because it has USEPA tolerances for residues on food (USEPA, 2007). H_2S is not a pesticidal a.i. and no studies were submitted by the registrant. It was included in the risk determination because of its toxicity at low doses, and because it is released as a gas in equimolar concentrations to CS_2, once STTC is prepared. All H_2S studies were obtained from the open literature.

Among many parameters, the STTC-risk assessment involved hazard identification (hazard ID), and human worker and bystander exposure estimates (where possible) (Reeve, 2012; Silva, 2012). A detailed occupational exposure assessment for each compound is in Reeve (2012) and dietary exposure is in Silva (2012). The Hazard ID included a review of reproduction, developmental, and developmental neurobehavioral (developmental neurotoxicity [DNT]) studies in animals and epidemiological studies from all available data sources, including open literature and submitted studies performed under FIFRA: USEPA, 1997a, 1997b; Silva, 2012). The lowest dose that did not cause toxicity (no observed effect level: NOEL) was identified for each targeted exposure durations (acute, subchronic, and chronic), when possible. When an NOEL was not achieved and data are lacking, DPR may estimate a value by use of a default 10× uncertainty factor (UF) that is applied to the lowest observed effect level (LOEL; USEPA, 1996). When sufficient data are available and appropriate, a Benchmark Dose (BMD) analysis will be performed (Crump, 1995; USEPA, 2012).

RESULTS

STTC Pharmacokinetics

Since STTC (a solid) immediately hydrolyzes into CS_2 and H_2S, the pharmacokinetics of its products are discussed in their respective sections. All studies were performed with technical grade STTC (~99% pure). Exposure is primarily dermal and is more likely for workers than bystanders (people who are not directly involved with a pesticide application but who may be exposed to drifting and volatilized pesticide) at application sites. This is because of proximity to the pesticide during application of the solution and is based on how rapidly the reaction occurs.

STTC Hazard Identification for Developmental and Reproductive Toxicity

Gavage studies performed in rats and rabbits. *Salamon (*1986a*):* STTC was administered by gavage to mated female Sprague–Dawley rats (25/dose) at 0 (deaerated water), 150, 400, 450, or 500 mg/kg/day on gestation days (GDs) 6 through 15. There was mortality: 0, 0, 5, 6, and 10 dams at 0, 150, 400, 450, or 500 mg/kg/day, respectively (only observable effect to dams; NOEL = 150 mg/kg/day). The developmental NOEL was above 500 mg/kg/day (no treatment-related fetal effects observed).

*Salamon (*1986b*):* STTC was administered by gavage to mated New Zealand White rabbits (15/dose) at 0 (deaerated water), 75, 150, or 185 mg/kg/day on GD 7 through 19. Deaths during dosing occurred at 150 mg/kg/day (2), at 185 mg/kg/day (4), and 1 female at 150 mg/kg/day aborted on GD 22. Temporary clinical signs (convulsions, prostration, lethargy, increased/labored respiration) were observed in dams shortly after dosing for 6 of 14 at 150 mg/kg/day and 10 of 14 at 185 mg/kg/day. The maternal NOEL was 75 mg/kg/day. There were no treatment-related effects on fetuses (developmental NOEL >185 mg/kg/day).

STTC Developmental and Reproductive Toxicity Conclusions

STTC administered to rats and rabbits did not include fetal developmental effects even at doses highly toxic to dams. Human reproductive or developmental epidemiological studies have not been reported on STTC from any route of exposure.

Carbon Disulfide

In the United States, there are several tolerances for commodities after CS_2 soil fumigation and insect control in stored grain (USEPA, 2007). CS_2 emissions in air are reported annually by the California Air Resources Board. Currently, DNT is the key CS_2 toxicological endpoint of concern for infants and children. No FIFRA Guideline studies for this endpoint were submitted by the registrant as it was not requested by the USEPA before 2008 (USEPA, 2008).

CS_2 Pharmacokinetics

In vivo and in vitro studies suggest that absorbed CS_2 is metabolized by the cytochrome P-450 system (Bond and DeMatteis, 1969; Bond et al., 1969; Freundt et al., 1974, 1975; Beauchamp et al., 1983). In addition to undergoing biotransformation (Fig. 1), CS_2 reacts directly with the sulfhydryls of glutathione and cysteine to generate the highly polar metabolites, thiazolidine-2-thione-4-carboxylic acid and 2-oxothiazolidine-4-carboxylic acid. Increased amounts of thiazolidine-2-thione-4-carboxylic acid have been detected in the urine of workers and other individuals who were exposed to CS_2. Dithiocarbamates

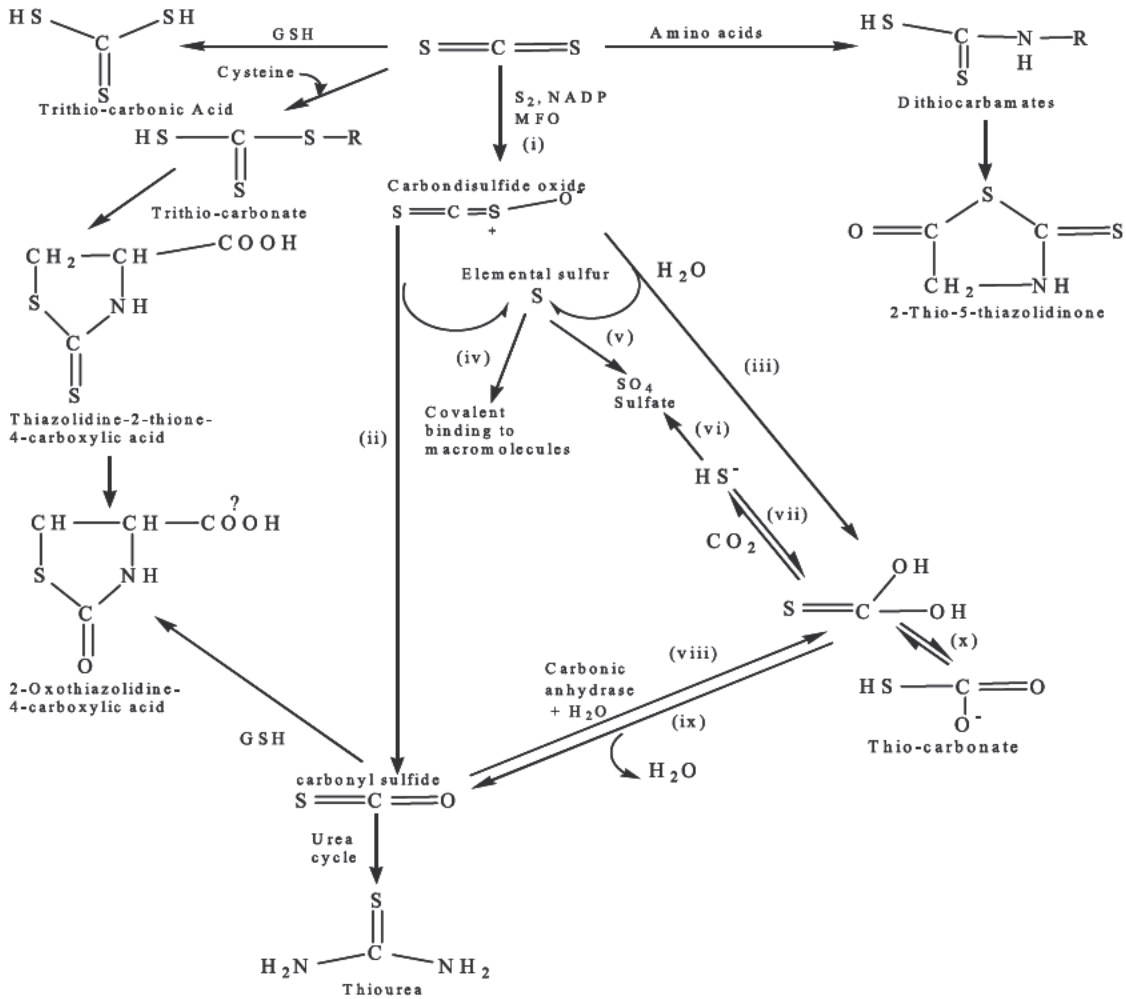

Fig. 1. Metabolism of CS_2 (Beauchamp et al., 1983).

generated by CS_2 reaction with amino acids have been shown to chelate metals (e.g., Zn^{++} and Cu^{++}) and inhibit enzymes (Beauchamp et al., 1983).

In humans and animals, CS_2 is absorbed mainly by inhalation (Teisinger and Soucek, 1949; Moorman et al., 1998). Although a qualitative animal study has shown that CS_2 vapor can be absorbed dermally (Cohen et al., 1958), no quantitative investigations could be found. Dermal exposure of humans to aqueous solutions of CS_2 resulted in absorption through the skin (Dutkiewicz and Baranowska, 1967).

Absorbed CS_2, because of its lipophilic nature, is distributed primarily to the brain and liver. In humans, the oxidation of sulfur to inorganic sulfate contributes little to CS_2 metabolism when compared to animals (Djerassi and Lumbroso, 1968); however, information on human CS_2 metabolic pathways or metabolites is limited. A common pathway between humans and animals results in dithiocarbamate formation which is known to contribute, in part, to CS_2 neurotoxicity (McKenna and DiStefano, 1977). Excretion of CS_2 metabolites is mainly via the kidneys. Unmetabolized compound can be excreted in the breath or urine (<1%).

CS_2 Hazard Identification for Developmental and Reproductive Toxicity

Most studies described below were performed via whole body inhalation. Less likely routes of exposure (oral and intraperitoneal [i.p.]) were included since reproduction and developmental effects of CS_2 were investigated. Oral and i.p. studies however were used for the purpose of risk assessment when dermal studies were either unacceptable or unavailable.

CS_2 Reproduction and DNT Studies (Whole Body Inhalation Table 2)

Tabacova et al. (1978, 1981, 1983), Tabacova and Balabaeva (1980), and Lehotzky et al. (1985) reported DNT effects of CS_2 in rat studies. None included positive controls for behavioral effects and some were without historical controls, which limited their use for risk determination. However, data were suitable for identifying potential adverse effects that could be further examined in future studies.

Tabacova et al. (1978)*:* Pregnant Wistar rats (32/dose) were exposed via inhalation to 0, 16, 32, and 64 ppm for

8 hr per day GD 0 to 21 (F0). Eighteen rats were killed at term and the rest were allowed to litter. Postnatal viability, body weight, appearance (postnatal day [PND] 21, 30, and 90), and behavior (Hall's open field test: PND 1), as well as oxygen consumption in liver, kidney, and brain tissue preparations and indices of lipid metabolism were measured. No animals were exposed to CS_2 after the F0 GD 21. Some of the F1 offspring were mated to produce the F2 generation and their reproductive capability was studied as was the in utero and postnatal development of the F2 progeny as described above. *F0 effects:* At 64 ppm, there was a statistically significant (three- to fivefold) increase in preimplantation loss and total embryonic lethality. *F1 effects:* At 32 and 64 ppm, there was a statistically significant (three- to fivefold) increase in preimplantation loss as well as a decrease in fetal body weight (not dose related: ↓2, 7, and 5% at 16, 32, and 64 ppm, respectively) and postnatal body weight (↓11, 19, and 18% at 16, 32, and 64 ppm, respectively). Body weight effects were reversed by PND 21. Pup viability was decreased at 64 ppm. There was a dose-related increase in external malformations (hydrocephalus, clubfoot, and tail deformations) at ≥32 ppm and generalized edema and hypognathia increased at 64 ppm. Visceral malformations (hydrocephalus) affected fetuses at 3.3, 26, and 31% at 16, 32, and 64 ppm, respectively (no per litter or historical control data). Other visceral malformations at ≥32 ppm were intracranial hematoma, renal hypoplasia, and hydronephrosis (no per litter or historical control data). Histological examination showed mild parenchymatous dystrophy and decreased glycogen content of fetal hepatic cells at 64 ppm (no data shown). Elevated oxygen consumption of kidney, liver, and brain tissues and an increase of free fatty acids (FFA) in the liver were found at 64 ppm. Behavioral changes were shown as decreased exploratory activity and increased "emotional activity" (not defined by authors), primarily in females and persistent up to PND 90 in all treated groups. There were no effects on the reproductive capabilities of the F1. *F2 effects:* Authors stated that F2 fetuses had congenital malformations of the same type and incidence as those observed in the previous generation; however, no data were shown for F2. Postnatally, there were behavioral changes in F2 similar to those observed in the F1 (no data shown for either generation). Maternal NOEL was 16 ppm based on effects at ≥32 ppm. Pup LOEL = 16 ppm based on behavioral changes observed at all doses. An NOEL of 1.6 ppm (NOEL = LOEL 16 ppm ÷ 10× UF) was estimated. Although there are numerous deficiencies in this study, results support the potential benefits of further examination the developmental and neurobehavioral effects of CS_2 after in utero inhalation exposure.

