Interim: September 2009

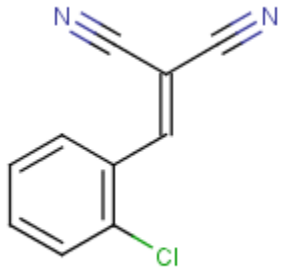
ACUTE EXPOSURE GUIDELINE LEVELS (AEGLs)

FOR

TEAR GAS (CS)

(CAS Reg. No. 2698-41-1)

$C_{10}H_5ClN_2$



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PREFACE

Under the authority of the Federal Advisory Committee Act (FACA) P. L. 92-463 of 1972, the National Advisory Committee for Acute Exposure Guideline Levels for Hazardous Substances (NAC/AEGL Committee) has been established to identify, review and interpret relevant toxicologic and other scientific data and develop AEGLs for high priority, acutely toxic chemicals.

AEGLs represent threshold exposure limits for the general public and are applicable to emergency exposure periods ranging from 10 minutes to 8 hours. Three levels – AEGL-1, AEGL-2 and AEGL-3 — are developed for each of five exposure periods (10 and 30 minutes, 1 hour, 4 hours, and 8 hours) and are distinguished by varying degrees of severity of toxic effects. The three AEGLs are defined as follows:

AEGL-1 is the airborne concentration (expressed as parts per million or milligrams per cubic meter [ppm or mg/m^3]) of a substance above which it is predicted that the general population, including susceptible individuals, could experience notable discomfort, irritation, or certain asymptomatic, non-sensory effects. However, the effects are not disabling and are transient and reversible upon cessation of exposure.

AEGL-2 is the airborne concentration (expressed as ppm or mg/m^3) of a substance above which it is predicted that the general population, including susceptible individuals, could experience irreversible or other serious, long-lasting adverse health effects or an impaired ability to escape.

AEGL-3 is the airborne concentration (expressed as ppm or mg/m^3) of a substance above which it is predicted that the general population, including susceptible individuals, could experience life-threatening health effects or death.

Airborne concentrations below the AEGL-1 represent exposure levels that could produce mild and progressively increasing but transient and nondisabling odor, taste, and sensory irritation or certain asymptomatic, non-sensory effects. With increasing airborne concentrations above each AEGL, there is a progressive increase in the likelihood of occurrence and the severity of effects described for each corresponding AEGL. Although the AEGL values represent threshold levels for the general public, including susceptible subpopulations, such as infants, children, the elderly, persons with asthma, and those with other illnesses, it is recognized that individuals, subject to unique or idiosyncratic responses, could experience the effects described at concentrations below the corresponding AEGL.

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SUMMARY

Tear Gas (o-Chlorobenzylidenemalonitrile; CAS No. 2698-41-1) is a white crystalline powder with a pepper-like odor. It was first synthesized by Corson and Stoughton in 1928 (thus the abbreviation "CS") (Corson and Stoughton, 1928; U.S. Army et al., 2005). It was developed in the 1950s as a replacement for the chemical incapacitant CN (1-chloroacetophenone) used by police because CS was a much more potent irritant than CN, but was significantly less toxic (WHO, 1970; Hu et al., 1989; Colgrave and Creasey, 1975). It was adopted for use by the military shortly after, and was widely used in Vietnam (Hu et al., 1989; WHO, 1970). It is currently used as an incapacitating agent both by military and law enforcement personnel (HSDB, 2008). It is reported that an aerosol concentration of 4 mg/m^3 will disperse the majority of rioters within 1 minute, and 10 mg/m^3 will deter trained troops (Upshall, 1973). With the exception of more severe cutaneous reactions, recovery from exposure is generally rapid upon exposure to fresh air, generally within 30 minutes after exposure (Ballantyne, 1977). CS may be manufactured through carbonyl condensation by combining o-chlorobenzaldehyde and malononitrile (HSDB, 2008). Recent production data were not located.

The AEGL-1 values were based on human exposure to 1.5 mg/m^3 for 90 minutes (Punte et al., 1963). All four subjects could tolerate the exposure, but experienced eye and nose irritation and headache. One subject developed nasal irritation within 2 minutes, three subjects developed headache (at 45, 50, and 83 minutes), and all four experienced ocular irritation (at 20, 24, 70, and 75 minutes). A modifying factor of 10 was applied to reduce the point-of-departure from a LOAEL to a NOAEL for AEGL-1 effects. An intraspecies uncertainty factor of 3 was applied because contact irritation is a portal-of-entry effect and is not expected to vary widely among individuals. The intraspecies UF of 3 is also supported by the fact that responses of volunteers with jaundice, hepatitis, or peptic ulcer or those that were 50-60 years old were similar to those of "normal" volunteers when exposed to a highly irritating concentration of CS for short durations (Punte et al., 1963; Gutentag et al., 1960). An interspecies uncertainty factor of 1 was applied because the study was conducted in humans. Time scaling was not applied in the development of the AEGL-1 values, because the critical effect (irritation) is a function of direct contact with the tear gas and is not likely to increase with duration of exposure at this level of severity (NRC, 2001).

The AEGL-2 values were based on human exposure to 1.5 mg/m^3 for 90 minutes (Punte et al., 1963). All four subjects could tolerate the exposure, but experienced eye and nose irritation and headache. One subject developed nasal irritation within 2 minutes, three subjects developed headache (at 45, 50, and 83 minutes), and all four experienced ocular irritation (at 20, 24, 70, and 75 minutes). An intraspecies uncertainty factor of 3 was applied because contact irritation is a portal-of-entry effect and is not expected to vary widely among individuals. The intraspecies UF of 3 is also supported by the fact that responses of volunteers with jaundice, hepatitis, or peptic ulcer or those that were 50-60 years old were similar to those of "normal" volunteers when exposed to a highly irritating concentration of CS for short durations (Punte et al., 1963; Gutentag et al., 1960). An interspecies uncertainty factor of 1 was applied because the study was conducted in humans. Time scaling was not applied in the development of the AEGL-2 values, because the critical effect (irritation) is a function of direct contact with the tear gas and is not likely to increase with duration of exposure at this level of severity (NRC, 2001).

AEGL-3 values were based on the threshold for lethality at each AEGL-3 exposure duration calculated using the probit-analysis based dose-response program of ten Berge (2006). Rat lethality data of McNamara et al.(1969); Ballantyne and Calloway (1972); and Ballantyne and Swantson (1978) were used in the calculation, and the threshold for lethality was set at the LC_{01}. The rat data indicated a time-scaling value of 0.704 ($C^{0.704} \times t = k$). The 4-hour AEGL-3 value was adopted as the 8-hour AEGL-3 value because time scaling yielded an 8-hour value inconsistent with the AEGL-2 values that were derived from a rather robust human data set. This is likely a result of the methodology (time-scaling to 8-hrs with an exponent 'n' of 0.704).

Inter- and intraspecies uncertainty factors of 3 each were applied (total 10) and are considered sufficient because clinical signs are likely caused by a direct chemical effect on the tissues. This type of portal-of-entry effect is not likely to vary greatly between species or among individuals. The interspecies UF of 3 is supported by calculated LCt_{50} values of 88,480 mg min/m^3 for rats; 67,200 mg min/m^3 for guinea pigs; 54,090 mg min/m^3 for rabbits; and 50,010 mg min/m^3 for mice (Ballantyne and Swantson, 1978), values all well within a factor of two. The intraspecies UF of 3 is supported by the fact that responses of volunteers with jaundice, hepatitis, or peptic ulcer or those that were 50-60 years old were similar to those of "normal" volunteers when exposed to highly irritating concentration of CS for short durations (Punte et al., 1963; Gutentag et al., 1960).

The calculated values are listed in the table below.

S 1. **Summary of AEGL Values for Tear Gas**

Classification	10-min	30-min	1-h	4-h	8-h	Endpoint (Reference)
AEGL−1 (Nondisabling)	0.050 mg/m^3	0.050 mg/m^3	0.050 mg/m^3	0.050 mg/m^3	0.050 mg/m^3	NOAEL for Ocular/nasal irritation and headache in humans (Punte et al., 1963)
AEGL−2 (Disabling)	0.50 mg/m^3	0.50 mg/m^3	0.50 mg/m^3	0.50 mg/m^3	0.50 mg/m^3	Ocular/nasal irritation and headache in humans (Punte et al., 1963)
AEGL−3 (Lethal)	140 mg/m^3	29 mg/m^3	11 mg/m^3	1.5 mg/m^3	1.5 mg/m^3	Threshold for lethality (LC_{01}) in rats [McNamara et al.(1969); Ballantyne and Calloway (1972); and Ballantyne and Swantson (1978)]

1. INTRODUCTION

Tear Gas ($C_{10}H_5ClN_2$; CAS No. 2698-41-1) is a white crystalline powder with a pepper-like odor. It was first synthesized by Corson and Stoughton in 1928 (thus the abbreviation "CS") (Corson and Stoughton, 1928; U.S. Army et al., 2005). It was developed in the 1950s as a replacement for the chemical incapacitant CN (1-chloroacetophenone) used by police because CS was a much more potent irritant than CN, but was significantly less toxic (WHO, 1970; Hu et al., 1989; Colgrave and Creasey, 1975). It was adopted for use by the military shortly after, and was widely used in Vietnam (Hu et al., 1989; WHO, 1970; Smith and Greaves, 2002). It is currently used as an incapacitating agent both by military and law enforcement personnel (HSDB, 2008). It is reported that an aerosol concentration of 4 mg/m^3 will disperse the majority of rioters within 1 minute, and 10 mg/m^3 will deter trained troops (Upshall, 1973). With the exception of more severe cutaneous reactions, recovery from exposure is generally rapid upon exposure to fresh air, usually within 30 minutes after exposure (Ballantyne, 1977).

Because CS is stable when heated and has a low vapor pressure, it requires a means of dispersement (Blain, 2003). Different forms of dispersement include the combination of CS with a pyrotechnic compound in a grenade or canister, generating a smoke or fog, and dispersement of a fine powder as an aerosol (Smith and Greaves, 2002; WHO, 1970). CS1 is a micronized powder formulation of CS containing 5% silica gel for dissemination by an explosive burst or dusting apparatus, and CS2 is the same as CS1 except that the CS1 is microencapsulated with silicone to improve its weather resistance and flow properties (WHO, 1970).

In controlled studies investigating the toxicological properties of CS aerosol, CS was disseminated from a 2% to 10% solution in methylene chloride or acetone by means of a pneumatic atomizing nozzle assembly (Owens and Punte, 1963; Punte et al., 1963; Gutentag et al., 1960) or by thermal dispersion by spraying the molten chemical (Punte et al., 1963; Gutentag et al., 1960; Punte et al., 1962).

CS may be manufactured through carbonyl condensation by combining o-chlorobenzaldehyde and malononitrile (HSDB, 2008). Recent production data were not located.

Hydrolysis of CS produces malononitrile and *o*-chlorobenzaldehyde (NTP, 1990). Hydrolysis of CS is relatively rapid, with a half-life of about 15 minutes at a pH 7, but CS reacts faster with an alkaline solution, having a half-life of about 1 minute at a pH of 9 (Blain, 2003).

When released to the air, CS will exist in both vapor and aerosol form (HSDB, 2008). CS in the vapor phase will be degraded by reaction with photochemically produced hydroxyl radicals, with an estimated half-life of 110 hours, and CS in the particulate phase will be removed by wet and dry deposition.

Chemical and physical properties are presented in Table 1.

TABLE 1. Chemical and Physical Properties		
Parameter	**Value**	**Reference**
Synonyms	CS; o-Chlorobenzylidenemalonitrile; 2-Chlorobenzalmalononitrile; o-Chlorobenzylidenemalononitrile; ((2-Chloro-phenyl)methylene)propanenitrile; 2-Chlorobmn; Alonitrile; beta,beta-Dicyano-o-chlorostyrene; Propanedinitrile, ((2-chlorophenyl)methylene)-	O'Neil et al., 2001
Chemical formula	$C_{10}H_5ClN_2$	O'Neil et al., 2001
Molecular weight	188.6	O'Neil et al., 2001
CAS Reg. No.	2698-41-1	O'Neil et al., 2001
Physical state	White crystalline solid	O'Neil et al., 2001
Solubility in water (g/L)	Sparingly soluble 2.0×10^{-4} M	O'Neil et al., 2001; ACGIH, 1991
Vapor pressure	3.4×10^{-5} mm Hg	
Vapor density (air =1)	6.5	U.S. Army et al., 2005
Density (solid)	bulk: 0.24-0.26 g/mL; crystal: 1.04 g/mL	U.S. Army et al., 2005
Melting point	95º-96ºC	ACGIH, 1991
Boiling point	310ºC to 315ºC	U.S. Army, 2005
Henry's Law Constant (atm-m^3/mol)	1.0×10^{-8}	HSDB, 2008
Volatility	0.71 mg/m3 @ 25ºC	U.S. Army et al., 2005
Stability/reactivity	Combustible material; may burn but does not ignite readily	U.S. Army et al., 2005
Conversion factors	1 ppm = 7.71 mg/m^3 1 mg/m^3 = 0.13 ppm	Calculated: $\frac{ppm \times M.W.}{24.45} = mg/m^3$

2. HUMAN TOXICITY DATA

2.1. Acute lethality

No human acute lethality data were located.

2.2 Nonlethal acute toxicity

2.2.1. Experimental studies

In a review article**,** Blain (2003) reported a TC_{50} (defined as the concentration required to obtain no more than a perceptible effect on 50% of the population exposed to the gas for 1 minute) of 0.004 mg/m^3 for ocular irritation and 0.023 mg/m^3 for airway irritation. An ICT_{50} (the concentration intolerable to 50% of the population for 1 minute) was also reported. No further details were presented.