*Tabacova and Balabaeva (*1980*):* Pregnant Wistar rats were administered CS_2 in air (8 hr/day) through GD 0 to 21, at 0.01 and 3.2 ppm. Effects of in utero exposure were examined in F1 offspring (9–11 litters/dose) until PND 21. Sporadic increases in pup mortality (PND 10–21), decreases in eye opening (PND 18), and decreases in viability index (PND 21) occurred at 3.2 ppm. Delays in visual, auditory, and behavior (surface righting, horizontal motor activity, and air righting) development occurred at 3.2 ppm. Pup behavior was returned to normal by PND 21. The pup NOEL was 0.01 ppm based on effects in pups at 3.2 ppm. Maternal effects were not described. Since there was a 320-fold difference between the low and mid-doses and there were insufficient data to perform a BMD the actual no effect level may be somewhat higher than 0.01 ppm.

*Tabacova et al. (*1981*):* CS_2 was administered in air to pregnant "albino" (unspecified strain) rats (20/dose) at 0, 0.01, and 3.2 ppm (8 hr/day) during GD 0 to 21. At term, dams received C-sections (13–14/dose) and indices of prenatal development were measured in addition to lipid metabolism, P-450, aniline hydroxylase, and aminopyrine *N*-demethylase (APND) activities in maternal and fetal livers. The remainder of the treated dams (6–7/dose) littered normally and postnatal development of F1 was examined to PND 90. A battery of tests for assessment of survival, morphological, neurophysiological development, and behavior was performed as well as the metabolic indices described above. Hexobarbital sleeping time was evaluated on 6 to 8 pups/dose on PND 7, 14, 21, and 90 as an index for the mixed function oxidase (MFO/P-450) status. P-450 in liver was determined PND 30 and the indices of lipid and energy metabolism in liver homogenates at PND 90. After reaching maturity, the F1 animals were mated within each dose group (no CS_2 exposure) to examine potential reproductive effects after having received treatment in utero. No overt maternal toxicity was observed. Hexobarbital sleeping time was prolonged to PND 21 at 3.2 ppm (subsequently reversed) accompanying decreased P-450 at PND 30. FFA was elevated 35% in liver at PND 90 at 3.2 ppm indicating effects to MFO. Postnatal visual (PND 18) and auditory sensory function (PND 14; startle response), and incidence of eye opening on PND 18 at 3.2 ppm were inhibited. Air righting (PND 18), surface righting (PND 1), and gait defects (PND 7) were observed but were reversed later in life at 3.2 ppm. There was a transitional decrease in duration of inactivity on PND 9 and motor activity effects on PND 14. Behavior and MFO activity were the most sensitive indicators of toxicity in pups. The maternal NOEL was >3.2 ppm (no effects observed) and the pup NOEL was 0.01 ppm based on effects described above. Since, as in Tabacova and Balabaeva (1980) above, there was a 320-fold difference between the low and mid-doses and there were insufficient data to perform a BMD the actual no effect level may be somewhat higher than 0.01 ppm.

*Tabacova et al. (*1983*):* CS_2 was administered in air throughout the 21-day "albino" (unspecified strain) rat F0 and F1 gestations (30–32/dose/generation) at 0, 0.01, 3.2, 32, and 64 ppm (8 hr/day). Some F0 and F1 dams received C-sections to examine fetuses while others were allowed to litter. F1 pups reared to maturity were mated to produce the F2 generation. CS_2 exposure was resumed during F1 GD 0 to 21 to half the mated F1 and but not to the other half. At 0.01 and 3.2 (6–8/dose), indices of lipid and energy metabolism (FFA, triglycerides, phospholipids, cholesterol, lipid peroxides [tested at 0.01 and 3.2 only], oxygen consumption) were determined in F0 and F1 maternal liver, placenta, and F1 and F2 fetal liver homogenates. DNA, APND, and aniline hydroxylase were determined in maternal (F0 and F1) and fetal (F1 and F2) livers. F1 and F2 pups were subjected to a battery of tests that included physical development, functional maturation, and behavior. Additionally,

hexobarbital sleeping time was measured on representative animals from each litter on PND 7, 14, 21, and 90 to provide activities in vivo for the oxidative enzyme systems described above. *Maternal effects:* There was decreased F0 and F1 maternal body weight gain, decreased placental oxygen consumption and increased liver FFA at term at 64 ppm. F1 maternal lipid peroxides in the placenta were increased and APND in liver at term was decreased in F1 at 3.2 ppm, the highest dose tested. All F0 and F1 parental effects were reversed after treatment was discontinued. *F1 and in utero-exposed F2 pup effects:* Percent of F1 malformed pups increased at ≥32 ppm (dose related: hydrocephalus, generalized edema, club foot, tail deformations, hypognathia) and in F2 pups at ≥ 3.2 ppm (not dose related). F2 fetuses at term showed decreased phospholipids and triglycerides in liver at 64 ppm. Hexobarbital sleeping time was increased in F1 PND 7 and 14 at 3.2 ppm (highest dose tested; reversed PND 21) and was observed at these time points in the F2 pups at 3.2 ppm whose F1 mothers had received CS_2 treatment during gestation (reversed PND 21). F1 and F2 pups had increased effects on postnatal motor and coordination functions (crossing narrow paths and open-field tests) at 3.2 ppm PND 14 and 21. These tests examined more than one neurophysiological function simultaneously: locomotion, coordination, orientation, exploration. Increased locomotor defects (unbalanced gait, hindlimb weakness, spinning, tremor) were observed at 3.2 ppm but they were not dose related and may have been within normal range (no positive controls). *F2 pups without in utero exposure:* These effects were not observed in the F2 pups not treated with CS_2 in utero. The maternal and pup NOEL was 0.01 ppm based on increased malformations and neurobehavioral effects in pups treated in utero effects at ≥3.2 ppm. As in the above studies by Tabacova, there was a 320-fold difference between the low and mid-doses and there were insufficient data to perform a BMD, the actual no effect level may be somewhat higher than 0.01 ppm.

Lehotzky et al. (1985): CS_2 was administered to Lati:CFY pregnant female rats in air at 0 ($n = 4$), <3.2 ($n = 3$), 225 ($n = 4$), and 642 ($n = 2$) ppm from GD 7 through 15 (6 hr/day). Pups were given a behavioral test-battery with open field behavior and behavioral patterns on PND 23, 36, and 90 days. On PND 21 auditory startle reaction, righting response and placing reflex were measured. Motor coordination at PND 24 was measured. Dams had increased clinical signs (tremor, muscle weakness) and mortality (33%) at 642 ppm. Perinatal pup mortality (35% at 225 ppm; 50% at 642 ppm) was increased and "most" survivors were hyperirritable (number not provided) at ≥225 ppm. Pup mean body weights were decreased at ≥ 225 ppm on PND 21 (16% at 225 ppm; 37% at 642 ppm). Eye opening (not dose related) and the auditory startle were delayed (↓2-, 3.5-, and 5-fold at 3.2, 225, and 642 ppm, respectively). Motor coordination, open field behavior and behavior patterns were affected at ≥225 ppm. There was a significant difference in avoidance conditioning (learning ability) of male pups between control and 642 ppm ($p < 0.01$) from PND 7 to 14 and at 3.2 and 225 ppm ($p < 0.01$) from PND 10 to 14 (reversed for all doses at PND 15). The immature gait, motor incoordination, decreased open field activity, and altered behavioral patterns on PND 21 and 36 were reversed PND 90. The dam NOEL was 3.2 ppm and the pup LOEL was 3.2 ppm based on the above temporary effects at 642 ppm. Since a pup NOEL was not achieved in this study and there were insufficient data to perform a BMD analysis, an estimation of 0.32 ppm was made by applying a 10× UF to the LOEL (3.2 ppm ÷ 10× UF; USEPA, 1996).

Whole Body Developmental and Reproduction Inhalation Studies Performed in Rats (Table 2)

*Tabacova et al. (*1979*):* CS_2 was administered in air to pregnant "albino" (unspecified strain) rats (18/dose) at 0, 16, 32, and 64 ppm (6 hr/day), and at 320 and 640 ppm (4 hr/day) during GD 0 to 21. CS_2 crossed the placental barrier and the ratio of its concentration in the maternal blood and fetus ranged from 5:1 to 16:1, depending on dose. Maternal clinical signs of toxicity were observed at 64 ppm (decreased body weight gain, tremor, bloody nasal secretion, vaginal bleeding, marked morphological changes in liver and placenta), along with a significant increase in preimplantation lethality (100% at 640 ppm) and decrease in fetal weight at birth. Increased FFA and glutamate oxaloacetate transaminase and decreased oxygen consumption in liver, kidney, and placenta, increased lactate dehydrase, alkaline phosphatase, and liver RNA were observed in pregnant animals compared with nonpregnant females which the authors claim supports the fact that pregnancy enhances the toxicity of CS_2. Some doses in this study were highly toxic. The maternal and pup NOEL was 16 ppm based on the effects described above at ≥32 ppm.

*Holson (*1992*):* CS_2 was administered in air to female Sprague–Dawley Crl:CDBR rats (24 control and 15/dose) at 0, 125, 250, and 500 ppm (6 hr/day) for 14 days before mating; through mating and through to GD 19 (Holson, 1992). Males, neonates, and pups were not exposed to CS_2. Females were necropsied following weaning and pups were necropsied on PND 42. *Maternal effects:* At 500 ppm, there were decreased body weights, body weight gain, and food consumption during gestation. Food consumption was increased at 500 ppm during PND 3 to 6 indicating CS_2 did not interfere with maternal food consumption during lactation. There was an increase in gestation length at ≥250 ppm (control = 21.7 days; 250 ppm = 22.1 days, $p < 0.05$; 500 ppm = 22.3 days, $p < 0.01$). At 250 ppm, the gestation length was within historical control data for WIL Research Labs (21.9 days) and the slight increase was due to one dam with a gestation length of 23 days. Two females at 500 ppm had eyes and extremities pale in color and decreased activity. One had a prolonged delivery (e.g., parturition initiated with delivery of nine pups PND 1; three pups PND 2; one pup PND 3). Three dams had total litter loss (11 and 3 pups) PND 1 and on PND 3 (10 pups) at 500 ppm. *Pup effects:* Pups showed a statistically significant increase in number of dead fetuses/litter, a decrease in live birth index and a decrease in viability on PND 1 and viability on PND 4 at 500 ppm. The maternal NOEL was 125 ppm based on effects at ≥250 ppm. The pup NOEL was 250 ppm based on increased malformations with severe maternal toxicity at 500 ppm.