A group of male volunteers was exposed to CS aerosol with a mass-median diameter of 0.9 microns (94 ± 15 mg/m^3; 4% larger than 10 micron) or of 60 microns (85 ± 16 mg/m^3; 4% smaller than 20 microns) to assess differences in ocular and respiratory responses to different particulate sizes of CS (Owens and Punte, 1963). Six volunteers were chosen from a group of approximately fifty based on their ability to best tolerate CS. Subjects wore tightly fitted

goggles and a nose and mouth respirator designed to protect against particle sizes less than one micron, and were exposed individually in a wind tunnel with a constant air speed of 5 mph. The exposure protocol was designed such that either eyes only, the respiratory system only, or eyes and the respiratory system could be exposed to either the small or large particles. The wind tunnel was elevated to a height of five feet, and a rubber-lined port was installed in the bottom of the duct enabling the subject to insert his head into the airstream of the tunnel and remove it quickly after the exposure. CS was disseminated from a 2% solution in methylene chloride by means of a pneumatic atomizing nozzle assembly. CS exposure concentrations were determined by collection of air samples using filter paper placed on air sampling probes located around the head area (one on top and one on each side near eye level), followed by extraction with ethanol and measurement with ultraviolet spectrophotometry. A modified cascade impactor was used to measure the CS aerosol containing the small particles, while the larger particles were sized microscopically, measuring and counting the various particles in the pre-ground material prior to dissemination. Tolerance time was defined as the time at which a subject could no longer remain in the atmosphere containing the compound and left the exposure chamber, and recovery time was defined as the time after the exposure when the subjects were able to sort and arrange a series of twenty-four playing cards from which the corner numbers were removed. Control values were determined before each test. The results indicate that small particles are more effective in rapidly producing eye irritation (Table 2). It is hypothesized that the onset of ocular response is faster with small particles because of the ease of solubility of the small particles in the eye fluid, while the onset of irritation would be delayed for the large particles due to the slowness of the large particle solubility. Once begun, however, the irritation process would continue for a longer period with the large particles compared to the small particles. Respiratory effects were more severe for the small particles (no volunteers could withstand exposure for more than 30 seconds) and required more time for recovery than the large particles. The difference in response is due to the fact that the smaller-sized particulates are able to penetrate more deeply into the respiratory tract. When both the eyes and the respiratory system were exposed to CS, the respiratory response predominated with exposure to the small particulates, while the ocular response predominated with exposure to the large.

TABLE 2. Results of human exposure to one or sixty micron CS aerosols: Tolerance and recovery time

Exposure Condition	% Subjects able to tolerate a 60-sec exposure		Recovery Time (sec)	
	Small particles [a]	Large particles [b]	Small particles [a]	Large particles [b]
Eyes	40	100	91	280
Respiratory system	0	67	51	9*
Eyes and respiratory system	16	85	52	188

Taken from Owens and Punte, 1963

[a] Measured concentration of 94 ± 15 mg/m^3

[b] Measured concentration of 85 ± 16 mg/m^3

A group of 4-6 volunteers was exposed to CS aerosol in a wind tunnel (8x8x8 ft; fixed wind speed of 5 mph) (Punte et al., 1963; Gutentag et al., 1960). Volunteers were both military and civilian. Each volunteer's medical history was recorded, and they were given a pre-exposure and post exposure physical examination. Volunteers were classified as "normal" or were placed in one of four special categories: those with hypertension (diastolic of 80-110 mm Hg or normal blood pressure reading with a history of hypertension; pre-exposure tests included EKG, chest X-ray, NPN, and urinalysis); those with hay fever, drug sensitivity, or bronchial asthma (volunteers with asthma had normal chest X-ray before exposure); those with a history of

jaundice, hepatitis, or a history of peptic ulcers without gastrointestinal bleeding; and those that were 50-60 years of age. Subjects classified as "normal" were further categorized into untrained men with or without protective masks or trained men with or without protective masks. The trained men had previous exposure with CS, while the untrained men did not. The CS was dispersed either from a 10% solution in acetone or methylene dichloride with a spray nozzle (mass mean diameter 3.0 or 1.0 micron, respectively) or by thermal dispersion (spraying the molten chemical; mass mean diameter 0.5 micron). Airborne samples of the aerosol were collected at various points in the wind tunnel. Particle size was characterized using a 6-stage modified cascade impactor, and exposure concentrations were measured using u.v. spectrophotometry. The subjects did not report any noticeable difference in symptoms when exposed to chemically dispersed CS compared to thermal dispersion. Groups of 3-6 untrained men without masks were exposed to CS in acetone, and the time to incapacitation was recorded (time at which the subject could no longer tolerate the exposure). Times ranged from 53 to >120 seconds at exposure to 5 mg/m^3; 19-43 seconds at exposure to 12 mg/m^3; to 5 seconds at exposure to 442 mg/m^3. When groups of 1-7 trained men were exposed, times ranged from 37 to >120 seconds at 4 mg/m^3; 18-41 seconds at 10 mg/m^3; to 12-25 seconds at 141 mg/m^3. To compare the effects of hyperventilation on exposure symptoms, untrained subjects ran for approximately 100 yards before exposure. Exercising subjects could not tolerate CS as well as normally breathing subjects: groups of three subjects exposed to 10, 13, or 39 mg/m^3 could tolerate CS up to 13, 13, and 9 seconds, respectively. While eye irritation was minimal, chest symptoms were more pronounced and recovery time was slightly prolonged (by 1-2 minutes). The reactions of subjects with jaundice, hepatitis, or peptic ulcer or those that were 50-60 years old were similar to those of "normal" subjects. Subjects with a history of drug allergies or sensitivities, hay fever, or asthma also tolerated exposure to CS at a level comparable to the "normal" subjects, but this group had a higher percentage of individuals with more severe chest symptoms, with many of them laying prostrate on the ground for several minutes. However, no wheezing or rhonchi were heard, and recovery was as rapid as that seen in other exposure groups. When subjects were exposed to CS at temperatures ranging from 0° up to 95°F, tolerance to the chemical was slightly reduced at the high temperature of 95°F. It was unclear if the decrease in tolerance was an actual effect of the exposure, the uncomfortable climate, or a combination of both. The increase in skin burning symptoms with the increased temperature was ascribed to an increase in perspiration.

As part of the study described above, the potential for development of tolerance to CS was investigated by exposing a group of 4 subjects to 1.5 mg/m^3 of CS for 90 minutes in a 20,000 L chamber (Punte et al., 1963). No data were provided regarding the monitoring of the CS aerosol. During the exposure, subjects were allowed to smoke, read, play cards, etc. During the exposure, only one subject noted nose irritation (noted 2 minutes into exposure), while three subjects reported headaches (starting at 45, 50, and 83 minutes) and all four subjects reported eye irritation (starting at 20, 24, 70, and 75 minutes). In the second part of the experiment, the four subjects were exposed to 1.5 mg/m^3 of CS for 40 minutes, after which enough additional CS aerosol was added to attain an airborne concentration of 11 mg/m^3 in about 10 minutes. Although the subjects had not been told of the increase in concentration, they all left within 2 minutes due to respiratory irritation. It was estimated that the exposure concentration ranged from 4.3 to 6.7 mg/m^3 when the subjects left the chamber. In the third part of the experiment, the subjects were exposed to 6 mg/m^3 of CS which was attained over 10 minutes. Symptoms reported by the subjects included nose and throat irritation, chest burning, sneezing, eye irritation

and lacrimation, headache, and skin irritation. Three of the four subjects reported that the exposure was unbearable at 18, 20, and 29 minutes, with chest symptoms being the reason the subjects left the chamber. The remaining subject was able to tolerate the agent, and the exposure was terminated at 40 minutes. The investigators attempted to enter the chamber without the benefit of the gradual increase in exposure concentration, and were unable to remain in the chamber. In the fourth experiment, a concentration of 6.6 mg/m^3 was attained over 30 minutes. It was noted that the usual signs and symptoms developed, but to a lesser degree. One of the subjects had to leave after 2 minutes of exposure due to a violent cough, but he returned to the exposure after his cough had ceased upon exposure to fresh air. He remained in the exposure chamber for the duration of the 60 minute exposure.

To assess the potential effect of CS exposure on ventilation, cardiac frequency, and breathing pattern, a group of 11 healthy soldier volunteers was exposed to CS aerosol (particle diameter of 1 micron) at a concentration that was progressively increased from 0.2 mg/m^3 up to 1.3 mg/m^3 (Cole et al., 1977; Cotes et al., 1972). The exact exposure duration was not provided, but appeared to be approximately 80 minutes. CS aerosol was produced by saturating the exposure chamber the evening before the exposure, followed by flushing with air to remove all of the gas except that adsorbed onto the walls and equipment. During exposure, pyrotechnic generators were ignited to progressively raise the concentration of CS throughout the exposure session. Subjects wore woolen or denim battle dress covered with cotton coveralls, boots, and gaiters. ECG electrodes were applied to the chest, and subjects wore a full respirator into the chamber. For the commencement of exposure, each subject removed his own respirator. During each exposure, each subject completed two 8-minute periods of exercise which consisted of cycling at 20W up to 120 W. During exercise, the subjects breathed through an oro-nasal mask and three-way valve box. Inspiration was from the chamber and expiration was through a 6 L capacity mixing bottle into a low resistance gas meter. Cardiac frequency was measured using the electrocardiograph, while a thermister in the valve box recorded respiratory frequency. A control exposure including exercise was conducted the day before and the day after the exposure to CS. A major difference between the control and CS exposures was that ventilation was continued throughout the control session but not the CS exposure session; therefore, the temperature was much higher in the CS exposure sessions compared to the controls (~24° C vs. 20.5° for controls). When first exposed to the CS aerosol, all subjects experienced intense discomfort including cough, lacrimation, and substernal pain. Discomfort was severe enough that two subjects withdrew (one before and one after the first period of exercise), and two additional subjects were unable to complete the first period of exercise due to coughing. It was noted that the coughing coincided with ignition of the CS generators. The discomfort disappeared with continuing exposure. Although cardiac frequency was increased during exposure to CS compared to control air, the difference was eliminated when the cardiac frequency was corrected for the increased ambient temperature (corrected to the arbitrary temperature of 20°C). The ventilation minute volume was reduced from exposure to CS compared to controls. The reduction appeared to be due to a decrease in respiratory frequency. The exposure was repeated using 17 volunteers (Cole et al., 1977; Cole et al., 1975). Exposure conditions were the same with the following exceptions: the CS candles were ignited between and not during periods of exercise, exposure concentrations were slightly higher (0.92 to 2.15 mg/m^3), and the subjects were seen on five consecutive half days sessions of which the first, third, and fifth sessions were for control observations, and the other two sessions were allocated one each for exposure to ammonia and to CS (the order of exposure changed between the

different weeks of the study). Results were generally identical to those observed in the first study. The only difference was that the reduction in the ventilation minute volume was the result of a diminution in tidal volume and occurred despite an increase in respiratory frequency.

To investigate the potential for the development of tolerance to CS, a group of 35 healthy male volunteers was exposed for 60 minutes to increasing concentrations of CS aerosol (Beswick et al., 1972). Exposures were conducted in a 100 m^3 chamber. The chamber was generally saturated an hour before the exposure, followed by air being blown through the chamber to remove the CS not absorbed on the walls and equipment. A number of parameters were assessed before and after exposure, including: a complete medical examination including a chest radiograph, collection of blood for hematology and clinical chemistry analysis, and respiratory function tests to assess peak flow, tidal volume, and vital capacity. A total of 10 different exposure trials were conducted, with no volunteers exposed twice. The exposure concentration was kept relatively constant for the first three trials (0.56-0.86 mg/m^3; 0.71-0.78 mg/m^3; 0.31-0.74 mg/m^3, respectively). For the seven remaining trials, exposure concentrations were increased by a factor of 2, 3, or 4 during the exposure period, with the highest ending concentration being 2.3 mg/m^3 (concentration ranges for the 7 trials were: 0.8-1.4; 0.84-2.3; 0.7-2; 0.63-2.3; 0.57-2.1; 0.42-1.8; and 0.45-1.7 mg/m^3). Chamber concentrations were measured at 10 minute intervals. For the exposures, two to eight volunteers entered the chamber wearing full respirators and protective coveralls. CS was generated and allowed to mix for 3 minutes before removal of the respirator. Symptoms from all volunteers were reported during individual interviews after exposure. One subject left the exposure chamber after 8 minutes of exposure with complaints of severe stinging of the eyes, throat irritation, cough and dyspnea, salivation, and nausea, while another left at 55 minutes of exposure due to vomiting. All other subjects remained in the chamber for the entire 60 minute exposure period. A table summarizing the symptoms of the exposed individuals is presented in Table 3. The predominant symptoms of exposure included salivation, eye irritation (stinging, watering), runny nose, and face stinging. Symptoms generally resolved within 10 minutes of leaving the chamber. To assess the development of tolerance, two of the trials (group IV in Table 3) consisted of four subjects that removed the respirator at the start of the exposure (with the CS concentration increasing with time), while the remaining four subjects did not remove the respirator until the last 5 minutes of exposure. The subjects that were exposed to CS throughout the entire exposure period were able to withstand the entire 60 minute exposure (concentrations increasing from 0.84-2.30 and 0.70-2.00 mg/m^3) except for the one individual that had to leave the chamber at 55 minutes due to vomiting. Of the subjects that removed their respirators the last 5 minutes of exposure, only one of eight subjects could remain in the chamber for more than one minute; five left within 30 seconds of removing their respirators. No exposure-related changes were observed in hematology or clinical chemistry parameters. Decreases in heart rate after exposure ceased were ascribed to the sense of relief each volunteer felt at the finish of an uncomfortable experience, and the increase in systolic blood pressure observed in individuals when exposure commenced was due to the abrupt onset of discomfort; continued exposure resulted in normal blood pressure readings. No abnormalities were noted in measurements of respiratory functions, but it was noted by the author that the sample size was small and thus may not be representative. It was concluded that the main effects of CS are due to local irritation of exposed nerve endings, and any systemic changes noted are due to stress.

TABLE 3. Symptoms of 34 volunteers exposed to CS aerosol for 60 minutes

Symptoms	Grouping of subjects for assessment of symptoms [a] (nominal fold increase in concentration during exposure)						
	I (steady)	II (x 2)	III (x 2)	IV (x 3) [b]	V (x 4)	Total	Notes
Number exposed	5	8	5	8	8	34	-
Eyes: Stinging	5	8	4	7	8	32	No connection between severity and conc., but duration may be less with constant conc.
Watering	5	8	5	7	7	32	
Nose: Stinging	3	4	1	6	4	18	Effects in nose generally diminished in subjects except those exposed to CS conc. increasing 3- or 4-fold during exposure period
Running	3	7	3	8	7	28	
Peppery feeling	2	4	2	4	5	17	
Blocked	1	3	2	3	2	11	
Mouth: Irritation	2	4	-	6	3	15	Copious production so severe that when subject was spitting, appeared to be vomiting
Salivation	5	8	5	8	8	34	
Throat: Irritation	4	5	3	6	5	23	-
Dry	-	1	-	6	1	8	
Chest: Burning	2	2	-	3	1	8	More severe effects appeared to be consequent upon deep breaths which all men who held their breath were eventually forced to take; coughing was generally sporadic
Tight	1	3	2	2	3	11	
Dyspnea	-	2	2	2	3	9	
Cough	5	2	4	3	4	18	
Nausea	2	3	1	3	2	11	Likely due to swallowing of large quantity of saliva; 1 subject in Group V vomited within first 5 min and left chamber but returned to chamber for duration of exposure; the subject in Group IV vomited at 55 min of exposure
Vomiting (during exposure)	-	-	-	1	1	2	
Face stinging	5	7	5	7	8	32	Appeared to be of shorter duration when CS conc. remained constant; appeared to be most unpleasant in shaved regions
Headache	2	1	2	1	-	6	3 persisted throughout exposure; 3 cases occurred post-exposure; appeared to be due to irritation of the frontal sinuses

Data taken from Beswick et al., 1972

[a] I. 0.78-0.77 mg/m^3 (5 exposed)
II. 0.56-0.86 mg/m^3 (3 exposed); 0.31-0.74 mg/m^3 (5 exposed; One volunteer left exposure at 8 min. and is not included)
III. 0.8-1.4 mg/m^3 (5 exposed)
IV. 0.84-2.3 mg/m^3 (4 exposed); 0.7-2.0 mg/m^3 (4 exposed)
V. 0.63-2.3 mg/m^3 (2 exposed); 0.57-2.1 mg/m^3 (2 exposed); 0.42-1.8 mg/m^3 (2 exposed); 0.45-1.7 mg/m^3 (2 exposed)

[b] To assess development of tolerance, 4 subjects removed respirators at start of exposure, while the other four removed respirators at the end; the subjects that removed respirators at beginning of exposure were able to withstand the entire 60 min. exposure except for one individual that had to leave the chamber at 55 minutes due to vomiting. Of the subjects that removed their respirators the last 5 minutes of exposure, only 1 of 8 could remain in the chamber for more than 1 min., 5 five left within 30 sec. of removing their respirators.

Three groups of volunteers were exposed to various concentrations of CS aerosol to investigate any potential effects of CS exposure on visual acuity (Rengstorff, 1969). The first exposure comprised a group of 10 male volunteers exposed to CS-2 aerosol (CS treated with Cab-o-sil 5 and hexamethyldisilaxane) at concentrations of 0.1 to 1.7 mg/m^3. The exposure was conducted in a wind tunnel suspended 4.5 feet above the floor; the volunteer sat on a chair at the end of the wind tunnel and put his head through a rubber aperture in the tunnel until he could no longer tolerate the exposure or for a maximum of 10 minutes. A powder dispenser disseminated specific concentrations of CS-2 at a MMD of 0.8 microns into air at a wind speed of 4.5 mph. An Orthorater was used to measure the binocular far and near visual acuity of the subjects before and after exposure. The second and third exposures were to CS aerosol and were conducted in a

circular steel chamber. CS aerosol (MMD of 0.9 micron) was disseminated from methylene dichloride solution using a thermal generator, and introduced into the chamber as a uniform cloud. Subjects wore protective masks for the first five minutes they were in the chamber, and then removed their masks for the commencement of exposure. The second exposure was comprised of 34 volunteers, and an Orthorater was again used to measure the binocular far and near visual acuity before and after exposure. A summary of the amount of time volunteers from the Exposure 2 group could tolerate exposure to CS is provided in Table 4. The third exposure comprised 22 volunteers who had a baseline acuity of 20/20 and who could remain in the exposure chamber for 10 minutes. Binocular acuity was measured using a Snellen visual acuity projector before, during, and a few minutes after exposure. The Snellen chart contained a row of 20/30, 20/25, and 20/20 letters. No exposure-related changes in visual acuity were noted from the exposures except those due to the inability of some subjects to keep their eyes open due to the intense eye irritation. Visual acuity returned to normal for all subjects several minutes after exposure to CS.

TABLE 4. Summary of exposure time of volunteers: subjects exposed to CS until they could no longer tolerate the exposure or for a maximum of 10 minutes

Concentration (mg/m^3)	Exposure time in seconds (number of volunteers)
0.4	135 (1) 420 (1) 435 (1) 600 (4)
0.6	30 (1) 35 (2) 38 (1) 40 (1) 65 (1) 68 (1) 102 (1) 105 (1) 135 (1) 600 (7)
0.9	600 (6)
1.0	35 (1) 40 (1) 45 (1) 50 (1)

Data taken from Rengstorff, 1969

To assess the effect of CS exposure on respiration, a group of six volunteers (four familiar with CS exposure) were exposed to various concentrations of CS (3 micron) in a wind tunnel while a portable breathing device monitored respiration (Craig et al., 1960). The subjects remained in the tunnel until the exposure became intolerable (see Table 5). Notable coughing was observed in the subjects exposed to 15 mg/m^3 for 61 seconds or to 150 mg/m^3 for 12 seconds. Based on the recordings made during exposure, it was concluded that although the breathing pattern of the volunteers was disrupted, adequate ventilation was maintained. Therefore, the incapacitation of CS is attributed to the unpleasant sensations of exposure rather than to any degree of respiratory failure.

TABLE 5. Summary of exposure time of volunteers: subjects exposed to CS until they

could no longer tolerate the exposure	
Concentration (mg/m^3)	**Exposure time in seconds**
5	110 +
12	24
15	61 +
64	15 +
80	12
150	12 +

Data taken from Craig et al., 1960
+ Previous experience with CS exposure

A group of 38 U.S. Marines was exposed to a cloud of CS dispersed by a thermal canister as part of a training exercise to test the ability and speed of the trainees in donning their gas masks (Thomas et al., 2002). The exposure occurred after six days of strenuous training with minimal sleep and reduced food consumption, and was followed by a 1.5 mile run. Temperature and relative humidity at the time of exposure were approximately 24°C and 91%, respectively. Clinical signs and symptoms began to develop 36-84 hours post exposure during and after periods of strenuous exercise (one became symptomatic after a 1,000-m pool swim at 36 hours post exposure; seven became symptomatic after a second swim consisting of a 1000-m open ocean swim 60 hours post exposure, and one became symptomatic after a third swimming event of a 1500-m open ocean swim 84 hours post exposure). A total of nine Marines were affected, with four Marines requiring admission into intensive care. Effects of exposure included dyspnea upon exertion, hemopytosis (ranging from frank blood to blood-tinged sputum), cough, rales, reduced arterial blood gas (range of 60-68), and infiltrates visible on chest radiograph. Signs and symptoms had resolved by 72 hours, and lung function before and after exercise challenge had returned to normal one week post exposure. An approximate recreation of the exposure with CS concentrations estimated by air sampling at the same location revealed CS concentrations ranging from less than quantifiable up to approximately 17 mg/m^3.

McDonald and Mahon (2002) propose that the pulmonary symptoms in the Marines described above by Thomas et al. (2002) were not the result of CS exposure, but rather the result of water aspiration or swimming induced pulmonary edema (SIPE). The conclusions were based on the fact that all became symptomatic immediately after swimming, there was a rapid resolution of symptoms, and there was no evidence of airway dysfunction. Delayed pulmonary effects of CS exposure are unusual, and there were no other reports of such symptoms in Marines even though approximately 200,000 Marines have been exposed to CS since 1996 under similar field conditions.

2.2.2. Case reports

The effects of exposure to CS are generally of an acute nature. However, reactive airways dysfunction syndrome (RADS) was reported in two individuals exposed to CS. One case involved a healthy 21-year-old female exposed for 5-10 minutes to CS smoke at a nightclub (Hu and Christiani, 1992). Immediately following exposure, she exhibited the typical signs and symptoms of CS exposure including tightness and burning in her chest and coughing. Physical examination at a hospital and chest radiography were normal, and she was released. She continued to experience coughing and shortness of breath, and by 4 weeks post exposure, she

had reduced forced expiratory volume in 1 second (FEV_1; 68% of predicted) and forced vital capacity (78%). Cough and shortness of breath were still present during the 2-year follow-up, and were made worse by exertion, cold air, and some environmental pollutants. The second case report described exposure to a riot-control agent containing 1% CS and 1% oleo resin capsicum; effects of exposure to the capsicum cannot be excluded (Roth and Franzblau, 1996). A healthy 53-year-old male was exposed for at least 30 seconds, and immediately experienced symptoms of mucous membrane irritation, cough, and chest tightness. Wheezing and shortness of breath continued for months after exposure, and were severe enough to require hospitalization. Pulmonary function test results indicated reversible and fixed obstructive pulmonary disease.

A 4-month-old infant exposed to CS for 2-3 hours developed pneumonitis and persistent leukocytosis (Park and Giammona, 1972). The infant was exposed when a CS tear gas canister was fired into a house to subdue an adult. Upon hospitalization, the infant had copious nasal and oral secretions and was sneezing and coughing. A chest X-ray demonstrated that the lungs were clear, but laboratory testing revealed leukocytosis. The infant developed severe respiratory distress by the second day of hospitalization, with pulmonary infiltrates evident on X-ray by day 7. The pulmonary infiltration began to decrease on day 15, and the lungs were clear on day 17. White blood cell counts were elevated throughout hospitalization, finally decreasing when the patient was discharged from the hospital.

CS is a common riot-control agent in Britain, and there are consequently reports that describe typical symptoms following exposure to CS in a confined space, such as a night club (Breakell and Bodiwal, 1998) or bus (Karagama et al., 2003), use by police on individuals for self defense (Euripidou et al., 2004), or under conditions of large-scale riot control (Anderson et al., 1996; Himsworth, 1969). Symptoms of exposure included but were not limited to: eye irritation, lacrimation, blurred vision, burning sensations sometimes accompanied by first degree burns, cough, headache, shortness of breath, chest pain, sore throat, retching, vomiting, and salivation (Breakell and Bodiwal, 1998; Karagama et al., 2003; Euripidou et al., 2004; Anderson et al., 1996; Himsworth, 1969). In general, the symptoms resolved rapidly; however, there were reports of effects lasting longer that that predicted. It is noted that the hand-held spray canisters used by the police contain CS dissolved in methyl isobutyl ketone, an industrial solvent and denaturant (Euripiou et al., 2004; Gray, 2000). It has therefore been proposed that the ketone combined with the CS may result in more long lasting adverse effects than CS preparations not containing the solvent.

2.3. Developmental/Reproductive Toxicity

The National Teratology Information Service collected outcome data on 30 pregnant women who were exposed to CS gas: 12 women during the first trimester, 11 during the second trimester, and 7 during the third trimester (McElhatton et al., 2004). Acute maternal toxicity (transient symptoms of ear, nose, and throat irritation) was noted by 50, 82, and 57% of the exposed women, respectively. Pregnancy outcome was not adversely affected by exposure. Birth weight was within the normal range except for one female baby weighing less than 2500 g. Only one infant had a congenital anomaly (hypospadia), and this anomaly has a background incidence of 1 in 1000 live born male infants. No concentration or duration exposure parameters were described.

2.4. Genotoxicity

No data were located.

2.5. Summary

CS is a potent irritant, with symptoms of exposure including lacrimation, blepharospasm, erythema of the eyelids, chest tightness, coughing, nasal irritation and discharge, salivation, throat irritation, nausea, vomiting (from swallowing excess saliva), and cutaneous irritation (ranging from stinging to contact irritation or allergic dermatitis). It is reported that an aerosol concentration of 4 mg/m^3 will disperse the majority of rioters within 1 minute, and 10 mg/m^3 will deter trained troops (Upshall, 1973). With the exception of more severe cutaneous reactions, recovery from exposure is generally rapid upon exposure to fresh air, generally within 30 minutes after exposure (Ballantyne, 1977).

Quantitative human inhalation exposure data showing exposure time to intolerance are summarized in Table 6. Many studies investigated the time to intolerance, which is the time at which a subject could no longer remain in the atmosphere containing CS and had to leave the exposure. Tolerance ranged from 5 seconds at 442 mg/m^3 to 12-25 seconds at 141 mg/m^3 to 9 seconds at 39 mg/m^3 to > 90 minutes at 1.5 mg/m^3 (Punte et al., 1963; Gutentag et al., 1960). Tolerance to low concentrations of CS could be increased when exposure occurred under conditions in which the exposure concentration was increased over time (Punte et al., 1963; Beswick et al., 1972). A study investigating the differences in respiratory and ocular responses to different particulate sizes of CS found that small particles are more effective in producing eye and respiratory irritation, while recovery time from ocular irritation was greater for large particles (due to the delay in the onset of irritation for the large particles due to the slowness of the large particle solubility), and recovery time from respiratory irritation was greater for small particles (smaller sized particles can penetrate further into the respiratory tract) (Owens and Punte et al., 1963).

Pregnancy outcome was not affected in a prospective case study of 30 pregnant women who were exposed to CS gas and experienced transient symptoms of ear, nose, and throat irritation (McElhatton et al., 2004). No other reproductive or developmental toxicity data in humans were available. Data were not available on repeat-exposure toxicity, genotoxicity, or carcinogenicity.