Beliles et al. (1980): CS_2 was administered in air to female CRS:COBS CD(SD)BR rats (30/dose/group) at 0 (air), 20, and 40 ppm (7 hr/day; 5 days/week) for 3 weeks before gestation (Groups 1–2, air; Groups 3–4, 20 ppm CS_2) through GD18, or during GD 0 to 18 (Groups 7–8, 40 ppm CS_2), or during GD 6 to 18 (Groups 5–6, 20 ppm CS_2; Groups 9–10, 40 ppm CS_2). Maternal body weights were decreased in Group 8 (GD 0–18, 40 ppm CS_2), but the report mentioned that this group had lower bodyweights at treatment initiation. Maternal lung and liver weights were increased in Group 6 (20 ppm) but the effect was likely incidental (not treatment related) since it was not observed at 40 ppm in any group. The maternal NOEL was 20 ppm based on effects observed at 40 ppm. The fetal NOEL was >40 ppm (no treatment-related effects).

*Zenick et al. (*1984*):* CS_2 was administered in air to adult male Long-Evans Hooded rats (14/dose) at 0 and 600 ppm (6 hr/day, 5 days/week) for 10 weeks to study endocrinologic and spermatogenic effects. One week pretest and at weeks 1, 4, 7, and 10, males were placed with ovariectomized, hormonally primed females and copulatory behaviors were scored. Results showed alterations in copulatory behavior (decreased mount latency at week 10, decreased ejaculation latency at 4, 7, and 10 weeks), and decreases in semen sperm counts (7 and 10 weeks). Males had decreased body weights at week 10, compared to controls. There were no effects on testosterone (T), follicle stimulating hormone (FSH), luteinizing hormone (LH), on sperm counts in the cauda epididymis or on histology of epididymis or testes. Only one dose was used and no NOEL obtained, limiting the usefulness of data for risk determination.

*Tepe and Zenick (*1984*):* CS_2 was administered in air to adult male Long-Evans Hooded rats at 0 (n = 29), 350, and 600 ppm (n = 15/dose) for 10 weeks (5 hr/day, 5 days/week). *Experiment #1:* After 10 weeks of exposure, the rats were terminated and LH, FSH, and T levels were assessed; testes were examined for histopathology and sperm count. At 600 ppm, body weights, epididymal sperm counts, and T levels were decreased without changes to reproductive organ weights or gonadotropin levels. *Experiment #2:* Males were exposed to 0 or 600 ppm CS_2 to determine if monitoring hormone levels and sperm status in the same male over time might increase the sensitivity of detecting a toxic reaction. After the 10-week treatment, males were mated with ovariectomized, hormone-primed females. Coital behavior and semen were monitored 1 week pretest and at 1, 4, 7, and 10 weeks of exposure. At 600 ppm CS_2, there were shorter times to mount, to ejaculate (with decreased sperm counts) and plasma testosterone was depressed. The NOEL was 350 ppm based on effects observed at 600 ppm.

*Zhao et al. (*1997*):* CS_2 was administered in air to pregnant female rats (unspecified strain) at 32 ppm (4 hr/day) on GD 7 to 8. At GD 9.5, the embryos were removed and explanted to serum taken from untreated rats employing the whole embryo culture. Growth of embryos (10 treated, 17 controls) was monitored for 44 hr. After treatment there were decreases in yolk sac diameters, crown-rump lengths, head length, number of somites, protein (μg/embryo), and morphologic score (limb bud and bronchial bar formation). The incidence of failure to complete axial rotation (50%) was significantly higher than for controls (5.9%). The results suggested that the effects of CS_2 on cultured embryos of treated pregnant dams were similar to those in vivo; embryonic toxicity occurred but not teratogenicity.

Whole Body Inhalation Studies Performed in Rabbits (Table 2)

*Denny (*1991*):* CS_2 was administered via inhalation to mated New Zealand White (Hra:SPF) rabbits (24/dose) at 0, 60, 100, 300, 600, or 1200 ppm (6 hr/day) during GD 6 to 18. Animals were terminated on GD 29 and fetuses were examined. Dams had increases in clinical signs (e.g., ataxia) and decreased food/water intake at ≥600 ppm. There were also increases in pre- and postimplantation loss, dead fetuses, and early and late resorptions at ≥600 ppm. Decreases in fetal weight and live litter size, along with increased litters with both visceral and skeletal malformations occurred at ≥600 ppm. The dam and fetal NOEL was 300 ppm based on effects observed at ≥600 ppm.

Studies Performed by Gavage or i.p. CS_2 Exposure Routes (Table 2)

Rat gavage (Jones-Price et al., 1984a*):* CS_2 was administered by gavage to mated (COBS) CD7(SD)BR rats (23–27/dose) at 0 (corn oil), 100, 200, 400, and 600 mg/kg/day from GD 6 to 15 (sacrifice was on GD 20). Maternal absolute/relative body weights were decreased at ≥200 mg/kg/day. At ≥400 mg/kg/day, relative liver/body weights and clinical signs were increased. Fetal body weights were decreased at ≥200 mg/kg/day. The maternal and fetal NOEL was 100 mg/kg/day based on effects observed at ≥200 mg/kg/day.

Rabbit gavage (Jones-Price et al., 1984b): Mated New Zealand White rabbits (23–28/dose) were gavaged with CS_2 at 0 (corn oil), 25, 75, and 150 mg/kg/day from GD 6 to 19. Maternal body weight and body weight gain decreased and mortality increased at 150 mg/kg/day. Clinical signs were observed acutely in dams postdosing at ≥75 mg/kg/day. There was an increase in relative and absolute maternal liver weights and a decrease in gravid uterine weights at ≥75 mg/kg/day. Fetuses showed a slight decrease in body weight at >75 ppm (statistically significant in Test for Linear Trends; $p < 0.01$). At 150 mg/kg/day, there was also an increase in percent litters with nonlive fetuses, in live fetuses/litter, in percent litters with affected fetuses, in percent fetuses malformed/litter, in percent males malformed/litter, and in percent litters with resorptions. The number of litters totally resorbed was increased in a dose-related manner. Fetuses showed an increase in percent nonlive fetuses/litter, percent resorptions/litter, and in percent fetuses affected/litter at all doses (LOEL for each effect = 25 mg/kg/day). BMD analyses (BMD Software version 2.3.1; Crump, 1995; USEPA, 2012) were performed by M. Silva for the following endpoints: %resorptions/litter, %affected/litter, and %nonlive/litter. The best fit was provided by the %nonlive/litter data (Table 1) yielding a BMD at the Lower Level ($BMDL_{05}$, confidence level 0.95)

Table 1
Fetal Effects of CS_2 after Gavage Exposure to New Zealand White Rabbit Dams on Gestational Days 6–19 (Jones-Price et al., 1984b)

Parameters	Carbon disulfide (mg/kg/day)				Historical controls[a]
	0	25	75	150	
Fetal effects					
No. of live fetuses examined[b]	196	143	148	78	528
No. of litters examined[c]	26	19	21	15	73, 76, 77
Ave fetal bwt (g)/litter	45.5 ± 7[@@#]	45.3 ± 8	41.6 ± 8	39.5 ± 7	46.6 ($n = 76$)
%nonlive fetuses/litter[d]	***13.7 ± 21***[@@@###]	***33.1 ± 34***[&]	***42.8 ± 35***[&&#]	***62.2 ± 29***[&&*]	***13.6 (n = 77)***
%affected/litter[c,d,e]	***18.4 ± 23***[@@@###]	***38.2 ± 34***[&]	***46.8 ± 33***[&&*]	***67 ± 3***[&&*]	***19 (n = 77)***
%fetuses malformed[b]	6.12	6.29	6.76	12.82	6.4 ($n = 77$)
%litters with malformations	26.9	31.6	28.57	60.0	34.2 ($n = 77$)
%resorptions per litter[e]	***12 ± 2***[@@@###]	***37.5 ± 35***[&]	***41.6 ± 35***[&&#]	***61.2 ± 29***[&&*]	***12.3 (n = 77)***
No. of litters totally resorbed	1	4	7	10	–

[a]Mean; no range presented.
[b]Only live fetuses were examined for malformations.
[c]Includes only litters with live fetuses.
[d]Nonlive = dead plus resorbed.
[e]Affected = nonlive plus malformed.
[@@,@@@]Different from controls at $p < 0.05$, 0.01, and 0.001, respectively (Test for Linear Trends).
[#,###]$p < 0.05$ and 0.001 Main Effect for Dose (ANOVA).
*$p < 0.001$ (Dunnett's Multiple Comparison Test).
[&,&&]$p < 0.05$ and 0.01, respectively (Williams' Test).
"–" – No data available.
"±" is the standard deviation.
Bold italics: Effects observed at LOEL for which Benchmark Dose analyses (BMD Software version 2.3.1; USEPA, 2012; Crump, 1995) were performed by M. Silva. Continuous, nonhomogeneous variance, restricted, CI 0.95 = $BMDL_{05}$ (SD × 0.5) for each data set was used. Hill model for %nonlive/litter was the best fit and the lowest $BMDL_{05}$ = 2.45 mg/kg/day; lack dose response $p < 0.0001$; constant variance $p < 0.07$; good variance model: $p > 0.3$; goodness of fit: 0.42; AIC = 632; scaled residual = 0.172. BMD analyses available on request (msilva@cdpr.ca.gov).

of 2.45 mg/kg/day.[i] The $BMDL_{05}$ for %affected/litter was 3.25 mg/kg/day by the same model but for %resorptions/litter data none of the models tested was a good fit. The fetal $BMDL_{05}$ for this study (%nonlive/litter = 2.45 mg/kg/day) is close to the estimated NOEL 2.5 mg/kg/day obtained by applying a default 10× UF to the LOEL (10× UF; LOEL ÷ 10; USEPA, 1996). This study was the best available for determination of an oral endpoint and could be used for both oral and dermal risk characterization. The maternal NOEL was 25 mg/kg/day based on body weight effects and mortality at ≥75 mg/kg/day.

Rat i.p. (Patel et al., 1999): Adult male Charles–Foster rats (10/dose) were treated i.p. with CS_2 at 0 (vehicle control = cotton seed oil), 25, 50, 100, and 200 mg/kg/day for 30 days. Body weight decreases, thickening and rupturing of seminiferous basement membrane, degeneration/disorganization of spermatogonial cells, fewer/absent sperm in the lumen were observed at ≥100 mg/kg/day. There were no histomorphological effects in epididymal tissue however there was a significant decrease in serum testosterone (ng/dl) at all doses (504.50 ± 162 SD, 224.30 ± 54*, 207.77 ± 73*, 207.77 ± 73*, 84 ± 93*, 31.60 ± 9.6* at 0, 25, 50, 100, and 200 ppm, respectively; *$p < 0.001$; ±SD) indicating a possible endocrine disrupting effect. A BMD analysis (BMD Software version 2.3.1; Crump, 1995; USEPA, 2012) was performed by M. Silva for decreased testosterone, since sufficient data were available. However, none of the models fit (see footnote). An estimated NOEL of 2.5 mg/kg/day was obtained by applying a default 10× UF to the LOEL (10× UF; LOEL ÷ 10; USEPA, 1996). Long-term exposure to high doses of CS_2 via an i.p. route is an unlikely scenario however effects in males are similar to those observed after inhalation exposure to adult male rats (Zenick et al., 1984; Tepe and Zenick, 1989; Table 2).

Rat i.p. (Kumar et al., 1999): Adult male albino Charles–Foster rats (5/dose) were treated i.p. with CS_2 at 0 (cotton seed oil), 25, 50, 100, and 200 mg/kg/day for 60 days. Effects of CS_2 on epididymis, adrenal weight, sperm count, and sperm head shape abnormality were studied. Sperm count was decreased at ≥100 mg/kg/day. Sperm head shape and abnormality (%) was increased at 200 mg/kg/day ($p < 0.001$) indicating potential for germ cell genotoxicity of CS_2 at high doses. The NOEL was 50 mg/kg/day based on sperm effects at ≥100 mg/kg/day.