TABLE 6. Summary of selected human acute inhalation toxicity/intolerance data

Concentration mg/m^3	Time to Intolerance (sec) [a]	Notes	Reference
94 [b] 85 [c]	< 60 < 60	6 subjects chosen for ability to tolerate CS	Owens and Punte, 1963
5 12 442	53 to ≥ 120 19-43 5	Untrained subjects; duration of exposure was maximum of 2 min.	Punte et al., 1963; Gutentag et al., 1960
4 10 141	37 to ≥ 120 18-41 12-25	Trained subjects (previous exposure to CS); duration of exposure was maximum of 2 min.	Punte et al., 1963; Gutentag et al., 1960
10 13 39	13 13 9	Untrained subjects; exercised before exposure	Punte et al., 1963; Gutentag et al., 1960
0.4 0.6 0.9 1	135 – 600 [e] 30-600 [e] 600 [e] 35-50	4 of 7 tolerated 10 min 7 of 17 tolerated 10 min 6 of 6 tolerated 10 min	Rengstorff, 1969
6 (attained over 10 min)	18 min 20 min 29 min	1 subject 1 subject 1 subject	Punte et al., 1963
0.78	60 min [f]	5 subjects: all remained in chamber for duration of exposure; tolerable but caused eye, nose, mouth, and throat irritation, nausea, chest discomfort, headache, and stinging of the face	Beswick et al., 1972
0.56-0.86 0.31-0.74	8 min 60 min [f]	9 subjects; conc. ↑ during exp.; 1 subject in 0.31-0.74 group left at 8 min (irritation); 8 subjects tolerated 60 min. exp. w/same signs as 0.78 group	
0.8-1.4	60 min [f]	5 subjects; all tolerated exposure w/ same signs as 0.78 group	
0.84-2.3 0.7-2.0 0.63-2.3 0.57-2.1 0.42-1.8 0.45-1.7	60 min [f]	16 subjects 1 subject vomited at 5 min, left chamber but returned for duration of exposure; 1 subject vomited at 55 min 14 subjects tolerated 60 min. exp. w/same signs as 0.78 group	
1.5	90 min [g]	Of 4 subjects exposed: 1 developed nose irritation (2 min. into exposure) 3 developed a headache (at 45, 50, and 83 min.) 4 had eye irritation (at 20, 24, 70, and 75 min.)	Punte et al., 1963

[a] Time at which the subject could no longer tolerate the exposure; duration given in seconds unless otherwise noted
[b] MMAD of 0.9 microns
[c] MMAD of 60 microns
[e] Exposure was for maximum of 10 minutes
[f] Exposure was for maximum of 60 minutes
[g] Exposure was for maximum of 90 minutes

3. ANIMAL TOXICITY DATA

3.1. Acute Lethality

3.1.1. Monkeys

Groups of eight immature male and female *Macaca mulatta* monkeys (3-4 kg) were exposed to a cloud of CS dispersed via an M7A3 CS grenade in a 20,000 L chamber at an average CS concentration of 900 mg/m^3 for 3 minutes, 1700 mg/m^3 for 5 minutes, 2850 mg/m^3 for 10

minutes, or 2500 mg/m^3 for 32 minutes (Striker et al., 1967). It was stated that the cloud was sampled and measured at various times, but further details were not provided. A group of eight monkeys served as controls; they were treated similarly to the exposed monkeys except they were not put into an exposure chamber. Monkeys were observed frequently for clinical signs during the first 72 hours after exposure. Chest radiographs were taken before exposure and at 2, 6, or 12 hours or 1, 3, 7, or 30 days post exposure. Monkeys were sacrificed at 12 hours or 3, 7, or 30 days after exposure. Clinical signs in monkeys exposed to 900 mg/m^3 for 3 minutes or 1700 mg/m^3 for 5 minutes were limited to blinking and a "fright reaction" noted immediately upon removal from the exposure chamber, quickly disappearing within a few minutes after the monkeys were moved to fresh air. Monkeys exposed to 2850 mg/m^3 for 10 minutes exhibited frequent blinking, labored respiration, coughing, oral and nasal discharge, occasional vomiting, and decreased activity and response to external stimuli, with one monkey additionally having copious eye discharge. The clinical signs were most severe by 12 hours post exposure and were generally resolved by 72 hours post exposure. Clinical signs in monkeys exposed to 2500 mg/m^3 for 30 minutes were severe and included prostration, dyspnea, copious oral and nasal discharge, and scleral congestion upon removal from the exposure chamber. A total of five monkeys died: four died three to twelve hours post exposure, and one died at 4 days post exposure. Dyspnea was most severe at 12 hours, while oral and nasal discharge and effects on the eyes were most severe by 24 hours post exposure. Radiographic findings were present only in this group, and included infiltrates that occurred by 3 hours post exposure but were most severe by 24 hours post exposure, and had cleared by 3 days post exposure.

Pathological examination of the monkeys exposed to 900 or 1700 mg/m^3 revealed mild pulmonary congestion, bronchorrhea, emphysema, and atelectasis within 12 hours post exposure, a disappearance of these changes at 72 hours post exposure, followed by a recurrence at 7 days and 30 days post exposure (Striker et al., 1967). Pathological lesions in monkeys exposed to 2850 mg/m^3 for 10 minutes were more severe and developed earlier. Pulmonary edema and congestion and bronchorrhea were present at 12 hours post exposure, progressing to purulent bronchitis and bronchopneumonia at 72 hours post exposure. At one week post exposure, acute pleuritis and interstitial pneumonitis were seen, while mucosal lesions and bronchopneumonia were resolving. Lesions were still present at 4 weeks post exposure, and included emphysema, atelectasis, and focal interstitial pneumonitis. In the 2500 mg/m^3 for 30 minute group, pathological examination of monkeys that died revealed severe pulmonary edema and congestion. Of the three surviving monkeys, one monkey each was sacrificed at 3, 7, or 30 days post exposure. The monkey at 3 days post exposure had considerable edema, but congestion was less prominent. At 7 days post exposure, emphysema involving all lobes and bronchiolitis were observed, but most of the edema had cleared. The monkey surviving to 30 days post exposure had small shrunken lungs, purulent mucoid material filling many small bronchioles, and distinct bronchiolitis.

McNamara et al. (1969) exposed groups of four monkeys (strain and sex not reported) to 7 different CS concentration-duration combinations. No further experimental details were available. Mortality data are summarized in Table 7.

3.1.2. Rats

Groups of ten rats were exposed to an aerosol of CS for exposure durations of 25-90 minutes (Punte et al., 1962). Animals were exposed in a dynamic inhalation chamber containing individual cages on racks. Aerosol was generated by passing dry nitrogen through an aspirator. Molten CS was maintained in a side-armed flask in an oil bath at 140-150°C. The aerosol was easily generated and liquid droplets recrystalized before entering the exposure chamber. Chamber concentrations were measured by drawing chamber air through filter paper for subsequent analysis by spectrophotometry. Samples for particle size determinations were collected by a Cascade impactor, and mass-median diameter was derived by use of stage calibrations based on the density of the compound; the particle size was about 1.5 microns (mass median diameter). Observations for clinical signs were made during and after exposure. Surviving animals were maintained for 14 days post exposure, at which time they were killed and subjected to histopathological examination. Immediately after the commencement of exposure, the animals became excitable and hyperactive, and lacrimation and salivation occurred within 30 seconds. Lethargy and dyspnea occurred after approximately 5-15 minutes of exposure. Dyspnea persisted for approximately an hour after exposure ceased, while all other signs subsided about 5 minutes after removal from the chamber. Histopathological examination revealed an increase in the number of Goblet cells in the respiratory tract and conjunctiva, necrosis in the respiratory and gastrointestinal tracts only if particles had impacted the surface, and an occasional animal with pulmonary edema and hemorrhage in the adrenal glands. The calculated LCT_{50} is 32,500 mg min/m^3. An unpublished report by McNamara et al. (1969) appears to provide data additional to those that have been published. Specific study details are not provided in this report, but one set of study results is consistent with those published by Punte et al. (1962). The report includes the mortality results of additional animal species exposed by inhalation to CS, as well as mortality data for CS dispersed by various methods. As discussed above, Punte et al. (1962) reported mortality data for rats, but the values were reported only in terms of mg min/m^3. Specific concentrations of CS (sprayed as molten agent) with corresponding exposure durations for these data are reported in McNamara et al. (1969) and are presented in Table 7.

Groups of 18 male albino SPF rats were exposed to pyrotechnically generated CS smoke in a 10 m^3 chamber (Colgrave and Creasey, 1975). The rats were exposed to 5871 ± 476 mg/m^3 of CS for 15 minutes, 6030 ± 590 mg/m^3 for 10 minutes, or to 6800 ± 1166 mg/m^3 for 5 minutes (averages and standard deviations calculated using data reported by Colgrave and Creasey; values were reported by authors as 6000, 6000, and 6400 mg/m^3, respectively). The CS was released from 4 four CS cartridges, each containing 12.5 g CS, 16 g potassium chlorate, 15 g lactose, and 7.5 g kaolin. The cloud of CS in the exposure chamber was sampled at approximately one minute intervals for the 10 and 15 minute exposures, and at 30 second intervals during the 5 minute exposure. The analytical method used to measure CS concentrations was not described. Survivors were killed at times ranging from 15 minutes to 2 days post exposure. All animals were necropsied, and selected tissues were analyzed by both light and electron microscope. Non-exposed controls served to establish the typical macroscopic and microscopic appearance of the particular strain used. Mortality occurred in 4 rats exposed to 5871 mg/m^3 for 15 minutes (death occurred by 24 hours post exposure), and in 2 rats exposed to 6030 mg/m^3 for 10 minutes (death occurred by 24 and 36 hours post exposure). All animals exposed to 6800 mg/m^3 survived to study termination at 2 days post exposure. Animals that died

following exposure to CS for 15 minutes developed marked pulmonary congestion with scattered alveolar hemorrhages and patchy edema. Survivors developed less marked pulmonary congestion and only occasional areas of edema and hemorrhaging. Rats that died following exposure to CS for 10 minutes also developed pulmonary congestion, but the severity was much less than that seen after the 15 minute exposure. Hemorrhages and edema were occasionally seen in the lungs of survivors. Examination of rats exposed to CS for 5 minutes revealed mild pulmonary congestion with occasional hemorrhage up to six hours post exposure. Rats killed at 12 hours to 2 days post exposure had no pulmonary findings except for one rat with moderate and extensive pulmonary congestion. Electron microscopic examination of the lungs from all exposed rats revealed changes in the epithelium and interstitium, with accumulation of fluid between the membrane layers and collagen-containing areas of the septum. Degenerative changes of the epithelium and endothelium led to rupture or dissolution of the capillary wall. The authors stated that the changes were similar in all exposed rats, with the changes varying only in the degree of severity. Damage was evident as early as 15 minutes post exposure, becoming more severe by 30 and 60 minutes post exposure.

Ballantyne and Callaway (1972), exposed groups of male and female Wistar-derived SPF rats to pyrotechnically generated CS smoke at a concentration of 750 mg/m^3 for 30 minutes, 480 mg/m^3 for 1 hour, or 150 mg/m^3 for 2 hours in a 10 m^3 exposure chamber. A group of control animals was also maintained, but no description of the treatment of the controls was provided (i.e., if they were exposed under similar conditions to clean air). The grenades used for the exposure contained CS (2 g), potassium chlorate (2.4 g), lactose (2.4 g), and kaolin (1.2 g). Although it was stated that the concentration of CS in the exposure chamber was sampled at the start of the exposure and at 6-minute intervals up to and including 57 minutes, no information was provided regarding the analytical technique. Groups of animals were sacrificed at 1, 10, and 28 or 29 days post exposure. Additionally, some of the animals exposed to 480 or 150 mg/m^3 were retained for up to 32 months to evaluate potential lasting toxicity and pathology (Marrs et al., 1983a). Animals that died or were sacrificed moribund after one month post exposure and those sacrificed at the termination of the study at 32 months were subjected to gross necropsy, and the heart, lungs, small intestine, liver, pancreas, spleen, kidneys, brain, gonads, and pituitary and adrenal glands were removed and processed for histological examination.

All animals exposed for 30 minutes to 750 mg/m^3 survived to the scheduled necropsy, and histopathological changes were observed only on post exposure day 1 (Table 8). One rat exhibited congestion of alveolar capillaries and a few scattered alveolar hemorrhages, while another rat had a few minute foci of renal tubular necrosis at the inner cortex. No pathological changes were noted in rats at post exposure day 10 or 28. Exposure to 480 mg/m^3 for one hour resulted in the mortality of some rats (Table 10), with the majority of the mortalities occurring on post-exposure days 1-2. Pathological changes in animals surviving exposure to 480 mg/m^3 were generally confined to post exposure day 1. Lesions in rats were limited to minimal pulmonary congestion and hepatic congestion in one rat, minimal pulmonary hemorrhage and hepatic necrosis in another rat, and mild pulmonary congestion in a third rat. Two rats showed mild pulmonary edema. A few rats killed at 10 days had healed lesions as evidenced by binucleate liver cells around centrilobular veins and immature epithelium in some renal tubules. No abnormal pathological changes were noted at day 29. Histopathological findings in rats that died were much more severe and included renal changes (mild to moderate necrosis of the cortex, moderate to severe necrosis of medulla, and some mild congestion); pulmonary changes

(mild to severe congestion, mild hemorrhage, and some mild edema) and hepatic changes (a few rats with mild congestion and mild to moderate necrosis; all rats had livers depleted of glycogen).

Exposure to 150 mg/m^3 for 2 hours resulted in no mortality. Pathological examination of surviving animals revealed lesions only on Day 1 post exposure. Lesions were confined to female animals and consisted of one rat with a few scattered alveolar hemorrhages, one rat with acute mucoid enteritis, and one rat with pneumonic consolidation of the right upper lung lobe.

Exposure to 480 mg/m^3 for 1 hour or to 150 mg/m^3 for 2 hours did not affect the lifespan of the rats, and no statistically significant increases were noted in non-neoplastic lesions in the exposed groups compared to controls (Marrs et al., 1983a). Common non-neoplastic lesions in male and female rats included changes in the lungs (engorgement, congestion, and inflammatory changes, pulmonary edema) and pyelonephritis of the kidney. Liver congestion was also a common finding. No exposure-related neoplastic lesions were evident in male rats. Female rats in the 150 mg/m^3 group exhibited an increased incidence of pituitary tumors; incidence was 26% for controls, 29% for 480 mg/m^3 for 1 hour, and 47% for 150 mg/m^3 for 2 hours. The increase was not statistically significant.

In another experiment, Ballantyne and Callaway (1972) exposed groups of 10 rats for 5 to 20 minutes to an approximate CS concentration of 4000 mg/m^3, followed by a 14-day observation period. An anti-riot grenade containing approximately 50 g of CS was ignited in a 10 m^3 static chamber and allowed to burn to completion. All animals that died and the survivors killed at the end of the 14-day post exposure period were subjected to gross and histological examination. Clinical signs during exposure could not be recorded because the aerosol generated in the chamber resulted in a complete lack of visibility. Upon removal from the chamber, animals exhibited signs of increased buccal and nasal secretion and dyspnea, particularly at the longer exposure durations. Mortality data are summarized in Table 9. No animals died during exposure. Necropsy of animals that died post-exposure revealed pulmonary edema and congestion, often with multiple, variable sized areas of hemorrhage, and the presence of mucus in the trachea and major bronchi. Histopathological examination of these animals revealed severe congestion of the alveolar capillaries and intrapulmonary veins and alveolar hemorrhage. Mucus was seen in some bronchi and bronchioles, and occasional areas of collapse and hemorrhage were seen distal to a completely occluded bronchiole. Moderate to marked pulmonary edema was also observed in several animals. No evidence of acute inflammatory cell infiltrate was observed in any of the lungs examined, suggesting that the CS aerosol produced direct injury to the pulmonary capillary endothelium. Circulatory failure evidenced as congestion of the liver, kidney, and spleen and dilation of the right ventricle was present in most of the animals that died. Animals that survived to 14 days post exposure did not have any residual pathology at necropsy.

Groups of twenty or twenty-one male Porton-Wistar rats were exposed by whole body inhalation to various concentrations of CS aerosol for durations of 10- to 60-minutes (Table 10) (Ballantyne and Swantson, 1978). Animals were exposed in a 1 m^3 dynamic flow chamber. The aerosol was generated by filling a Collision spray with molten CS (heated to 150ºC) and passing pure nitrogen into the air stream. The resultant aerosol was fed into the diluting air stream. Chamber atmosphere was sampled for one minute at five minute intervals by aspirating air through glass fiber discs held in double cone filters. A bubbler containing hydrochloric acid in

ethanol was connected in line to the glass filter to act as an additional trap. The contents of the bubbler were used to elute CS from the filter discs, and the concentration of CS in the resultant extract was measured by absorption spectrophotometry and compared against a prepared standard. Signs of toxicity included increased nasal and buccal secretions and increased rates of respiration upon removal from the chamber, which disappeared within approximately one hour post exposure. No animals died during exposure; deaths generally occurred within the first two days following exposure. A summary of mortality data is presented in Table 10. Necropsy findings in animals dying within 48 hours post exposure included pulmonary congestion and edema (with some animals also having multiple variable sized hemorrhages) and congestion of the trachea. Moderate amounts of mucus were also seen in the trachea. Histopathological examination of the lungs from these animals revealed moderate to marked congestion, inter- and intra-alveolar hemorrhaging, and excess secretions in the bronchioles and intrapulmonary bronchi. Examination of animals dying after 48 hours revealed similar findings with additional findings of early bronchopneumonia. Congestion of the liver, kidney, spleen, and small intestines were also frequently seen in animals dying from exposure. No abnormal findings were noted in animals surviving 14 days post exposure.

3.1.3. Mice

Groups of twenty mice were exposed to an aerosol of CS for exposure durations of 10-60 minutes (Punte et al., 1962). Experimental procedures, clinical signs, and necropsy results are similar to those described for rats in Section 3.1.2. The calculated LCT_{50} is 43,500 mg min/m^3. An unpublished report by McNamara et al. (1969) appears to provide data additional to those that have been published. Specific study details are not provided in this report, but one set of study results is consistent with those published by Punte et al. (1962). The report includes the mortality results of additional animal species exposed by inhalation to CS, as well as mortality data for CS dispersed by various methods. As described above, Punte et al. (1962) reported mortality data for mice, but the values were reported only in terms of mg min/m^3. Specific concentrations of CS (sprayed as molten agent) with corresponding exposure durations for these data are reported in McNamara et al. (1969) and are presented in Table 7.

Ballantyne and Callaway (1972) exposed groups of 10 mice for 5 to 20 minutes to an approximate CS concentration of 4000 mg/m^3, followed by a 14-day observation period. The experimental protocol, clinical signs, and necropsy findings are similar to those described in the rat study (Section 3.1.2). Mortality data are summarized in Table 9.

Groups of nineteen to forty male albino mice were exposed by whole body inhalation to various concentrations of CS aerosol for durations of 15- to 30-minutes (Table 10) (Ballantyne and Swantson, 1978). Experimental protocol, clinical signs, and necropsy findings are as described for the rat study in Section 3.1.2. Mortality data are summarized in Table 10.

3.1.4. Guinea Pigs

Groups of ten guinea pigs were exposed to an aerosol of CS for exposure durations of 5-40 minutes (Punte et al., 1962). Experimental procedures, clinical signs, and necropsy results are similar to those described for rats in Section 3.1. 2. The calculated LCT_{50} is 8,300 mg min/m^3. An unpublished report by McNamara et al. (1969) appears to provide data additional to those that have been published. Specific study details are not provided in this report, but one set of study results is consistent with those published by Punte et al. (1962). The report includes the mortality results of additional animal species exposed by inhalation to CS, as well as mortality data for CS dispersed by various methods. As described above, Punte et al. (1962) reported mortality data for guinea pigs, but the values were reported only in terms of mg min/m^3. Specific concentrations of CS (sprayed as molten agent) with corresponding exposure durations for these data are reported in McNamara et al. (1969) and are presented in Table 7.

Ballantyne and Callaway (1972) exposed groups of five guinea pigs for 5 to 20 minutes to an approximate CS concentration of 4000 mg/m^3, followed by a 14-day observation period. The experimental protocol, clinical signs, and necropsy findings are similar to those described in the rat study (Section 3.1.2). Mortality data are summarized in Table 9.

Groups of ten to twenty female Dunkin Hartley guinea pigs were exposed by whole body inhalation to various concentrations of CS aerosol for durations of 10- to 45-minutes (Table 10) (Ballantyne and Swantson, 1978). Experimental protocol, clinical signs, and necropsy findings are as described for the rat study in Section 3.1.2. Mortality data are summarized in Table 10.

3.1.5. Rabbits

Groups of four rabbits were exposed to an aerosol of CS for exposure durations of 30-90 minutes (Punte et al., 1962). Experimental procedures, clinical signs, and necropsy results are similar to those described for rats in Section 3.1.2, except that hyperactivity, salivation, and lachrymation were not reported. The calculated LCT_{50} is 17,000 mg min/m^3. An unpublished report by McNamara et al. (1969) appears to provide data additional to those that have been published. Specific study details are not provided in this report, but one set of study results is consistent with those published by Punte et al. (1962). The report includes the mortality results of additional animal species exposed by inhalation to CS, as well as mortality data for CS dispersed by various methods. As described above, Punte et al. (1962) reported mortality data for guinea pigs, but the values were reported only in terms of mg min/m^3. Specific concentrations of CS (sprayed as molten agent) with corresponding exposure durations for these data are reported in McNamara et al. (1969) and are presented in Table 7.

Ballantyne and Callaway (1972) exposed groups of five rabbits for 5 to 20 minutes to an approximate CS concentration of 4000 mg/m^3, followed by a 14-day observation period. The experimental protocol, clinical signs, and necropsy findings are similar to those described in the rat study (Section 3.1.2). Mortality data are summarized in Table 9.

Groups of five to ten female New Zealand white rabbits were exposed by whole body inhalation to various concentrations of CS aerosol for durations of 5- to 60-minutes (Table 10) (Ballantyne and Swantson, 1978). Experimental protocol, clinical signs, and necropsy findings are as described for the rat study in Section 3.1.2. Mortality data are summarized in Table 10.

3.1.6. Hamsters

Ballantyne and Callaway (1972), exposed groups of male and female golden hamsters once to pyrotechnically generated CS smoke at a concentration of 750 mg/m^3 for 30 minutes, 480 mg/m^3 for 1 hour, or 150 mg/m^3 for 2 hours in a 10 m^3 exposure chamber. The experimental protocol is identical to that discussed for rats in section 3.1.2.

All animals exposed for 30 minutes to 750 mg/m^3 survived to the scheduled necropsy, and histopathological changes were observed only on post exposure day 1 (Table 10) . Three hamsters exhibited a few scattered alveolar hemorrhages, with one of these hamsters also having congestion of alveolar capillaries. No pathological changes were noted at post exposure day 10 or 28. Exposure to 480 mg/m^3 for one hour resulted in the mortality of some hamsters (Table 10), with the majority of the mortalities occurring on post-exposure days 1-2. Pathological changes in animals surviving exposure to 480 mg/m^3 were generally confined to post exposure day 1. Pathological changes included eight hamsters with mild pulmonary congestion with four of these hamsters also exhibiting mild pulmonary hemorrhage. A hamster with no lung lesions had mild renal congestion and necrosis in the medulla, while another had only mild necrosis in the medulla. A few hamsters killed at 10 days had healed lesions as evidenced by binucleate liver cells around centrilobular veins and immature epithelium in some renal tubules. No abnormal pathological changes were noted at day 29. Histopathological findings in hamsters that died were generally similar to those in rats (section 3.1.2); however, the lesions were less severe in hamsters than in rats.

Exposure to 150 mg/m^3 for 2 hours resulted in the mortality of 2 male hamsters (Day 12 or 16), and necropsy revealed bronchopneumonia. Pathological examination of surviving animals revealed lesions only on Day 1 post exposure. Lesions were confined to female animals and consisted of one female with a few scattered alveolar hemorrhages and 2 females with a few scattered foci of acute renal tubular necrosis at the inner cortex.

Exposure to 480 mg/m^3 for 1 hour or to 150 mg/m^3 for 2 hours did not affect the lifespan of the hamsters, and no statistically significant increases were noted in non-neoplastic lesions in the exposed groups compared to controls (Marrs et al., 1983a). Common non-neoplastic lesions in male and female hamsters included changes in the lungs (engorgement, congestion, and inflammatory changes, pulmonary edema) and pyelonephritis of the kidney. No exposure-related neoplastic lesions were evident in male or female hamsters.

3.1.7. Dogs

McNamara et al. (1969) exposed groups of four dogs (strain and sex not reported) to 8 different CS concentration-duration combinations. No further experimental details were available. Mortality data are summarized in Table 7.

Following a 30-second exposure to 25 mg/m^3 of CS, a dog exhibited increased blood pressure, altered respiratory pattern, tachycardia, and increased femoral artery blood flow (Cucinell et al., 1971). In another exposure, two dogs were exposed for 23 minutes to 2600 mg/m^3 of CS. One dog survived, while the other dog died 52 hours post exposure. It was noted that following exposure to a lethal dose of CS, dogs recover partially, but then develop respiratory distress and die 48-70 hours post exposure.

TABLE 7. Mortality data in rats, mice, guinea pigs, rabbits, dogs, and monkeys following inhalation exposure to CS aerosol

Species	Concentration (mg/m^3)	Duration (min)	Mortality	Time to death (days) [a]
Rats [b]	560	25	1/10	1(1)
	543	35	2/10	1(2)
	489	45	3/10	2 (1), 3(1), 4(1)
	454	55	5/10	1 (3), 3(2)
	500	60	2/10	1(2)
	500	80	6/10	1(1), 2(2), 6(3)
	500	90	8/10	1(1), 3(2), 7(2), 11(3)
Mice [b]	1200	10	0/20	-
	1100	20	7/20	7(1), 8(3), 9(3)
	900	30	2/20	7(2)
	800	40	5/20	5(2), 9(3)
	740	50	5/20	5(1), 6(3), 7(1)
	683	60	14/20	5(4), 8(5), 9(4), 13(1)
Guinea pigs [b]	400	5	1/10	7(1)
	400	10	2/10	7(1), 8(1)
	400	15	4/10	1(2), 6(2)
	500	20	3/10	1(1), 6(1), 7(1)
	400	25	7/10	2(5), 7(1), 8(1)
	400	30	7/10	1(4), 5(1). 7(1), 9(1)
	425	40	8/10	1(7), 3(1)
Rabbits [b]	500	30	1/4	6(1)
	250	40	0/4	-
	267	45	0/4	-
	250	80	3/4	1(1), 2(1), 7(1)
	333	90	4/4	1(1), 2(1), 3(1), 8(1)
Dogs [c]	833	20	0/4	-
	649	30	1/4	12(1)
	508	36	2/4	5(1), 10(1)
	899	40	2/4	1(1), 2(1)
	520	45	2/4	1(1), 4(1)
	612	45	2/4	1(2)
	797	60	3/4	1(2), 3(1)
	909	60	2 /4	1(2)
Monkeys [c]	469	24	1/4	5(1)
	673	30	2/4	1(2)
	381	45	2/4	1(2)
	612	45	1/4	1(1)
	699	60	1/4	1(1)
	941	60	3/4	1(3)
	1057	60	2/4	1(2)

Data taken from McNamara et al., 1969

[a] Number in parenthesis indicates number of animal deaths on that day

[b] Source of CS the same

[c] Source of CS the same; except monkey exposure had MMD=2.0-3.2 μ; stated that u.v. analysis conducted at 260 mμ

TABLE 8. Summary of acute toxicity data in rats and hamsters

Conc. (mg/m^3)	Time (min)	Species	Sex	No. Exposed	No. of Deaths (%)	Animals Killed					
						Day 1		Day 10		Day 25 or 29	
						No. killed	No. w/ lesions	No. killed	No. w/ lesions	No. killed	No. w/ lesions
750	30	Hamster	M	24	0	8	3	8	0	8	0
		Rat	M	24	0	8	2	8	0	8	0
480	60	Hamster	M	47	16 (34)	8	4	2	0	9	0
			F	59	15 (25)	7	6	3	0	8	0
		Rat	M	60	6 (10)	6	2	2	0	8	0
			F	60	3 (3)	7	1	2	0	8	0
150	120	Hamster	M	58	2 (3)	8	0	6	0	6	0
			F	62	0	8	3	10	0	8	0
		Rat	M	60	0	8	0	8	0	8	0
			F	60	0	8	2	8	1	8	0

Data taken from Ballantyne and Callaway, 1972

TABLE 9. Summary of mortality of guinea pigs, rabbits, rats, and mice exposed to CS