CS_2 Developmental and Reproductive Toxicity Conclusions

Animal studies (Table 2). *Inhalation studies including neurobehavioral toxicity:* Developmental neurobehavioral toxicity, after in utero CS_2 exposure via inhalation

[i] Models tested (polynomial, linear, power, exponential, and Hill) were for continuous, nonhomogeneous variance, restricted at the 95th percentile (CL 0.95 = $BMDL_{05}$) and SD × 0.5 for each data set (USEPA, 2012). Hill model for %nonlive/litter was the best fit and the lowest $BMDL_{05}$ = 2.45 mg/kg/day; lack dose response $p < 0.0001$; constant variance $p < 0.07$; good variance model: $p > 0.3$; goodness of fit: 0.42; AIC = 632; scaled residual = 0.172.

Table 2
Developmental Toxicity of CS_2 in Animal Studies

Exposure	Major effects at and above the LOEL	NOEL	References[a]
Rat: whole body reproduction and developmental inhalation studies with neurobehavioral evaluations (ppm)			
Wistar: 8 hr/d, F0 GD 0–21	Dam: ↑preimplantation loss; ↑total embryonic lethality Fetal: ↓fetal body weights; ↓pup viability; ↓pup weights to PND 21; ↑external and visceral malformations; edema and hypognathia; liver pathology; ↑kidney, liver, and brain oxygen consumption; ↑behavior changes.	Dam: 16 Fetal/pup (estimated)[a]: 1.6	1
Albino: 8 hr/d, GD 0–21	Fetal: ↑pup mortality (PND 10–21), ↓eye opening (PND 18 [%]), and viability index (PND 21 [%]); delays visual, auditory, and behavior (motor activity and surface and air righting)	Fetal: 0.01	2
Albino: 8 hr/d, GD 0–21	F0 dam: no overt maternal toxicity F1 fetal/pup/adult: ↑hexobarbital sleeping time; ↓P-450; ↑FFA; ↓visual, startle response, eye opening, air righting, surface righting, and gait defects (behavior effects reversed)	Dam/fetal/pup: 0.01	3
Albino: 8 hr/d F0 GD 0–21 F1 GD 0–21	F0 and F1 females: ↓bodyweight gain, ↓placental O_2 consumption, ↑placental lipid peroxides, ↓liver APND, and ↑FFA (all effects reversed posttreatment) Pup effects: ↑F1 and F2 pups % malformed: hydrocephalus, generalized edema, club foot, tail deformations, hypognathia); ↓F2 liver phospholipids and triglycerides; ↑ F1 and F2 pups hexobarbital sleeping time (reversed PND 21); ↑F1 and F2 postnatal motor and coordination effects; ↑locomotor defects (unbalanced gait, hindlimb weakness, spinning, tremor)	Dam/fetal/pup: 0.01	4
Lati:CFY; 6 hr/d; GD 7–15; observed to PND 90	Dam: ↑tremor, muscle weakness, and mortality Pup: ↑pup mortality with hyperirritability in survivors; eye opening and auditory startle delayed; impaired motor and open-field activity; altered behavioral patterns PND 21 and 36 (reversed PND 90); ↑ latency of conditioned avoidance response (reversed PND 15)	Dam: 3.2 pup(estimated)[a]: 0.32	5
Rat: Whole Body Developmental and Reproduction Inhalation Studies (ppm)			
Albino: 4–6 hr/d, GD 0–21	Dam: ↓bwt gain; ↑tremor, bloody nasal secretion, vaginal bleeding, liver, and placenta pathology; ↑FFA, GOT, lactate dehydrase, alkaline phosphatase, and liver RNA; ↑preimplantation lethality; ↓O_2 consumption in liver, kidney, and placenta Fetal: ↓fetal birth weight	Dam/fetus:16	6
Crl:CD® (SD)BR: 6 hr/d; 14 d premate-GD 19	Dam: ↑dystocia; ↑clinical signs; ↓body weight, ↓weight gain, ↓food consumption Fetal: ↑mortality and malformations; ↓live birth index and viability LD1 and 4	Dam: 125 Fetal: 250	7
CRS:COBSCD (SD)BR; 7 hr/d; 5 d/wk; 3 wk premate through gestation	Dams: ↑lung and liver weights; ↓body weight Pup: No effects	Dam: 20 Fetus: >40	8
Long-Evans Hooded: 6 hr/d, 5 d/wk, 10 wk	Adult males: ↓body weight, altered copulatory behavior (↓mount latency, ↓ejaculation latency, ↓sperm counts), ↓serum testosterone	Adult male: 350	9, 10
GD 7–8; cultured embryos	Embryos: ↓yolk sac diameters, crown-rump lengths, head length, number of somites, protein, limb bud, and bronchial bar formation; ↑incidence of failure to complete axial rotation (50%)	Embryo: >32	11
Rabbit: Whole Body Developmental Inhalation Study (ppm)			
NZW (Hra:SPF) 6 hr/d; GD 6–18	Dams: ↑pre- and postimplantation loss and fetal loss; ↓food/water intake; ↑clinical signs (e.g., ataxia); affected hematology parameters Fetal: ↓body weight; ↓live litter size; ↑litters with both visceral and skeletal malformations	Dam/fetal: 300	12
Rat: Developmental Gavage Study (mg/kg/day)			
COBS CD(SD)BR GD 6–15	Dams: ↓body weight and body weight gain; ↑relative liver weight; ↑clinical signs Fetal: ↓body weight	Dam/fetal: 100	13

Continued

Table 2
(Continued)

Exposure	Major effects at and above the LOEL	NOEL	References[a]
Rabbit: Developmental Gavage Study (mg/kg/day)			
New Zealand White GD 6–19	Dams: ↓bwt/bwt gain; ↑mortality; ↑relative/absolute liver wt; ↓gravid uterine wt; ↑clinical signs	Dam: 25	14
	Fetal: ↓bwt; ↑%nonlive fetuses/litter, %litters with nonlive fetuses; %resorptions/litter; %litters with resorptions; %fetuses affected/litter; %litters with affected fetuses, %fetuses malformed/litter, %males malformed/litter	Fetal ($BMDL_{05}$)[b]: 1.53	
Rat: Intraperitoneal Studies (mg/kg/day)			
Charles–Foster 30 d	Adult male: ↓body weight, thickening, and rupturing of seminiferous tubule basement membrane, degeneration/disorganization of spermatogonial cells, fewer/absent sperm in the lumen; ↓serum testosterone (ng/dl); ↑sperm head shape and abnormality (%); ↓sperm count	Adult male: (estimated)[a] 2.5	15
Charles–Foster 30 d	Adult male: ↓sperm count; ↑sperm head shape and abnormality (%)	Adult male: 50	16

APND, aminopyrine *N*-demethylase; $BMDL_{05}$, Benchmark Dose Lower value (confidence level [CL] = 0.95); bwt, body weight; d, day; F0, 1st parental generation; F1, offspring of F0 and 2nd parental generation; F2, offspring of F1 parents; FFA, free fatty acids; GD, gestation day; GOT, glutamate oxaloacetate transaminase; hr/d, hours per day; NOEL, no observed effect level; NZW, New Zealand White; %, percent; wk = week; wt, weight.
1. Tabacova et al. (1978); 2. Tabacova and Balabaeva (1980); 3. Tabacova et al. (1981); 4. Tabacova et al. (1983); 5. Lehotzky et al. (1985); 6. Tabacova et al. (1979); 7. Holson (1992); 8. Beliles et al. (1980); 9. Zenick et al. (1984); 10. Tepe and Zenick (1989); 11. Zhao et al. (1997); 12. Denny (1991); 13. Jones-Price et al. (1984a); 14. Jones-Price et al. (1984b); 15. Patel et al. (1999); 16. Kumar et al. (1999).
[a]A NOEL was estimated from the lowest dose (LOEL) by use of a 10× uncertainty factor (USEPA, 1996): Estimated NOEL = (LOEL ÷ 10).
[b,c] Hill and Polynomial models, respectively: continuous, nonhomogeneous variance, restricted (CL = 0.95 = $BMDL_{05}$; USEPA, 2012) for continuous data BMD calculations ($P_0 = 0.05$).

to rat dams, appears to be the most sensitive endpoint for developmental or reproductive toxicity. Studies where neurobehavioral effects were tested in pups had the lowest NOELs. Tabacova et al. (1978, 1981, 1983) and Tabacova and Balabaeva (1980) showed developmental delays and neurobehavioral effects at <3.2 ppm after exposure in utero over one or two generations. Two studies (Tabacova et al., 1978, 1981) showed toxicity in fetuses/pups (estimated NOEL = 1.6 ppm and NOEL = 0.01, respectively) below the maternal NOEL, indicating a possible adverse effect via in utero exposure. However, this was not observed in Tabacova et al. (1983), where both the maternal and pup NOELs were 0.01 ppm. Most studies by Tabacova had a 320-fold difference between the low and mid-doses (0.01 and 3.2 ppm); however, in Tabacova et al. (1978), the low dose was 16 ppm providing an estimated pup NOEL of 1.6 ppm. Based on the results of studies by Tabacova, the actual NOEL for DNT effects may be between 0.01 and 1.6 ppm.

Lehotzky et al. (1985) also tested DNT effects in pups after in utero CS_2 exposure when dams received inhalation treatment. Male pups showed transitionally decreased avoidance conditioning at all doses in the absence of low-dose effects in dams. The maternal NOEL was 3.2 ppm but the estimated fetal/pup NOELs for was 0.32 ppm. This value is five times less than the one estimated from Tabacova et al. (1978) but it is still 32 times greater than the NOELs achieved in others by Tabacova (Table 2).

There were numerous deficiencies and inconsistencies in these studies (e.g., malformations at 3.2–16 ppm in Tabacova et al., 1978, 1983 but none observed at <64 ppm with the same exposure regimen). Studies testing neurobehavioral effects were from the open literature and were performed without the positive controls currently required by FIFRA Guidelines (Tabacova et al., 1978; Tabacova and Balabaeva, 1980; 1981, 1983; Lehotzky et al., 1985; USEPA, 1998). Studies providing the lowest NOELs had numerous deficiencies as would be expected since they are older or not designed according to FIFRA Guidelines. In general, there was a lack of maternal observations, no positive controls, statistical significance of reported behavioral effects was not stated, chemical purity was not stated, exposure methods not explained and effects that were not dose related may have been within normal range (no historical data); there were no positive. But as a whole, results indicated that CS_2 exposure in utero, or perinatally for one or more generations can affect motor coordination, auditory and visual development, and other behaviors in rat pups at low doses. The lowest NOELs were from studies by Tabacova and Balabaeva (1980), Tabacova et al. (1981, 1983) (0.01 ppm) but again, these studies had numerous deficiencies. However, the low NOELs from four studies where DNT effects were tested (Tabacova and Balabaeva, 1980; Tabacova et al., 1981, 1983; Lehotzky et al., 1985) indicated there is potential toxicity at very low exposure.

Other developmental or reproductive effects were observed at higher doses in rat and rabbit inhalation studies where neurobehavioral assessments were not performed (Table 2; Tabacova et al., 1978, 1981,1983; Tepe and Zenick, 1984; Zenick et al., 1984; Denny, 1991; Holson, 1992). In

general, dams had indications of toxicity (increases in: clinical signs, preimplantation loss, gestation length, total litter loss, and number of stillborn per litter; decreases in: body weight/weight gain during gestation and lactation, food intake during gestation). The NOEL range in rat dams was 16 to 125 ppm. Differences could be due to parameters tested as well as strain of rat used for testing. Rabbit dam and fetal NOEL was 300 ppm and effects were similar to those observed in rat (Table 2). Results suggest that fetal/pup effects (↓viability, body weight; ↑malformations) may have been due to maternal toxicity, since their NOELs were either equal or greater than those of the dams. In support, Zhao et al. (1997) showed that cultured embryos from dams treated with CS_2 GD 7 to 8 via inhalation behaved in vitro similarly to those in vivo. Their results indicated that embryonic toxicity but not teratogenicity had occurred.