Conc. (mg/m^3)	Exp. Duration (min)	Mortality (No. died/No. exposed)			
		Rat	Mouse	Guinea pig	Rabbit
3950	5	0/10	1/10	1/5	0/5
4760	5	0/10	0/10	0/5	0/5
4250	10	1/10	0/10	5/5	0/5
4330	10	1/10	4/10	3/5	2/5
4150	15	0/10	3/10	3/5	2/5
5167	15	7/10	3/10	5/5	2/5
4000	20	9/10	8/10	5/5	4/5
4300	20	8/10	6/10	5/5	5/5

Data taken from Ballantyne and Callaway, 1972

TABLE 10. Summary of mortality data in rats, rabbits, and guinea pigs following inhalation exposure to CS

Species	Average Concentration (mg/m^3)	Duration (min)	Mortality (No died/No. exposed)	% Mortality	LCT_{50} (mg min/m^3 ± 95%)
Rat (male)	1802	10	0/20	0	88,480 (77,370-98,520)
	1806	45	8/20	40	
	1911	45	9/20	45	
	2629	60	20/21	95	
	2699	60	20/20	100	
Mouse (male)	1432	15	1/40	3	
	2753	20	17/40	43	50,010 (42,750-60,220)
	2333	30	10/19	53	
	2400	30	17/40	43	
	2550	30	24/36	67	
Guinea pig (female)	2326	10	2/20	10	67,200 (59,200-78,420)
	2380	15	2/10	20	
	1685	25	10/20	50	
	2310	20	8/20	40	
	1649	30	11/20	55	
	1302	45	9/11	82	
	2041	30	13/20	65	
	2373	30	10/19	53	
Rabbit (female)	846	5	0/10	0	54,090 (42,630-70,400)
	836	10	0/10	0	
	1434	10	0/10	0	
	1802	10	1/5	20	
	2188	15	2/10	20	
	2380	15	3/8	38	
	1407	30	4/10	40	
	1653	30	2/10	20	
	1309	45	4/5	80	
	2118	45	9/10	90	
	2133	60	7/8	88	
	3066	60	8/9	89	

Data taken from Ballantyne and Swantson, 1978

3.2. Nonlethal Acute Toxicity

3.2.1. Mice

An RD_{50} of 4.0 mg/m^3 (95% C.I.: 3.3-5.2 mg/m^3) (reported as: 0.52 ppm; 95% C.I.: 0.429-0.677 ppm) was reported for male Swiss-Webster mice (Kane et al., 1979).

3.2.2. Rabbits

To investigate whether CS exposure can cause diarrhea, four rabbits were exposed to thermally-generated pure CS in a 10 m^3 chamber (Ballantyne and Beswick, 1972). The exposures were as follows: one rabbit each was exposed to 58 mg/m^3 for 30 minutes, 46 mg/m^3 for 20 minutes, 54 mg/m^3 for 12 minutes, or 17 mg/m^3 for 17 minutes. Animals were placed singly in cages with removable trays lined with several layers of filter paper arranged to collect

stool samples. The number of stool pellets passed, their total weight, and water content were recorded for several day before and after exposure. Exposure to CS did not result in an increased incidence of diarrhea.

Two rabbits were exposed in a static chamber to the entire contents of a 3 ounce unit containing 71.5 grams of CS (Gaskins et al., 1972). The unit required 20 seconds to fully dispense. Both rabbits became unconscious after approximately two minutes of exposure and were moved to fresh air. The rabbits had regained their righting reflex approximately 10-20 minutes post exposure and were almost completely recovered by one hour post exposure, with the only effect still visible being moderate eye wetness. Gross necropsy of the rabbits two weeks post exposure did not reveal any abnormalities. Another two rabbits were exposed to 23.2 g of CS during dispersion of a CS unit requiring about ten seconds to completely discharge. The dispensed CS formed a cloud in the chamber. The rabbits tried to avoid the spray as it was dispensed, and then sat quietly with their eyes tightly closed for the remainder of the five minute exposure. No abnormalities were observed in the eyes or skin of the exposed rabbits.

3.3. Repeat Dose Studies

3.3.1. Rats

Groups of five male and five female F344/N rats were exposed to CS2 concentrations of 0, 1, 3, 10, 30, or 100 mg/m^3 for 6 hours/day, 5 days/week for 2 weeks (NTP, 1990). (CS2 contains 94% CS, 1% hexamethyldisilizane, and 5% Cab-o-Sil®). All rats exposed to 30 or 100 mg/m^3 died before study termination. Rats from all CS2 exposure groups exhibited adverse clinical signs, ranging from erythema and blepharospasm at the lower concentrations and progressing to dacryorrhea, mouth breathing, listlessness, and mouth breathing at the high concentrations. Rats in the 1 mg/m^3 group gained more weight over the exposure period compared to controls, but generally decreased body weight was noted at exposure concentrations of 3 mg/m^3 and higher.

Groups of ten male and ten female F344/N rats were exposed to 0, 0.4, 0.75, 1.5, 3, or 6 mg/m^3 of CS2 6 hours/day, 5 days/week for 13 weeks (NTP, 1990). One male rat exposed to 6 mg/m^3 died; all others survived to study termination. Clinical signs of eye irritation (partial or complete eyelid) were noted in all CS2 exposure groups, and rats exposed to 6 mg/m^3 of CS2 developed erythema of the extremities that persisted overnight. Rats exposed to 1.5 mg/m^3 and higher gained significantly less weight over the study period compared to controls; final mean body weight was 17-44% lower than that of controls for males and 10-24% lower for females. An approximate 46% reduction in thymus weight relative to body weight was noted in male and female rats exposed to 6 mg/m^3. Concentration-related histopathological changes included focal erosion with regenerative hyperplasia and squamous metaplasia of the respiratory epithelium. Acute inflammation and hyperplasia of the respiratory epithelium were also noted.

One group of 56 male rats was exposed for five minutes/day for five days to a mean CS concentration of 1470 or 1770 mg/m^3, while another group of 49 male rats was exposed for 80 minutes/day for nine days to a mean CS concentration of 12.5 or 14.8 mg/m^3 (Ballantyne and Callaway, 1972). Exposures to the thermally-generated CS aerosol (mass mean diameter of 1-2

μm) were conducted in a 1 m^3 chamber, with chamber air sampled continuously throughout exposure at a rate of 1 liter per minute using a double cone filter. The samples were analyzed for CS content (further details not provided). Groups of 3-5 survivors were sacrificed at 1, 6, and 24 hours and 2, 3, 4, 5, 7, 10, 14, and 21 days after the final exposure, and subjected to gross and microscopic examination. All animals survived the 5-minute exposures. Histopathological examination revealed minimal congestion of the alveolar capillaries at one or six hours post exposure in 2/5 rats; a few scattered alveolar hemorrhages at two days post exposure in 1/4 rats; and scattered patches of bronchopneumonia at 7, 8, 10, and 18 days post exposure in 1/5, 1/3, 1/3, and 2/5 rats, respectively. Pathological changes in control rats included scattered alveolar hemorrhages in 2/11 rats, and subacute mucoid enteritis in 1/11 rats. Five of the 49 rats exposed for 80 minutes/day to 12.5 or 14.8 mg/m^3 died: one after the 7^{th} exposure, two after the 8^{th} exposure, and two died five days after the final exposure. Necropsy revealed widespread acute bronchopneumonia. Histopathological examination of the surviving animals revealed lesions up to five days post exposure; no lesions were reported in rats examined after five days post exposure.

3.3.2. Mice

Groups of five male and five female $B6C3F_1$ mice were exposed to CS2 concentrations of 0, 1, 3, 10, 30, or 100 mg/m^3 for 6 hours/day, 5 days/week for 2 weeks (NTP, 1990). All mice exposed to 10 mg/m^3 and greater died before study termination. Mice from all CS2 exposure groups exhibited adverse clinical signs, ranging from erythema and blepharospasm at the lower concentrations and progressing to dacryorrhea, mouth breathing, listlessness, and mouth breathing at the high concentrations. Mice exposed to 1 mg/m^3 gained more weight over the exposure period compared to controls, but generally lost body weight at exposure concentrations of 3 mg/m^3 and higher.

Groups of ten male and ten female $B6C3F_1$ mice were exposed to 0, 0.4, 0.75, 1.5, 3, or 6 mg/m^3 of CS2 6 hours/day, 5 days/week for 13 weeks (NTP, 1990). All mice exposed to 6 mg/m^3 died and 1 male and 1 female mouse from the 3 mg/m^3 group died during the second week of exposure. The clinical signs of closed or partially closed eyes during exposure were noted in mice from all exposure groups through week 6, with the mice exposed to 3 mg/m^3 again exhibiting eye closure during weeks 12 and 13. Concentration-related decreases in body weight compared to controls were noted in all exposure groups; final mean body weights of mice in the 3 mg/m^3 group were 13% lower for males and 9% lower for females. Exposure-related histopathological changes were observed in mice exposed to 1.5 mg/m^3 and higher and included focal inflammation and squamous metaplasia (primarily in the nasal turbinates) and inflammation of the vomeronasal organ.

3.3.3 Rats, Mice, Guinea Pigs, Rabbits

Groups of 5-10 guinea pigs, 5 rabbits, 10 rats, and 10-20 mice were exposed for 5 hours/day for 1 to 7 successive days to an approximate CS concentration of 30-40 mg/m^3 (Ballantyne and Callaway, 1972). For the exposure, an anti-riot grenade containing 0.5 to 0.75 g CS was ignited every 30 minutes in a 10 m^3 static chamber to maintain the nominal concentration. The authors stated that concentrations were determined by continuous sampling throughout the animal

exposure, but no other details were provided. Animals were removed to fresh air following each exposure, and were maintained for a 14-day post exposure period after their last exposure. All animals that died and the survivors killed at the end of the 14-day post exposure period were subjected to gross and histological examination. A summary of the mortality data is presented in Table 11. The description of clinical signs was limited to a statement that rabbits and rats exhibited more rhinorrhea and lacrimation than did mice, while guinea pigs showed few clinical signs apart from occasional sneezing during the first hour of exposure. Necropsy of animals that died revealed moderate to marked congestion of the alveolar capillaries and intrapulmonary veins and inter- and intra-alveolar areas of hemorrhage, and many of the animals that died also had congestion of the liver, kidney, and small intestine. Moderate pulmonary edema was noted in a "few of the animals." No residual pathology was noted in animals that survived to study termination.

TABLE 11. Summary of mortality of guinea pigs, rabbits, rats, and mice exposed to CS for 5 h/day for up to 7 days

Species	Duration		Conc. (mg/m^3)	Mortality (No. died/No. exposed)
	hr/day	No. days		
Guinea pig	5	1	44.7	0/5
		3	36.0	2/5
		4	34.2	3/10
		6	35.2	2/5
		7	43.7	10/10
Rabbit	5	3	36.0	1/5
		5	34.2	2/5
Rat	5	1	37.0	1/10
		3	36.0	9/10
		5	34.2	7/10
Mouse	5	1	40.0	0/10
		2	38.8	0/10
		3	36.0	1/10
		4	31.9	10/10
		5	56.4	16/20

Data taken from Ballantyne and Callaway, 1972

3.4. Developmental/Reproductive Toxicity

Groups of 22-24 pregnant Porton strain rats or 12 pregnant New Zealand White rabbits were exposed to CS aerosol for 5 minutes/day over GDs 6-15 or GDs 6-18, respectively, and were sacrificed on GD 21 or GD 30, respectively (Upshall, 1973). CS aerosol with a particle size of 1-2µm was generated by melting pure crystalline CS at 120°C using a Collison spray. A preliminary study investigated exposure to 0 or 20 mg/m^3 CS, followed by a subsequent concentration-response study at concentrations of 0, 6, 20, or 60 mg/m^3. Rat controls were recaged and moved out of their normal environment during the test group exposure, while rabbit controls were exposed to a siliconized silica aerosol at 60 mg/m^3. Additional control groups of pregnant rats were exposed to a particulate aerosol (60 mg/m^3 of Neosil) or to water aerosol to evaluate the stress of aerosol exposure. At sacrifice, cesarean section was performed, and fetuses were evaluated for skeletal or visceral abnormalities. In addition, the lungs, liver, kidneys, and adrenal from the rabbit dams in the concentration-response study were evaluated histologically. No definitive effects of treatment were noted. In the preliminary rat study,

exposed rats exhibited a decrease in maternal weight gain compared with controls (-23%), but a clear concentration-response was not observed in the main study (-23%, -12%, and 15% for the 6, 20, or 60 mg/m^3 groups, respectively). Fetal weight appeared to decrease with increasing concentration in the main rat study (3.3, 3.2, and 3.1 g, respectively, vs. 3.5 for controls), but the fetal weights were comparable to the rat fetal weights recorded in other studies. No other statistically significant effects were observed. No exposure-related effects were noted in exposed rabbits or their offspring. Although the exposure concentrations were sufficient to cause extreme irritation, clinical signs in exposed rats and rabbits were not reported.

3.5. Genotoxicity

In general, CS was not mutagenic to *Salmonella typhimurium*. Mutations were not induced with or without the presence of S9 at CS concentrations of 12.5-800 μg/plate in strains TA97a, TA98, TA100, TA102, or TA104 (Meshram et al., 1992); of up to 1.5 mg/plate in strains TA98, TA100, TA1535, TA1537 (Wild et al., 1983); of 10 μg/plate up to 2 mg/plate in strains TA98, TA1535, TA1537, or TA1538 (Däniken et al., 1981); or at CS2 concentrations of 3.3-333 μg/plate in strains TA98, TA100, TA1535, or TA1537 (NTP, 1990). Equivocal responses for CS and CS2 were reported in strain TA100 only without S9 (Däniken et al., 1981; NTP, 1990), and for CS2 in strain TA97 but only with 30% S9 (NTP, 1990). Cytotoxicity was observed starting at doses of 200 μg/plate, but the presence of 30% S9 generally reduced the cytotoxicity.