CS_2 was highly toxic to the male rat reproductive system (changes in copulatory behavior, ↓semen sperm counts, ↓body weight, ↓epididymal sperm counts, ↓T levels) after inhalation exposure (Tepe and Zenick, 1984; Zenick et al., 1984). However, the NOEL of 350 ppm was higher than was observed in dams or fetuses/pups showing that male rats are less sensitive to CS_2 via inhalation for the limited parameters tested.

Gavage studies: CS_2 gavage treatment to pregnant rabbits showed effects on fetuses at all doses ($BMDL_{05}$ = 2.45 mg/kg/day) at a maternal NOEL of 25 mg/kg/day (↓body weight; ↑mortality at ≥75 mg/kg/day; Table 1; Jones-Price et al., 1984b). Similar effects did not occur after gavage treatment in pregnant (COBS) CD7 (SD) BR rats where the dam and fetal NOEL was 100 mg/kg/day (Jones-Price et al., 1984a). Maternal toxicity was evident (decreased body weight and increased liver weights and clinical signs) and fetuses experienced decreased body weights at >200 mg/kg/day.

The rabbit study (Jones-Price et al., 1984b) was the best available for determination of an oral NOEL. Since CS_2 has tolerances on crops, a dietary risk determination, as well as an aggregate exposure (occupational + dietary/dermal) was performed (Silva, 2012). An oral NOEL can be used for occupational exposure assessment when a dermal study is not available as was the case for CS_2.

i.p. studies: Studies performed by i.p. (Kumar et al., 1999; Patel et al., 1999) showed similar effects in the male reproductive tract to those seen after CS_2 inhalation exposure (Zenick et al., 1984; Tepe and Zenick, 1984). This not a likely route of exposure but it is noteworthy that effects are not necessarily route-specific. The i.p. estimated NOEL for decreased testosterone (2.5 mg/kg/day; Patel et al., 1999) was similar to that obtained for rabbit fetuses (2.45 mg/kg/day; Jones-Price et al., 1984b) after gavage treatment in dams. The two rat studies could support a no effect level of approximately 2.5 mg/kg/day for oral or dermal exposure.

Evidence for CS_2 Human Reproductive Toxicity after Inhalation Exposure (Table 3)

Risk of CS_2 exposure to workers in viscose factories worldwide is well documented by Gelbke et al. (2009). Epidemiological studies documenting effects in males and female workers under factory conditions were performed to evaluate the current CS_2 occupational exposure limit (OEL: vary from 1 to 10 ppm). Current international OELs for CS_2 (see Table 6) are lower than those held previous to 1970. The effects of the lowered OELs have also been examined (e.g., Cirla et al., 1978; Meyer, 1981; Selevan et al., 1983). In these occupational environments, actual CS_2 exposure was often unknown and had to be estimated based on company records, and/or job description. Despite the uncertain exposure data, many epidemiological studies have been reported evaluations of reproductive and endocrine parameters in both men and women. Although authors did not specifically mention co-exposure with H_2S, it is highly likely it occurred.

*Cirla et al. (*1978*):* Effects of CS_2 were examined in workers at a viscose rayon factory in Italy. Estimated concentrations ranged from "very light" (n = 14; <19 ppm for <4 years), "light" (n = 73; ≤19 ppm), "heavy" (n = 82; 38.4–76.8 ppm; higher peak values possible in the past; last 3 years <19 ppm), "heavy/light" (n = 47; 57.6–76.8 ppm; last 12 years <19 ppm), control subjects (unexposed workers in "clean" depts.). Radioimmunologic assay of FSH and LH in blood showed a statistically significant decrease in those with "heavy" exposure in the past (no effect on T). FSH and LH + FSH were decreased in groups with "very light," "light," and "heavy" exposure. Declared sexual intercourse/month in married workers was decreased in all exposed groups compared to controls. Sexual intercourse was also significantly decreased in the specific age range 31 to 45 for "very light" and "light" CS_2 exposure. Data suggest CS_2 has a primary effect on hypophyseal activity such that even after discontinuation of heavy exposure, sexual activity may remain decreased despite hormone levels returning to normal (LOEL = 19 ppm).

*Cirla and Graziano (*1981*):* CS_2-exposed (n = 50; 3.2–8 ppm for 3–12 years) and nonexposed male subjects (n = 50) in a viscose rayon factory in Italy were examined for endocrinological functions (glycemia, thyroxine, FSH, LH, T). There was no evidence of adverse effects from exposure at ~8 ppm for up to 12 years. The NOEL was 8 ppm.

*Meyer (*1981*):* Semen quality in workers exposed to CS_2 for 1 to 15 years (>10 ppm; n = 18; 2–10 ppm; n = 27; <2 ppm; n = 22; no measurements available; n = 19) and in control subjects (n = 89) from a Tennessee rayon filament plant was analyzed by the National Institute of Occupational Safety and Health (NIOSH). No statistically significant difference between the control and CS_2-exposed groups was observed. According to the authors, the lack of effects on semen parameters may be due to regulations calling for lower CS_2 exposure levels in factory conditions than were previously allowed. Additionally, CS_2 exposures (i.e., length of employment) may have been too brief to induce measurable effects.

*Selevan et al. (*1983*):* Workers (married male hourly employees) in a Tennessee rayon filament company exposed to CS_2 as well as unexposed workers (0.2 ppm background) were selected for this study performed by NIOSH. The wives of the CS_2-exposed (n = 236) and unexposed workers (n = 304) were examined for pregnancy outcome (patterns of fetal loss, births, time between live births), health, and demographics. The men within each

Table 3
CS_2 Human Reproductive Effects

Design	Major effects	NOEL/LOEL	References[a]
M workers: 0, <19–77 ppm; 3–21 years	↓FSH, ↓LH, ↓LH + FSH (T unaffected) and ↓declared sexual intercourse/month ("light" and "heavy" exposure)	LOEL = 19 ppm	1
M workers: 0, 3.2–8 ppm for 3–12 years	No effects on any parameters examined, including FSH, LH, and T	NOEL = 8 ppm	2
M workers: <2 to >10 ppm; ~1–15 years	No association between semen quality and exposure to CS_2 under factory exposure conditions	NOEL > 10 ppm	3
M workers: 0, <5 ppm, >5 ppm for ≥ 1 year	OR for length of exposure to CS_2 (as estimated by time since first employment) and fetal loss were small but statistically significant (range: 1.14–1.18).	NOEL < 5 ppm	4
F/M workers and wives of exposed M: 0.54–4.47 ppm for ~1–15 years	↑Overdue delivery in wives of the exposed male workers; ↑menstrual disturbance in exposed female workers (average 7-year exposure ≥ 2.08 ppm) RR = 1.92	Female worker NOEL = 1.0 ppm	5
F workers: 0, 0.54–4.74 ppm for ~1–15 years	↑Incidence in dose-related menstrual disturbance (irregular cycle or unusual bleeding)	NOEL = 2.1 ppm	6
M workers: 0, ≤10 ppm 19.3 years; or formerly exposed	Six-year follow-up of cohort: no exposure-related effects on endocrine function: hypophysis, or gonadal function	NOEL < 10 ppm	7
M workers: ~3.2 ppm	↓Number and length of sexual encounters; ↓sperm quantity, ↓acrosomal membrane integrity rate, vitality, and density; ↑liquefaction time and sperm abnormality	LOEL = 3.2 ppm	8
M workers: 1–40 ppm; 1–36 years	↑LH, ↑FSH; ↑free T index (suggested pituitary–gonadal axis effects); ↓sex hormone binding globulin	LOEL = 10 ppm	9
In vitro human sperm/hamster egg fusion; 1, 5, or 10 μmol per liter	↑Aberrant rate (%), ↑average breaks; ↑incidence in numerical aberration; ↑structural chromosome aberration; ↑total abnormalities	NOEL = 5 μmol per liter	10

bwt, body weight; d, day; F, female; FSH, follicle stimulating hormone; LH, luteinizing hormone; LOEL, lowest observed effect level; M, male; NOEL, no observed effect level; OR, odds ratio; %, percent; RR, relative risk; T, serum testosterone.
[a]1. Cirla et al. (1978); 2. Cirla and Graziano (1981); 3. Meyer (1981); 4. Selevan et al. (1983); 5. Liang et al. (1988); 6. Zhou et al. (1988); 7. Takebayashi et al. (2003); 8. Ma et al. (2010); 9. Wagar et al. (1981); 10. Le and Fu (1996).

group did not differ in number of years employed but "exposed" were younger than "nonexposed." CS_2 level associated with each pregnancy was estimated using work histories of the father for the 4 months before the estimated date of conception allowing time for expression of effects in sperm. Interviewed were 90.7% of wives for the exposed population and 85.5% of wives of control subjects. Pregnancies analyzed (546 Caucasians) occurred during 1950 to 1978. Exposure to males whose wives experienced pregnancies was CS_2 at "none" (~0.2 ppm), <5 ppm (low) or >5 ppm (high). Assignment of exposure status was the major problem in data analysis and interpretation of results. Odds ratios (ORs) for the effect of exposure using three logistic regression analysis models (Model #1 exposure = "any" and "none"; Model #2 = actual level; Model #3 = "none, low and high") on fetal loss was less than one (ORs range: 0.06–0.70). Selevan et al. were not able to determine whether decreased fetal loss was due to exposure level or recall bias. The OR for length of exposure to CS_2 (as estimated by time since first employment using the above exposure models) and fetal loss were small but statistically significant (range: 1.14–1.18). The frequency of births in wives' whose husbands were exposed to CS_2 at "none," < 5 ppm or >5 ppm at the time of conception were compared to those expected based on person-years accumulated in each category. Although there were fewer births per person-years for the exposed populations, these deficits were not significantly different than those observed in the unexposed population. The spacing of live births (or birth interval) was examined and differences in exposed and unexposed intervals were minor and nonsignificant. The authors concluded there is not an effect of CS_2 at the levels of exposure examined (NOEL > 5 ppm).

Wagar et al. (1981): Serum levels of LH, FSH, T, and sex hormone binding globulin (SHBG) were determined for male viscose rayon factory workers (n = 69; mean age 40 years) exposed to CS_2 for 1 to 36 years (mean = 12.5 years) at 4 to 20 ppm (1960s), 1 to 13 ppm (1970s), and 1 to 20 ppm (1980) in Finland. There were 22 male control subjects (mean age = 39 years). FSH was significantly higher but SHBG was significantly lower overall in CS_2-exposed men. For the subgroup of men age < 39 years (1–9 years CS_2), LH, FSH, and free T index were increased and SHBG

was decreased. This age group FSH was increased after ≥10 years of CS_2 exposure. Men age ≥40 years old had increased FSH and LH only after ≥10 years. Results indicate that exposure levels of CS_2 < TLV (where TLV is threshold limit value) (10 ppm in 1983) may affect the hormonal balance in the pituitary–gonadal axis. The major deficiency was the small sample size.

Liang et al. (1988): Menstrual problems, pregnancy/birth outcome, and teratology of occupational exposure to CS_2 (≥1–15 years) in males and females were examined. Female viscose rayon factory workers (337) in China exposed to CS_2 at 1.0, 2.08, and 4.74 ppm (range: 0.54–4.74 ppm) from 1970 to 1985 (<3.2 ppm in the factory since 1980) and 397 nonexposed subjects were examined by questionaire about menstrual history and related questions. Married female exposed (389 pregnancies) and nonexposed workers (293 pregnancies) as well as wives of exposed (293 pregnancies) and nonexposed male workers (203 pregnancies) were interviewed for pregnancy progression and outcome. No associations between exposure at <3.2 ppm and effects on the course and outcome of pregnancy occurred, although a higher rate of overdue delivery was observed among wives of the exposed male workers. There was an increased risk (RR = 1.92 [where RR is relative risk]) of menstrual disturbance (painful, profuse, and/or irregular, or too little bleeding) among female workers (35%) with an average 7-year exposure to ≥2.08 ppm (NOEL ~0.992 ppm). At higher CS_2 levels, there was an exposure–response relationship.