Other *in vitro* genotoxicity testing was generally positive. CS induced sister chromatid exchanges (SCE) and chromosomal aberrations in Chinese hamster (CHO) ovary cells both with and without S9 at CS2 concentrations of 6 μg/mL and greater (NTP, 1990). Trifluorothymidine resistance in mouse L5178Y lymphoma cells was induced in the absence of S9 at a CS concentration of 2.5 μg/mL (McGregor et al., 1988; NTP, 1990). V79 Chinese hamster cells exposed to 19, 38, or 75 μM of CS in culture for 3 hours and evaluated 6 days later showed reduced survival (by ~ 20, 30, and 80%, respectively; values are read off of graph), and exhibited a concentration-related increase in the frequency of mutants resistant to 6-thioguanine (mutations induced approximately 4 to 5-fold above controls at the highest concentration) (Ziegler-Skylakakis et al., 1989). Exposure to CS also increased the frequency of micronuclei by approximately 2-fold at 19 μM of CS up to 18-fold at 75 μM of CS as measured 24 hours after exposure, but did not induce DNA repair synthesis as assessed using the BrdUrd density-shift method. A concentration-dependent increase was observed in spindle cell disturbances, particularly C-metaphases (chromosomes completely scattered in cytoplasm and often highly contracted), when cells were exposed to 5, 9, 19, or 38 μM of CS for three hours (Schmid and Baucher., 1991). The C-mitotic effect was also reflected in the appearance of a metaphase block and the disappearance of other mitotic figures (prophases, ana-telophases). When a differential staining technique was applied to allow for visualization of the spindle apparatus and chromosomes, a concentration-dependent increase in the number of mitoses with abnormal spindles was again observed, particularly apolar mitoses (mitotic figures without any signs of polar spindle configurations) (Salassidis et al., 1991). Further investigation into the mechanism of CS-induced c-mitotic spindle damage found that exposure of cells to 38 μM of CS for 20 hours or 3 hours followed by 20 hours of recovery resulted in an increase in the number of aneuploid cells and in the polyploid index (Schmid and Bauchinger, 1991). The number of aneuploid cells and the polyploid index were increased to a much greater extent by exposure to the metabolite *o*-chlorobenzaldehyde as compared to CS, suggesting that this metabolite may

play a role in the induction of spindle damage. A comparison of the effectiveness of various exposure conditions revealed that cells exposed to CS at concentrations of up to 38 μM for 20 hours exhibited a concentration-dependent increase in the number of S-cells and the frequency of chromatid-type aberrations (single breaks, isolocus breaks and exchanges, and gaps); exposure for 3 hours followed by a 20-hour recovery period resulted in similar effects but was not as effective; and no effects were observed when cells were incubated with the supernatant from the 3 hour exposure (Bauchinger and Schmid, 1992). It is noted that the cell cycle time of the V79 cell line used is approximately 8-10 hours; therefore, the cells had time to run through one or two S-phases.

Genotoxicity testing *in vivo* was generally negative. CS did not bind to DNA in the liver or kidneys of rats i.p. injected with 13 mg/kg of radiolabeled CS and evaluated 8 or 75 hours after dosing, but did bind to nuclear proteins in these organs (Dänkien et al., 1981). CS did not cause an increase in sex-linked recessive mutations in germ cells of male Drosophila when administered in the feed at concentrations of 5×10^{-4} M to 2.6×10^{-3} M for three days (Wild et al., 1983), and did not increase micronucleated polychromatic erythrocytes in the bone marrow of NMRI mice administered CS by intraperitoneal injection of 19 or 38 mg/kg or by oral administration of 113 or 226 mg/kg (Wild et al., 1983). It is noted that the oral dose of 226 mg/kg killed 10 of 13 exposed mice.

3.6. Chronic Toxicity/Carcinogenicity

Groups of fifty $B6C3F_1$ mice and fifty F344/N rats/sex were exposed for 6 hours/day, 5 days/week for 105 weeks to target CS2 concentrations of 0, 0.75, or 1.5 mg/m^3 (mice) or 0, 0.075, 0.25, or 0.75 mg/m^3 (rats) (NTP, 1990). Rats exposed to 0.75 mg/m^3 of CS2 developed histopathological changes in the respiratory and olfactory epithelium of the nasal passage and inflammation and proliferation of the periosteum of the turbinate bones. No neoplastic effects were present. Lesions seen in the nasal cavity of exposed mice included inflammation in the anterior middle portions of the nasal passage, and focal hyperplasia and/or squamous metaplasia of the respiratory epithelium. No other adverse effects of exposure were noted. Female mice exhibited a statistically significant, exposure-related reduction in the incidences of hyperplasia and adenomas of the pituitary gland pars distalis (rates of the adenomas in the 0, 0.75, and 1.5 mg/m^3 groups: 16/47, 5/46, and 1/46, respectively). Lymphomas in female mice also occurred with a significant negative trend (21/50, 12/50, and 8/50, respectively).

Groups of 75 male SPF Porton strain mice, 50 male Porton Wistar derived rats, and 50 Dunkin Hartley guinea pigs were exposed to nominal concentrations of 0, 3, 30, or 300 mg/m^3 CS aerosol (mass mean diameter of 3-4 μm) for 1 hour/day, 5 days/week for up to 55 exposures (11 weeks) in mice and up to 120 exposures (24 weeks) in rats and guinea pigs (Marrs et al., 1983b). Exposure to the high concentration resulted in excessive mortality in mice and guinea pigs within days of the start of exposure; therefore, exposure to the high concentration was discontinued after three exposures in mice, and after five exposures in rats and guinea pigs (the actual mortality at days 3 and day 5, respectively, were not provided) (Marrs et al., 1983b). During the first month of the experiment, 17% of the mice and 46% of the guinea pigs exposed to the high-concentration died. Mice exhibited a significant trend ($p<0.001$) in the incidence of early death with concentration of exposure. The authors also reported a significant trend

($p<0.001$) in the incidence of early death with concentration of exposure in guinea pigs; however, most of the mortality in guinea pigs occurred during the first month. Post mortem examination of ten guinea pigs that died during exposure revealed acute alveolitis in seven of the animals, with mild alveolitis present in the other three examined. The cause of death in mice dying during exposure could not be determined. The author noted that toxic signs were not usually observed: death occurred suddenly and without warning. No cause of death could be ascribed to animals that died during the observation period. CS exposure did not affect the growth of rats or guinea pigs, but did result in a concentration-related decrease in the growth of mice. No definitive, exposure-related histological findings were observed in mice, rats or guinea pigs at study termination. No exposure-related neoplasms were identified.

3.7. Summary

Clinical signs noted in the acute and repeated-dose animal studies suggest that CS is highly irritating. The majority of the acute inhalation exposure data in animals focused primarily on lethality as an endpoint, and death was generally caused by pulmonary edema and congestion. Kidney damage was also occasionally noted, but may have been secondary to anoxia. Genetic toxicology results were mixed. The responses in the *Salmonella* gene mutation test were generally negative, as were results of *in vivo* genotoxicity assays. CS induced trifluorothymidine resistance in mouse L5178/TK lymphoma cells in the absence of S9, and induced both SCEs and chromosomal aberrations in CHO cells in the presence and absence of S9. No developmental toxicity was noted in rats or rabbits, and there was no evidence of carcinogenicity in rats or mice.

4. SPECIAL CONSIDERATIONS

4.1. Metabolism and Disposition

Absorption

One cat with a cannulated trachea was exposed to CS aerosol by an oral-nasal mask to assess absorption of CS by the upper respiratory tract (cannulation prevented access to the lower respiratory tract), while a second cat was exposed via a tracheal tube to assess absorption by the lower respiratory tract (Leadbeater, 1973). Blood concentrations of CS and its metabolites following exposure to the upper and lower respiratory tract were about 30% and 80%, respectively, of those measured in intact cats.

Toxicokinetics

The half-lives of CS, 2-chlorobenzylmalonitrile, and 2-chlorobenzaldehyde were measured *in vivo* in cats after the chemicals were administered directly into the femoral artery via a cannula, or in rabbits after CS was administered directly into the ear vein (Leadbeater, 1973; Paradowski, 1979). The half-lives in cats were 5.5, 9.5, and 4.5 seconds, respectively, regardless of whether just CS or all of the individual chemicals were administered, and in rabbits ranged from 19-25, 38-55, and 38-41 seconds, respectively. The *in vitro* half-lives in blood of cats, humans and rats were also measured: the half-lives of CS, 2-chlorobenzylmalonitrile, and 2-chlorobenzaldehyde in cats were 5, 470, and 70 seconds, respectively; in humans were 5, 660, and 15 seconds, respectively, and in rats were 7, 30, and 15 seconds, respectively (Leadbeater, 1973). The *in vitro* half-life of CS in the blood of rabbits was higher at approximately 60 seconds; the authors postulated the increase in the half-life compared to rats, cats, and humans could be due to the much greater concentration tested in rabbits (Paradowski, 1979).

CS incubated with rat liver homogenate for 5 minutes (ethanol-buffer; pH 7.4; 37ºC) resulted in a 59% decrease in the initial amount of glutathione, with 26% of the depletion occurring spontaneously (non-enzymatically) (Rietveld et al., 1986). Binding to glutathione *in vivo* was confirmed by enhanced urinary thioether excretion in rats following i.p. administration of CS (Rietveld et al., 1983; 1986). The thioether was identified as 2-chlorobenzylmercapturic acid.

Metabolism

Metabolism of CS appears to be qualitatively similar in species. *In vivo*, CS can be hydrolyzed to 2-chlorobenzaldehyde or malononitrile, or can be reduced to 2-chlorobenzyl malononitrile (see Figure 1.) (Paradowski, 1979; Leadbeater, 1973). The 2-chlorobenzaldehyde can then be oxidized to 2-chlorobenzoic acid for subsequent glycine conjugation, or reduced to 2-chlorobenzyl alcohol for ultimate excretion as 2-chlorobenzyl acetyl cysteine or 2-chlorobenzyl glucuronic acid. The malononitrile can break down to cyanide, ultimately being excreted as thiocyanate. The reduction of CS to 2-chlorobenzyl malononitrile is a relatively minor pathway; 2-chlorobenzyl malononitrile can be conjugated with glycine, or can be hydrolyzed to 2-chlorophenyl 2-cyanoproprionate.

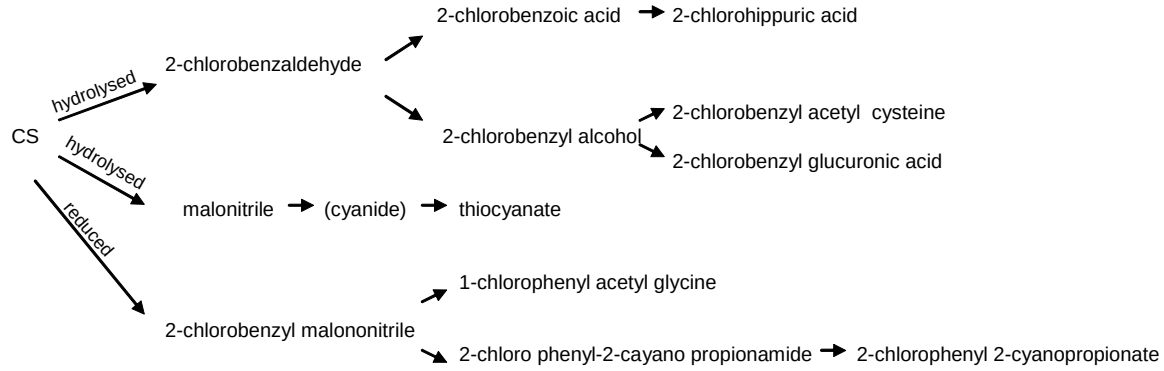


Figure 1. Summary of predominant metabolic pathways of CS in rats as proposed by Rietveld et al., 1983; Paradowski, 1979; Leadbeater, 1973.

Radiolabeled CS was administered to rats intravenously (i.v.) (0.08, 0.8, and 80 µmol/kg of ^{3}H-ring labeled; 0.8 and 80 µmol/kg of ^{14}C-cyanide labeled; or 0.8 and 80 µmol/kg of (^{14}C=C) side-chain labeled CS) or intragastrically (80, 106, and 159 µmol/kg of ^{14}C-cyanide labeled CS) (Brewster et al., 1987). The major urinary metabolites recovered in rats up to 96 hours after i.v. or intragastric administration of CS were 2-chlorohippuric acid (49% of dose), 2-chlorobenzyl glucuronic acid (10%), 2-chlorobenzyl cysteine (8%), and 2-chlorobenzoic acid (8%), and minor metabolites included 2-chlorophenyl acetyl glycine (3%), 2-chlorobenzyl alcohol (1.6%), and 2-chlorophenyl 2-cyano propionate (1.6) (see Figure 1). In another investigation, the concentration of cyanide and thiocyanate in rat urine over a 24-hour period was measured in untreated rats, rats administered CS by i.v., or in rats exposed by i.p. or intragastric administration to the CS hydrolysis product malononitrile. Following CS and malononitrile administration, urinary cyanide levels remained at or below baseline levels, while thiocyanate levels generally increased as the CS or malononitrile dose increased. The percentage molar conversion to CS to thiocyanate was 21.5% at an i.p. dose of 212 µmol/kg and 30% at an intragastric dose of 212 µmol/kg, while it was 60% or more with a malononitrile i.p. dose of 80 µmol/kg or intragastric malononitrile dose of 212 µmol/kg.

Metabolism in rabbits is similar to that in rats. The predominant biotransformation pathway in the blood of rabbits administered high doses of CS by i.v. ($0.5LD_{50}$ to the LD_{50}) was hydrolysis of CS to 2-chlorobenzyaldehyde and malononitrile (~30-40%), with a minor pathway of reduction to 2-chlorobenzyl malononitrile (10%) (Paradowski, 1979). The authors stated that the remaining 50-60% of the administered CS disappeared from the blood by other means; no other explanation was provided. The liver is involved in the metabolism of CS as demonstrated by an increase in the half-lives of CS and its metabolites in blood of rabbits when the liver was excluded from the circulation. More of the CS was accounted for after dosing, with approximately 75% of the CS hydrolyzed to 2-chlorobenzyaldehyde and 15% reduced to 2-chlorobenzyl malononitrile. When the kidney was excluded from the circulation, no changes were observed in CS or metabolites in the blood.