*Zhou et al. (*1988*):* A retrospective cohort study performed in China examined menstrual status, term and outcome of pregnancy in 265 female factory workers exposed to CS_2 and 291 nonexposed female workers, as well as 530 pregnancies (off-site women). CS_2 concentration to which the workers were exposed for 15 years before the study was an average of 0.992, 2.08, and 4.74 ppm (range: 0.54–4.74 ppm). The results showed that exposed females had a higher incidence rate of menstrual disturbance (irregular cycle or unusual bleeding) than the nonexposed women (35.9 vs. 18.2%, RR = 2.0, $p < 0.01$), and an exposure–response relationship between the CS_2 level and the incidence rate of menstrual disturbance was revealed. Data indicate that exposure to CS_2 at about 4.74 ppm may affect the function of the female reproductive system (NOEL ~2.08 ppm).

Le and Fu (1996): The effect of CS_2 on human sperm chromosomal aberration was investigated using human sperm/hamster egg fusion technique. Sperm from nine healthy men were used to analyze 203 human sperm chromosomal complements. The sperm were treated in vitro with CS_2 at 0, 1, 5, and 10 μmol per liter or Pingyanmycin (40 μmol per liter = positive control) and incubated with female Golden Hamster oocytes. There was an increase in aberrant rate (%), and average breaks at 10 μmol per liter. Aberrations and structural chromosome aberrations (breaks, deletions, centric rings, fragments, and chromatid exchange) had a 1 and 5.9% incidence, respectively (total abnormalities = 6.9%). The NOEL was 5 μmol per liter, indicating that direct mutagenesis occurred only at the highest concentration.

*Takebayashi et al. (*2003*):* There was no relationship between occupational exposure to CS_2 at ≤10 ppm and endocrine dysfunction from a 6-year cohort study of Japanese rayon workers. CS_2-exposed subjects ($n = 432$ males) and referent subjects ($n = 402$ males) were initially examined in 1992 to 1993. Subsequently, 251 CS_2-exposed, 140 former-CS_2 exposed (three factories closed), and 359 referent workers received a follow-up survey (89.9% participated) in 1998 to 1999. Mean duration of exposure was 19.3 years at the end of the study. Effects on thyroid, hypophysis, and gonad function were examined (adjusting for age, smoking, and alcohol drinking). There were no exposure-related differences when the average internal and external CS_2 levels were below the OEL (≤10 ppm).

*Ma et al. (*2010*):* The effects of CS_2 exposure (3 years at ~3.2 ppm maximum allowable air concentration value for China) on sexual function (number of sexual encounters and length of sexual encounters) and semen quality in 80 CS_2-exposed males (categorized by job type) and 49 control subjects from the filature and cotton pulp departments of a fabric factory in China were studied. Semen samples were obtained from 43 of the exposed and 35 of the nonexposed subjects. Statistical adjustments were made for potential confounding factors (e.g., age or alcohol consumption). Exposed workers had decreased number of sexual encounters and length of sexual encounters was shorter compared with the control ($p < 0.001$), decreased sperm volume, total sperm count, longer liquefaction time, decreased acrosomal membrane integrity rate, viability and density, and increased sperm abnormality compared to controls ($p < 0.01$). Age (OR = 3.67) and job performed (OR = 0.08) were the most important factors affecting in sexual dysfunction. Duration of exposure affected sexual function and semen quality (not statistically significant).

CS_2 Developmental and Reproductive Epidemiology Conclusions

Reproductive effects in male and female workers (increased sperm abnormalities, loss of libido, and menstrual irregularities) after CS_2 exposure in viscose rayon factories conditions have been described (Cirla et al., 1978; Selevan et al., 1983; Wagar et al., 1981; Liang et al., 1988; Zhou et al., 1988; Ma et al., 2010). Based on the results of Liang et al. (1988), who reported increased menstrual disturbance in female workers at >2.08 ppm (NOEL = 1 ppm), Gelbke et al. (2009) stated that "…the reproductive capacity of female workers may not be adequately protected at exposures around 10 ppm," (acceptable exposure level in many industrial settings; Table 6). However, other studies performed worldwide did not find reproductive effects in men or women after factory exposure to CS_2 (Cirla and Graziano, 1981; Meyer, 1981; Takebayashi et al., 2003). The epidemiological studies varied according to design, but in general most industrial studies had small sample sizes and it was reportedly difficult to determine exposure to CS_2 when other chemicals, including H_2S, were also involved. Therefore, although data are useful in risk determination, the major deficiencies in these studies (insufficient exposure data, small sample sizes, broad exposure range over the years, co-exposure with H_2S) meant there was a great deal of uncertainty when calculating safe exposure levels.

Table 4
H_2S Reproduction and Developmental Whole Body Inhalation Studies in Sprague–Dawley Rat

Exposure	Effects	NOEL	References[a]
Dam: 6 hr/d; 7 d/wk; GD 6-PND 21	Dam: ↓F0 food consumption (1st week only); ↑liver cholesterol (6%), and dystocia Pup: ↑mortality, ↑brain cholesterol (reversed PND 21)	Dam/pup: 20 ppm	1
F0 F: -14 d -GD 19; F1 pup: PND 5–18; F0 M: 70 d; 6 hr/d; 7 d/wk	F0 parental: ↓F0 food consumed (M/F). Males: ↓body weights; ↑abnormal sperm%; seminiferous tubule degeneration and associated epididymal changes; ↑basal cell hyperplasia in olfactory mucosa, sensory neuron loss F1 pups: no effects	F0 adult: 10 ppm F1 pup: >80 ppm	2
Dam: 7 hr/d; GD 5-PND 21	Dam: ↑taurine concentration (tested only at 50 ppm) Pup: CNS effects: Purkinje dendrite structure and growth effects (↑nonsymmetrical growth pattern); ↓aspartate, glutamate, and GABA; ↑5-HT and NE; ↑taurine (reversed)	Dam/pup: 20 ppm	3

CNS, central nervous system; d/wk, days per week; F, female; F0, 1st parental generation; F1, 2nd parental generation or pups of F0 parents; GABA, γ-aminobutyric acid; GD, gestation day; hr/d, hours per day; 5-HT, serotonin; LOEL, lowest observed effect level; M, male; NOEL, no observed effect level; NE, norepinephrine; %, percent; PND, postnatal day.
[a]1. Hayden et al. (1990); 2. Dorman et al. (2000); 3. Hannah et al. (1989, 1990); Hannah and Roth (1991); Skrajny et al. (1992).

Hydrogen Sulfide

California's statewide ambient air quality standard for H_2S of 0.03 ppm (time-weighted average 1 hr) protects against nuisance odor (rotten egg smell) for the general public. OEHHA (1999, 2000) adopted 0.030 ppm as the acute reference exposure level and 0.008 ppm as the chronic reference exposure level for use in evaluating long-term emissions from Hot Spots facilities. H_2S is on California's TAC priority list and is regulated under the Children's Environmental Health Protection Act due to its neurotoxicity (Senate Bill 25, 1999; OEHHA, 2001).

A major issue with the STTC and similar pesticide risk determinations, such as the metam sodium risk assessment, is the lack of agricultural and bystander field exposure for H_2S (Rubin, 2004). In addition, most available epidemiological studies focused on acute respiratory effects or acute neurotoxicity rather than effects from subchronic or chronic low-dose exposures. But concerns remained over what the effects of longer term exposures (seasonal or subchronic) might be on human reproduction or development in an occupational setting.

Separate registrant-submitted studies for risk assessment were not required by DPR since H_2S is a nonpesticidal byproduct of STTC. A rationale for this decision may have been that exposure levels protecting for CS_2 toxicity would also be protective for H_2S. DPR, however, evaluated the H_2S animal reproduction and developmental toxicity studies reported in the open literature (summarized below) for the STTC-risk determination.

H_2S Pharmacokinetics

No quantitative studies were found concerning the absorption of inhaled H_2S; however, numerous toxicological case histories suggest that it is rapidly absorbed via this route. In animal processing facilities, sewers, sludge plants, and tanks where H_2S levels can be greater than 500 ppm, victims were reported as losing consciousness after only one or two breaths (ATSDR, 2006). The gas is rapidly absorbed in the lungs and metabolized by oxidation, methylation, and reactions with metalloproteins or disulfide-containing proteins (Figure 2; Beauchamp et al., 1984; Chou, 2003). H_2S inhibits the cytochrome c-oxidase protein complex within the mitochondrial electron transport chain which leads to anaerobic metabolism, severely decreased ATP production and the generation of lactic acid (Beauchamp et al., 1983; Deng, 1992). Nervous tissues, with their high oxygen demand, are especially sensitive to the disruption of oxidative metabolism and in the central nervous system (CNS) these effects may result in respiratory arrest (Ammann, 1986). NIOSH reported that H_2S exposure was the "primary occupational cause of unexpected death (ATSDR, 2006). While no physiologically based pharmacokinetic models have been reported, researchers have demonstrated that inhalation of H_2S causes inhibition of lung mitochondrial cytochrome oxidase (Khan et al., 1990). In another study by Nagata et al. (1990), rats, which inhaled H_2S, had detectable levels of sulfide in both the brain and lung. With the nonexposed controls, there was no sulfide detected in these tissues (Nagata, 1990).

H_2S Hazard Identification for Developmental and Reproductive Toxicity

Whole body inhalation developmental and reproduction studies performed in rats (Table 4). *Hayden et al. (1990):* H_2S was administered in air (7 hr/day) to Sprague–Dawley rats (8–15/dose; 29 controls) at 0, 20, 50, and 75 ppm from GD 6 to PND 21. Neonates/pups were not exposed to H_2S. Developmental parameters were measured as well as pup liver weights,

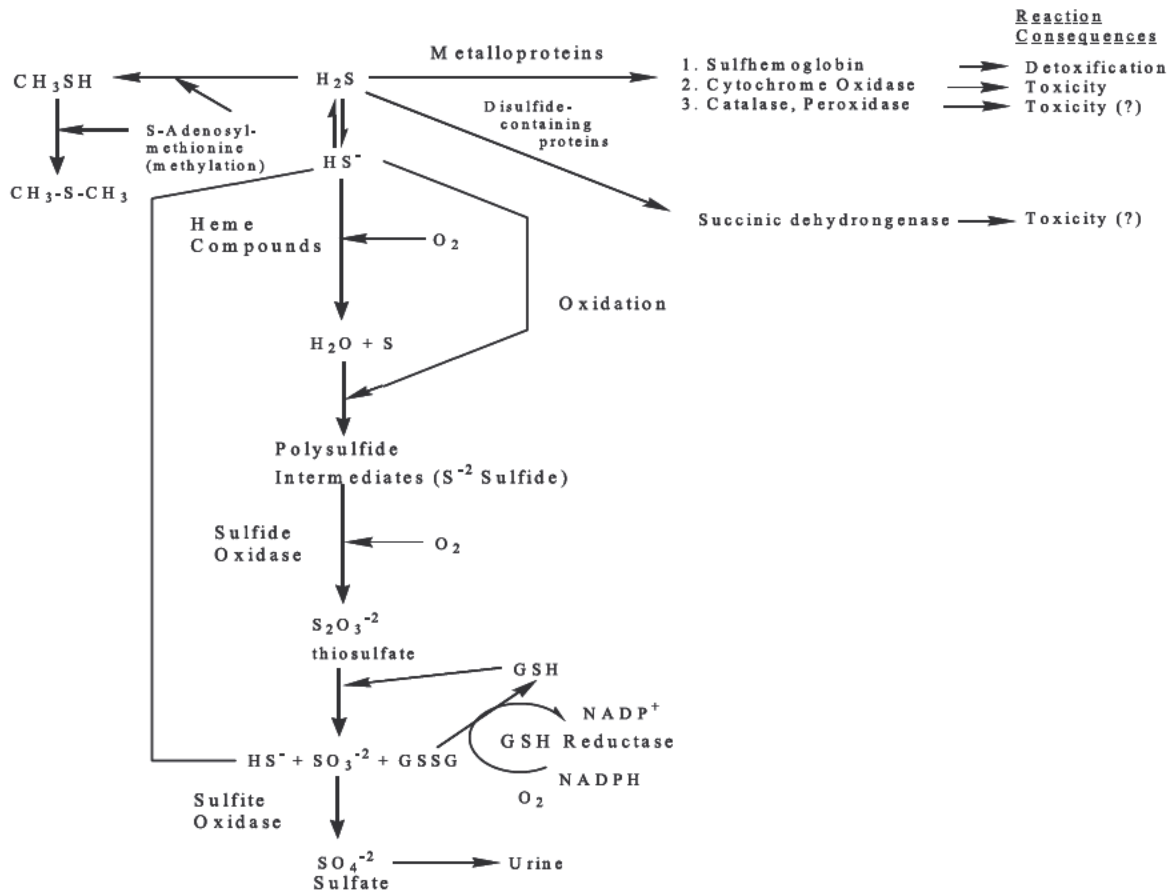

Fig. 2. Metabolic pathways of H_2S (adapted from Beauchamp et al., 1984).

brain weights, and motor development (righting reflex, assessed PND 1, 7, 14, and 21). Liver and brain protein, DNA, and cholesterol were assessed in pups on PND 1, 7, 14, and 21 and in dams PND 21. Food consumption was decreased in dams at ≥50 ppm (reversed by GD 13). Mean parturition times (dystocia) were increased at ≥20 ppm (not statistically significant) when compared to their matched controls. Extended labor time resulted in fetal death due to asphyxiation in a few cases. The viability of pups born to dams with greater than 200 min parturition time was decreased by 27%. Cholesterol levels in maternal liver were increased at 75 ppm PND 21. Brain cholesterol content (≥50 ppm; $p < 0.05$) in pups at PND 14 was increased (normal by PND 21). Cholesterol increases occurred (nondose related; reversed in pups) but were not accompanied by effects on liver or brain weights or other pathology in dams or pups. NOEL for pups and dams was 20 ppm based on the above results.

Dorman et al. (2000): H_2S administered in air to Sprague–Dawley rats (12/sex/dose) at 0, 10, 30, and 80 ppm (6 hr/day; 7 days/week) for 2 weeks before mating, through 2 weeks of mating to GD 19. Exposure of dams and pups resumed from PND 5 to 18. Litters were weaned PND 21. Adult F0 males were exposed for 70 consecutive days. F0 adults at 0 and 80 ppm had gross and histopathology examination on the reproductive tract. F1 pups were evaluated for effects on motor activity (PND 13, 17, 21, and 60), passive avoidance (PND 22 and 62), functional observation battery (FOB PND 60), acoustic startle response (PND 21 and 62), and neuropathology (1/sex/litter; PND 23 and 61). *F0 generation:* Food consumption was decreased in males and females at >30 ppm during exposure week 1 (premating). Male bodyweight decreased at 80 ppm and persisted throughout the study. There were no effects on the reproductive performance or on any pup parameters measured. At 30 (1/12) and 80 (1/11) ppm, males had an increase in abnormal sperm (29 and 73%, respectively, of all sperm analyzed). The abnormal sperm percentage was elevated compared to historical controls (Charles River Laboratories). Histologically males at 80 ppm had seminiferous tubular degeneration (including intratubular sperm statis, tubular mineralization, sperm granulomas, and multinucleated giant cells) and associated changes in the epididymis (degenerate sperm forms in lumen, aspermia, and oligospermia). One case each ($n = 12$) of epididymal sperm granulomas and unilateral necrosis of the cauda was detected at 80 ppm. The F0 parental NOEL was 10 ppm. *F1 observations and behavior:* There were no treatment-related effects on pup observations or on histopathology of brain (examined at six levels), spinal cord, or peripheral nerves. There were no treatment-related effects on motor activity or behavior (pup NOEL > 80 ppm).

Table 5
$CS_2 + H_2S$ Rat and Human Developmental and Reproductive Effects

Design	Major effects	NOEL/LOEL	References[a]
Sprague–Dawley rat inhalation			
$CS_2 + H_2S$; 6 hr/day GD 6–20	Dam and fetus: ↓body weight	LOEL $CS_2 + H_2S$ = 400 + 100 ppm	1
Epidemiology inhalation			
Male workers: CS_2 <9.6 ppm + H_2S 10.8 ppm; 10-36 years	↑LH, ↑FSH (suggested pituitary–gonadal axis effects); no effect on T	LOEL $CS_2 + H_2S$ ~10 + 10 ppm	2
Female bystanders: CS_2 < or >3.2 ppm + H_2S< or > 2.9 ppm	No effects on spontaneous abortions	NOEL $CS_2 + H_2S$ >3.2 + >2.9 ppm	3
Male workers: CS_2 1.3–37 ppm + H_2S 4–17 ppm	No effects on parameters measured (serum LH, FSH, prolactin, T levels)	NOEL $CS_2 + H_2S$ >37 + 17 ppm	4

FSH, follicle stimulating hormone; LH, luteinizing hormone; LOEL, lowest observed effect level; M, male; NOEL, no observed effect level; T, serum testosterone.
[a]1. Saillenfait et al. (1989); 2. Wagar et al. (1981); 3. Hemminki and Niemi (1982); 4. Vanhoorne et al. (1993).

Neurological Effects of H_2S

Hannah et al. (1989, 1990); Hannah and Roth (1991); Skrajny et al. (1992). A collection of articles (Hannah et al., 1989, 1990; Hannah and Roth, 1991; Skrajny et al., 1992) examining the effects of low H_2S doses on CNS chemistry in the frontal cortex and cerebellum of developing rats is presented below. The concern addressed in these studies is that at low doses, H_2S disrupts CNS monoamine production resulting in irreversible changes in the development of neurons in young animals.

*Hannah et al. (*1989*):* Pregnant Sprague–Dawley rats (8/dose) were treated with H_2S in air (7 hr/day) from GD 5 to PND 21 at 0 and 75 ppm to study the effects on levels of aspartate, γ-amino butyric acid (GABA), glutamate, glycine, taurine, norepinephrine (NE), and serotonin (5-HT) in the developing rat cerebrum and cerebellum. At PND 21, decreased aspartate, glutamate, and GABA in the cerebrum and aspartate and GABA in the cerebellum was observed. Authors suggested that these CNS alterations could have behavioral or structural developmental effects in offspring, but these were not examined in this study.

*Hannah et al. (*1990*):* Pregnant Sprague–Dawley rats (12/group) were treated with H_2S in air (7 hr/day) from GD 5 to PND 21 at 0 and 50 ppm to study the effects on maternal blood plasma taurine levels. Blood plasma taurine was increased over controls at birth and PND 21 (~30% each sampling time at 50 ppm). The increase was not time-related. The mechanism producing increased taurine levels was initiated before parturition resulting in the stable 30% increase between birth and weaning. Pup taurine was initially elevated in brain but returned to control levels by PND 21 (no data shown). This timing corresponded to establishment of the blood-brain barrier to taurine in rat pups (Hannah et al., 1989). Taurine transfers from the dam transplacentally and via milk and high initial levels in pup CNS may have been maternal in origin. High initial taurine levels in treated pup brains occurred at a time of maximum sensitivity of neuronal growth to both exogenous and endogenous factors. The authors speculated that the increased taurine in pup brains may affect neuronal development, however no pup data were shown and this article presents only preliminary information.

*Hannah and Roth (*1991*):* Pregnant Sprague–Dawley rats (10/dose) were treated with H_2S in air (7 hr/day) from GD 5 to PND 21 at 20 and 50 ppm to study the effects on developing cerebellar Purkinje cells in rats. Cerebellar Purkinje cells from pups were examined PND 7, 14, and 21. Results at ≥20 ppm showed pathological changes in the structure and growth of Purkinje cell dendrites (longer branches, nonsymmetrical growth, increased vertex path length, and number of branches the dendritic field) at both doses. Clinical signs were not described for dams or pups, however, so it is not known whether any of the CNS effects observed were manifest through behavioral alterations.

*Skrajny et al. (*1992*):* Pregnant Sprague–Dawley rats (20/dose) were treated with H_2S in air (7 hr/day) from GD 5 to PND 21 at 20 and 75 ppm to study the effects on serotonin (5-HT) and NE in developing rat CNS (cerebellum and frontal cortex). At 75 ppm, the levels of 5-HT (PND 14 and 21 both brain areas) and NE (PND 7, 14, and 21: cerebellum; PND 21: frontal cortex) were increased. At 20 ppm, H_2S 5-HT (PND 21: frontal cortex) was increased but NE was decreased (PND 14, 21: frontal cortex; PND 14: cerebellum). Clinical signs were not mentioned in any of the articles and it is not known how much effect toxicity had on pup behavior. Data are difficult to interpret for NE (PND 14: cerebellum), since a decrease is induced at 20 ppm but it is increased at 40 ppm (both points are statistically significant). This may represent background variability rather than a real effect of treatment. Authors speculate that it is due to alteration of "more than one biochemical process involved in monoamine turnover, including synthesis, release, or biotransformation." The NOEL for pups was 20 ppm, since the effects occurring to NE and 5-HT at the lower dose are questionable.

H_2S Developmental and Reproductive Toxicity Conclusions

There were no H_2S-induced developmental neurobehavioral effects in F1 pups from treated dams (Dorman et al., 2000). F1 pups showed no treatment-related systemic effects or nervous system histopathology at any dose (pup NOEL > 80 ppm). On the other hand, systemic effects in F0 adults (M/F: food consumption; M: body weight, abnormal sperm, abnormal sperm percentage, seminiferous tubular degeneration, changes in the epididymis, epididymal sperm granulomas, unilateral

Table 6
Regulated Parameters for Exposure to C_2S and H_2S

Agency	CS_2		H_2S	References
ACGIH	TLV-TWA: 1 ppm (neurotox; skin irritation) TLV-STEL: 2 ppm		1 ppm (neurotoxicity) 5 ppm	ACGIH (2010)
NIOSH	Odor threshold: 0.1–0.2 ppm REL: 8 hr TWA/day = 1 ppm 15-min ceiling = 10 ppm IDLH: 500 ppm		Odor threshold: 0.003–0.02 ppm 8 hr TWA/day = 1 ppm 10-min ceiling = 10 ppm IDLH: 300 ppm	NIOSH (2012)
Cal/OSHA	PEL: 8 hr TWA = 4 ppm STEL: 15 min TWA = 12 ppm COEL: 10 min = 30 ppm		8 hr TWA = 10 ppm STEL: 15 min TWA = 15 ppm COEL: limit: 10 min = 50 ppm	Cal/OSHA (2012)
OSHA	PEL: 8 hr TWA = 20 ppm APC: 30 min = 30 ppm		8 hr TWA = 10 ppm STEL: 15 ppm	OSHA (2012)
OEHHA	REL: acute 1 hr: 1.98 ppm (repro/develop)		Acute 1 hr: 0.03 ppm (respiratory)	OEHHA (1999, 2001)
Calif. EPA	REL: chronic: 0.3 ppm (neurotox/repro)		Chronic: 0.008 ppm (respiratory)	
United States EPA	Chronic inhalation BMC_{05} 6.86 ppm[a] UF: 30 (neurotox) RfC = 0.2 ppm	Acute oral NOEL = 11 mg/kg/d 300 (hepatotox) RfD = 0.1 mg/kg/d	Acute inhalation AEGL 10 min: 0.07 ppm 27 (respiratory) RfC = 0.0014 ppm	ATSDR (1996, 2002), IRIS (2003, 2012), NRC (2010)

AEGL, represent threshold exposure limits for the general public, applicable to 12 emergency exposure periods ranging from 10 min to 8 hr; AMP, acceptable maximum peak above the acceptable ceiling concentration for an 8-hr shift (for H_2S, 10 min once only if no other measurable exposure occurs); APC, acceptable peak concentration (CS_2); COEL, ceiling occupational exposure limit HEC: Human Equivalent Concentration = 2.54 ppm; IDLH, immediately dangerous to life or health; inhalation RfC, an estimate with uncertainty spanning perhaps an order of magnitude of a daily inhalation exposure of the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime; MRL, maximum respiratory level; PEL, permissible exposure limit (the maximum permitted 8-hr time-weighted average concentration of an airborne contaminant; REL, reference exposure level; RfD, derived from toxicity studies on animals and are based on the highest oral dose or level of exposure at which no adverse effects were observable. STEL, short-term exposure limit: 15 min (Cal/OSHA) or 30-min (OSHA) time-weighted average exposure which is not to be exceeded at any time during a workday even if the 8-hr time-weighted average is below the PEL; TWA, time-weighted average; TLV, threshold limit value; UF, uncertainty factor.
[a]Benchmark Concentration (CL = .95) reported by the United States EPA.

necrosis of the cauda) occurred at ≥30 ppm (NOEL = 10 ppm). The authors suggest "that H_2S is neither a reproductive toxicant nor a behavioral developmental neurotoxicant in the rat at occupationally relevant exposure concentrations (≤10 ppm)."

While monoamine effects in the CNS were observed in pups, behavior was not tested and clinical signs were not described in the studies by Hannah et al. (1989, 1990), Hannah and Roth (1991), and Skrajny et al. (1992). Without behavioral evaluaton it could not be determined whether taurine effects were detrimental in the long term on neuronal development in pups. Effects in pups, however, appeared to be secondary to increases in maternal blood taurine levels and returned to control levels by PND 21.

CS_2 + H_2S Co-Exposure Hazard Identification for Developmental and Reproductive Toxicity in Rat and Humans

The following studies document co-exposure of CS_2 + H_2S in rats (Saillenfait et al., 1989) and humans (Hemminki and Niemi, 1982; Wager et al., 1983; Vanhoorne et al., 1993) and their effects on development and reproduction. Many of the previously mentioned CS_2 epidemiological studies were likely also co-exposures with H_2S but exposure levels were not stated in the report.

Co-Exposure Study Performed in Rat (Table 5)

*Saillenfait et al. (*1989*):* Pregnant Sprague–Dawley rats (7–9/dose) were treated with H_2S in air at 0, 50, 100, and 150 ppm (6 hr/day) GD 6 to 20. Dams had decreased body weights and body weight gain at ≥150 ppm. Fetal body weights were decreased (4, 7, and 7% at 50, 100, and 150 ppm, respectively) but the effect was not dose related. Body weight and body weight gain were decreased in dams at ≥100 ppm. The decreased pup body weight at 150 ppm was likely due to maternal toxicity. These results were from a preliminary rangefinding study performed with H_2S as part of a study designed to test the effects of co-exposure of CS_2 (400 ppm) + H_2S (100 ppm) and CS_2 (800 ppm) + H_2S (100 ppm). Effects were observed as decreased body weight in dams and fetuses at both CS_2 + H_2S co-exposure doses. Maternal clinical signs were not mentioned and the doses eliciting effects for CS_2 and H_2S were sufficiently high as to be an unlikely exposure scenario (CS_2 + H_2S LOEL = 400 + 100 ppm, respectively).

Human Co-Exposure to $CS_2 + H_2S$ (Table 5)

*Wager et al. (*1981*):* Endocrine function in male viscose rayon factory workers in Finland ($n = 15$) exposed to CS_2 at <9.6 ppm + 10.8 ppm H_2S for 10 years (before 1950s: 20–40 ppm CS_2; 1960s: 10–30 ppm CS_2; exposure duration range: 10–36 years) was compared with 16 age-matched controls. FSH (exposed ≥ 5 years) and LH (exposed ~20 years) in the serum were significantly increased in the exposed group (7 > reference limit). Although the serum T values were not affected, the FSH and LH values were considered "latent primary gonadal insufficiency" by the authors. A major deficiency in this study is the small sample size (LOEL = CS_2 <9.6 ppm + H_2S 10.8 ppm).

Hemminki and Niemi (1982): Spontaneous abortion incidences in women in an industrial community in Finland were examined. Analyses were made in relation to the occupation of the women and to the level of $CS_2 + H_2S$ in the family's residential area. Information on abortions and births was obtained from the hospital discharge register; information on the women and their families was obtained from the files of the population and housing census. Two zones of CS_2 concentration (>3.2 ppm; $n = 42$ F; <3.2 ppm; $n = 41$ F) and two zones of H_2S concentration (>2.9 ppm; $n = 29$ F; <2.9 ppm; $n = 54$ F) were considered in the analysis. The results showed no statistically significant evidence for harmful effects of CS_2 or H_2S for the parameters measured and with a small sample size (LOEL CS_2 +H_2S ~10 + 10 ppm).

*Vanhoorne et al. (*1993*):* Male workers ($n = 117$) and control subjects ($n = 66$; not exposed to any toxic agent in the work environment) in a Belgian viscose rayon factory were exposed to CS_2 (1.3–37 ppm) + H_2S (4–17 ppm) for at least 1 year depending on job description. The work environment was relatively unchanged from 1932 to the time of data collection for this study. CS_2 cumulative exposure index was calculated for each individual. Serum LH, FSH, prolactin, and T levels were tested by radioimmunoassay. Univariate analysis of the hormones versus exposure showed significantly lower levels of prolactin in the exposed compared with the control subjects ($p = 0.01$). However, when adjusted for age, alcohol, smoking, body mass index, and stress level, the CS_2 cumulative exposure index was not associated with effects on any of the hormones assayed. According to the authors "This discrepancy from previous studies [such as those described above by the current author; Cirla et al., 1978; Wager et al., 1981; Selevan et al., 1983; Liang et al., 1988; Zhou et al., 1988; Ma et al., 2010] may result from differences in exposure (such as concurrent H_2S), in population selection or confounding bias ."

$CS_2 + H_2S$ Developmental and Reproductive Toxicity Conclusions

Each study had numerous deficiencies and data appear to be equivocal for effects on the reproductive system after $CS_2 + H_2S$ co-exposure. They focused primarily on CS_2 effects and were not specifically designed to investigate co-exposure with H_2S. Saillenfait et al. (1989) had no description of clinical observations beyond survival and body weight and there were no individual data. The study concluded that there was fetal toxicity associated with maternal toxicity (body weight decreases). The report also stated that 100 ppm H_2S "…was selected as the threshold level for maternal and fetal toxicity since a toxic level would hinder the detection of possible enhancement of CS_2's own effects." Since the doses were high and an NOEL was not achieved, it is difficult to draw conclusions from this co-exposure study.

In the epidemiology studies, the exposure data for workers and bystanders were inadequate and too few parameters were tested (references). (Wagar et al., 1981; Vanhoorne et al., 1993). Therefore, results should be interpreted with caution.

DISCUSSION

The STTC parent compound is not detrimental to development or reproduction in animal studies (Salamon, 1986a, 1986b, 1986c) but when prepared for pesticidal use (mixed, loaded, and applied), two highly toxic compounds (CS_2 [a.i.] and H_2S) are released in air. The primary route of CS_2 exposure is inhalation, but since there are dietary tolerances for CS_2 on several crops treated in California, oral developmental/reproduction studies were also evaluated. Results of animal studies suggest that endocrine disruption may result from low levels of exposure to CS_2. The most sensitive endpoint for CS_2 was DNT when dams were exposed via inhalation at <3.2 ppm (lowest NOEL = 0.01 ppm: Tabacova and Balabaeva, 1980; Tabacova et al., 1981; Tabacova et al., 1983; Lehotzky et al., 1985). H_2S, a known neurotoxin had no DNT effects at >80 ppm (Dorman, 2000).

CS_2 has been described previously as a suspected human developmental toxicant after reproductive effects (spontaneous abortions, premature births, birth defects, decreased sperm production, loss of libido, and menstrual irregularities) were reported (Cirla and Graziano, 1981; Meyer, 1981; Selevan et al., 1983; Zhou et al., 1988; Williams et al., 2000; Takebayashi et al., 2003). Epidemiological studies showed that women working in viscose rayon factories had menstrual disturbances after chronic CS_2 exposure in air (NOEL = 1 ppm; Table 3; Liang et al., 1988). Menstrual disturbances indicate the potential for endocrine disruption in female workers exposed occupationally to CS_2 at low levels.

Some of the effects on CS_2-treated male rats were similar to those of male factory workers, including effects on sexual behavior, sperm parameters, and endocrine hormone levels (FSH, LH, T) at low exposures (Tables 3 and 4. Animal hormone assays showed primarily decreased testosterone (Tepe and Zenick, 1984; Kumar et al., 1999; Patel et al., 1999). Human subjects, however, had inconsistent hormonal results. FSH, LH, and T were decreased, increased, or showed no effects depending on the study (Table 3). This could be due to many factors, including study design, sample size, exposure data, and testing methods. Inconsistency could also be due to co-exposure to unknown levels of H_2S, which was mentioned in several of the CS_2-focused studies (Gelbke et al., 2009).

H_2S was not a reproductive or developmental toxin based on the results of animal studies (Table 4) and epidemiological studies (ATSDR, 2006). Subchronic treatment in rat (70 days: Dorman et al., 1999; 90 days: Brenneman et al., 2000) showed olfactory neurotoxicity was the most sensitive endpoint with the lowest NOEL (10 ppm).

Due to the low odor threshold, humans can initially be alerted to H_2S exposure before being overcome. Clinical effects after acute H_2S exposure are unconsciousness, followed by apparent recovery (knockdown) and incoordination, memory and motor dysfunction, personality changes, hallucinations, and anosmia (ATSDR, 2006). These clinical effects are consistent with organic brain disease resulting from anoxia and may persist for several years after the initial H_2S exposure (Arnold et al., 1985; Tvedt et al., 1991a, 1991b; Reiffenstein et al., 1992; Kilburn and Warshaw, 1995; Snyder et al., 1995).

STTC is only one example of a multitude of sources, both environmental (not limited to pesticides) and industrial, which generate CS_2 and H_2S and may pose a human hazard through air and diet (ATSDR, 1996, 2006; OEHHA, 1999, 2001; Williams et al., 2000; Gelbke et al., 2009; Reeve, 2012). STTC byproducts were also associated with toxicity to terrestrial and aquatic organisms (USEPA, 2008). Worker exposure epidemiology studies examining effects of both byproducts focused mainly on CS_2 toxicity and do not address the likely co-exposure with similarly toxic H_2S. Co-exposure studies were preliminary at best. At this time, each byproduct is regulated separately (Table 6) but it would be worth further investigation to examine the developmental and reproductive toxicity of CS_2 and H_2S as a mixture and their long-term effects after low-dose exposures.

It is problematic that human exposure data for STTC or other pesticides generating CS_2 and H_2S are inadequate or unavailable (Reeve, 2012). Lack of data means risk assessors must resort to use of surrogate data, as was the case with STTC, CS_2, and H_2S, compounding the unreliability of the projected measurements. Yet numerous pesticides (metam sodium, metam potassium, and dazomet) currently registered in California generate CS_2 and H_2S as byproducts (DPR, 2012). Toxicology is important in identifying endpoints and generating no effects levels but human exposure data drive the margins of exposure for workers and bystanders. The USEPA required the registrant to provide a FIFRA Guideline CS_2 DNT inhalation study after reports of fetal effects at low doses in addition to a flux rate study of off-site byproduct movement due to lack of critical data (USEPA, 2008). These studies were not performed and pesticide product containing STTC was canceled. Nevertheless the potential for developmental and neurobehavioral effects from CS_2 and H_2S generated from other sources remains.

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