Maximum blood levels of CS and its derivatives in cats were attained 30 minutes after intragastric administration of 40 mg/kg of CS (Leadbeater, 1973). By 90 minutes post dosing, blood levels of 2-chlorobenzylmalonitrile and 2-chlorobenzaldehyde were still elevated, while CS levels had returned to zero. When anesthetized cats were exposed for 60 minutes via oral-nasal masks to CS aerosol (75 or 750 mg/m^3 of pyrotechnically generated CS aerosol, 750 mg/m^3 of pure CS aerosol from molten CS, or 62.5 mg/m^3 of CS aerosol generated from an aqueous suspension of micronized CS in acetic acid using a Collison sprayer), the levels of CS and 2-chlorobenzylmalonitrile rapidly reached steady values, while that of 2-chlorobenzaldehyde continued to increase. The blood levels resulting from exposure to 750 verses 75 mg/m^3 pyrotechnically generated CS did not result in a 10-fold decrease in the concentration of CS and its metabolites 2-chlorobenzylmalonitrile and 2-chlorobenzaldehyde: concentrations were reduced by 4.5, 7.7, and 5.9, respectively. Exposure of cats to 100 mg/m^3 of CS for 5 minutes/day for 4 days preceding exposure to 75 or 750 mg/m^3 of CS resulted in reduced blood concentrations of CS and its derivatives.

Rats receiving a single oral dose of 50-500 mg/kg of CS had lower blood levels of CS and its derivatives compared to cats (Leadbeater, 1973). CS was only detected in the blood of rats receiving the highest dose of 500 mg/kg of CS. As in cats, blood concentrations of 2-chlorobenzylmalonitrile and 2-chlorobenzaldehyde in rats did not increase in a dose-related manner. Rats breathing CS aerosol at concentrations of 14-245 mg/m^3 for five minutes had measurable amounts of CS and 2-chlorobenzylmalonitrile in the blood immediately after exposure, but 2-chlorobenzaldehyde was detected only in rats exposed to concentrations greater than 100 mg/m^3.

Available animal data suggest that CS should be absorbed by the human respiratory tract following inhalation exposure, and that CS should proceed via a similar metabolic pathway. However, humans are not able to tolerate as great an exposure as animals. Six healthy human males inhaled 0.5 to 1.5 mg/m^3 of CS over 90 minutes, and blood was drawn before and after exposure to measure CS and its derivatives (Leadbeater, 1973). Two men left the chamber within 20 minutes. CS and 2-chlorobenzaldehyde were not detected in the blood of any of the volunteers, and only a trace of 2-chlorobenzylmalonitrile was detected in the blood of one man who remained in the chamber for the entire exposure.

Distribution and Elimination

To evaluate the fate of CS in rats, radiolabeled CS was administered intravenously (^{3}H-ring labeled, ^{14}C-cyanide labeled, or (^{14}C=C) side-chain labeled) or intragastrically (^{14}C-cyanide labeled) and urine, feces, and CO_2 collected over 96 hours post dosing (Brewster et al., 1987). The majority of the administered dose was recovered in the urine, with recovery ranging from 44.4 to 100%. Recovery in feces up to 96 hours post dosing ranged from 1.2 to 23.4%, and recovery in CO_2 was minimal at 0 to 2.1%. When comparing the recovery of the three different radiolabels following i.v. administration, more radioactivity was recovered in the feces of the rats administered the (^{14}C=C) side-chain labeled CS (21 to 23%) compared to the other two labels (4 to 8%).

Male mice were administered ^{14}CN-CS by i.v., and were killed at selected time intervals and autoradiographed to evaluate distribution (Brewster et al., 1987). A significant amount of radioactivity was present in the gastrointestinal tract at five minutes post dosing. At one hour, significant amounts of radioactivity were present in the gastrointestinal tract, urinary bladder, mouth, and esophagus, with lesser amounts in the blood, liver, and salivary glands. At 24 hours, most of the residual radioactivity was present in the mouth, salivary glands, gastrointestinal tract, or urinary bladder.

4.2. Mechanism of Toxicity

CS is an SN_2 alkylating agent, and therefore reacts directly with nucleophilic compounds (Cucinell et al., 1971). Consequently, sulfhydryl-containing enzymes and other biological compounds are prime targets. Most notably, CS reacts rapidly with the disulfhydryl form of lipoic acid, a coenzyme in the pyruvate decarboxylase pathway. *In vitro*, CS reacted readily with cysteine, N-acetyl L-cysteine, glutathione, dithiothreitol, and lipoic acid, with first order reaction constants of 0.33, 0.42, 0.85, 4.88, and 10.4, respectively. CS incubated with rat liver homogenate for 5 minutes (ethanol-buffer; pH 7.4; 37ºC) resulted in a 59% decrease in the initial amount of glutathione, with 26% of the depletion occurring spontaneously (non-enzymatically) (Rietveld et al., 1986). Binding to glutathione *in vivo* was confirmed by enhanced urinary thioether excretion in rats following i.p. administration of CS; the thioether was identified as 2-chlorobenzylmercapturic acid (Rietveld et al., 1983; 1986). In another study, rats administered a dose 120% of the LD_{50} by i.p. injection became moribund (most likely due to the relatively slow generation of cyanide from the malononitrile metabolite) approximately 30 minutes after injection. Support for the role of cyanide in the CS-induced lethality was the observation that administration of thiosulfate intraveneously at this time reduced mortality by 65% compared to control rats (21 of 32 rats survived compared to 1 of 11 control rats). Intravenous administration of 8 mg/kg of CS in dogs resulted in a rapid drop in the plasma sulfhydryl concentration, returning to normal within approximately 3 hours (Cucinell et al., 1971).

4.3. Other Relevant Information

4.3.1. Species Variability

CS is a potent acute irritant and the mode of ocular and pulmonary toxicity is direct contact and its associated alkylating properties; therefore, the mechanism of action is not expected to vary greatly among species. Ballantyne and Swantson (1978) calculated LCT_{50} values of 88,480 mg min/m^3 for rats; 67,200 mg min/m^3 for guinea pigs; 54,090 mg min/m^3 for rabbits; and 50,010 mg min/m^3 for mice, values all well within a factor of two.

4.3.2. Susceptible Populations

CS is an irritant and the mechanism of toxicity is a direct contact effect; therefore, the mechanism of action is not expected to vary greatly between individuals. The reactions of volunteers with jaundice, hepatitis, or peptic ulcer or those that were 50-60 years old were similar to those of "normal" volunteers when exposed to highly irritating concentration of CS for short durations (Punte et al., 1963; Gutentag et al., 1960). Subjects with a history of drug allergies or sensitivities, hay fever, or asthma also tolerated exposure to CS and were similar to the "normal" subjects, but this group had a higher percentage of individuals with more severe chest symptoms, many of them laying prostrate on the ground for several minutes. However, no wheezing or rhonchi were heard, and recovery was as rapid as that seen in other exposure groups.

4.3.3. Concentration-Exposure Duration Relationship

The concentration-exposure time relationship for many irritant and systemically-acting vapors and gases can be described by the relationship $c^n \times t = k$, where the exponent, n, ranges from 0.8 to 3.5 (ten Berge et al., 1986). An analysis of the available rat, mouse, rabbit, guinea pig, dog, or monkey acute inhalation lethality data for derivation of the exponent 'n' was conducted using using the DoseResp software of ten Berge (2006). These analyses utilized the concentration-specific data contained in Tables 7, 9, 10 and 11, and produced the following exponent 'n' values and confidence limits:

Rat:	0.704 (0.543-0.865)
Mouse:	0.701 (0.509-0.892)
Rabbit:	0.658 (0.467-0.849)
Guinea pig:	0.559 (0.018-1.099)
Dog:	0.356 (-1.464-0.751)
Monkey:	0.187 (-0.281-0.656)

Details of the analysis are given in Appendix B.

5. DATA ANALYSIS FOR AEGL-1

5.1. Summary of Human Data Relevant to AEGL-1

Several studies describe irritation in humans (Table 6); however, the severity of effects is above the definition of AEGL-1.

5.2. Summary of Animal Data Relevant to AEGL-1

No animal studies were available for development of AEGL-1 values.

5.3. Derivation of AEGL-1

The AEGL-1 values will be based on human exposure to 1.5 mg/m^3 for 90 minutes (Punte et al., 1963). All four subjects could tolerate the exposure, but experienced eye and nose irritation and headache. One subject developed nasal irritation within 2 minutes, three subjects developed headache (at 45, 50, and 83 minutes), and all four experienced ocular irritation (at 20, 24, 70, and 75 minutes). Because the observed effects are above those defined by AEGL-1, a modifying factor of 10 will be applied to reduce the point-of-departure from a LOAEL to a NOAEL for AEGL-1 effects. An intraspecies uncertainty factor of 3 will be applied because contact irritation is a portal-of-entry effect and is not expected to vary widely among individuals. The intraspecies UF of 3 is also supported by the fact that responses of volunteers with jaundice, hepatitis, or peptic ulcer or those that were 50-60 years old were similar to those of "normal" volunteers when exposed to a highly irritating concentration of CS for short durations (Punte et al., 1963; Gutentag et al., 1960). An interspecies uncertainty factor of 1 will be applied because the study was conducted in humans. Time scaling was not applied in the development of the AEGL-1 values, because the critical effect (irritation) is a function of direct contact with the tear gas and is not likely to increase with duration of exposure at this level of severity (NRC, 2001). AEGL-1 values are presented in Table 12.

TABLE 12. AEGL-1 Values for Tear Gas

10-min	30-min	1-h	4-h	8-hour
0.050 mg/m^3	0.050 mg/m^3	0.050 mg/m^3	0.050 mg/m^3	0.050 mg/m^3

6. DATA ANALYSIS FOR AEGL-2

6.1. Summary of Human Data Relevant to AEGL-2

Four subjects exposed to 1.5 mg/m^3 tolerated a 90 minute exposure, but experienced clinical signs of irritation. One subject developed nasal irritation within 2 minutes, three subjects developed headache (at 45, 50, and 83 minutes), and all four experienced ocular irritation (at 20, 24, 70, and 75 minutes) (Punte et al., 1963). When a total of 35 subjects were exposed for 60 minutes to CS concentrations ranging from 0.31-2.3 mg/m^3, one subject left at 5 minutes due to vomiting but returned for the duration of the exposure, another vomited at 55 minutes of exposure (vomiting in both cases was ascribed to swallowing large amounts or saliva), and one subject voluntarily left the exposure at 8 minutes due to irritation (Beswick et al., 1972). Clinical signs noted during the 60-minute exposure included eye, nose, mouth, and throat irritation, nausea, chest discomfort, headache, and stinging of the face.

6.2. Summary of Animal Data Relevant to AEGL-2

Blinking, mild pulmonary congestion, and emphysema were noted in monkeys exposed to 900 mg/m^3 for 3 minutes or 1700 mg/m^3 for 5 minutes. Monkeys exposed to 2500 mg/m^3 for 32 minutes showed blinking labored respiration, coughing, oral and nasal discharge, vomiting, decreased activity, pulmonary edema, and congestion (Striker et al., 1967). Mice exposed to 40

mg/m^3 for 5 hours had rhinorrhea and lacrimation, and guinea pigs exposed to 45 mg/m^3 for 5 hours showed occasional sneezing during the first hour of exposure.

6.3. Derivation of AEGL-2

The AEGL-2 will be based on human exposure to 1.5 mg/m^3 for 90 minutes (Punte et al., 1963). All four subjects could tolerate the exposure, but experienced eye and nose irritation and headache. An intraspecies uncertainty factor of 3 is applied because contact irritation is a portal-of-entry effect and is not expected to vary widely among individuals. The intraspecies UF of 3 is also supported by the fact that responses of volunteers with jaundice, hepatitis, or peptic ulcer or those that were 50-60 years old were similar to those of "normal" volunteers when exposed to a highly irritating concentration of CS for short durations (Punte et al., 1963; Gutentag et al., 1960). An interspecies uncertainty factor of 1 is applied because the study was conducted in humans. Time scaling is not applied in the development of the AEGL-2 values. The critical effect (irritation) is a function of direct contact with the tear gas and is not likely to increase with duration of exposure at this level of severity (NRC, 2001). AEGL-2 values are summarized in Table 13 and calculations are in Appendix A.

TABLE 13. AEGL-2 Values for Tear Gas

10-min	30-min	1-h	4-h	8-h
0.50 mg/m^3	0.50 mg/m^3	0.50 mg/m^3	0.50 mg/m^3	0.50 mg/m^3

These values are supported by the data of Beswick et al. (1972). When a total of 35 subjects were exposed for 60 minutes to CS concentrations ranging from 0.31-2.3 mg/m^3, one subject left at 5 minutes due to vomiting but returned for the duration of the exposure, and another vomited at 55 minutes of exposure (vomiting in both cases ascribed to swallowing large amounts or saliva). One subject voluntarily left the exposure at 8 minutes due to irritation; this subject was exposed in the range of 0.56-0.86 mg/m^3, and the AEGL-2 values are below this exposure range. Although clinical signs of irritation were noted, five subjects exposed to a constant 0.78 mg/m^3 CS for 60 minutes all remained in the chamber for the entire exposure. Again, the AEGL-2 values are below this exposure concentration.

7. DATA ANALYSIS FOR AEGL-3

7.1. Summary of Human Data Relevant to AEGL-3

No human studies were available for development of AEGL-3 values.

7.2. Summary of Animal Data Relevant to AEGL-3

Animal lethality data are available for rats, mice, rabbits, guinea pigs, dogs, and monkeys exposed to varying concentrations of tear gas for varying time periods (McNamara et al., 1969; Ballantyne and Calloway, 1972; Ballantyne and Swantson, 1978). Exposure durations ranged from 5 to 300 minutes and concentrations ranged from 37 to 5176 mg/m^3. Mortality incidences ranged from 0 to 100%, depending on concentration-duration pairings. The experimental parameters are summarized in Tables 7, 9, 10, and 11.

7.3. Derivation of AEGL-3

Using the rat, mouse, rabbit, guinea pig, dog, and monkey data sets of McNamara et al.. (1969); Ballantyne and Calloway (1972); and Ballantyne and Swantson (1978) presented in Tables 7, 9, 10, and 11, the threshold for lethality at each AEGL-3 exposure duration was calculated using the probit-analysis based dose-response program of ten Berge (2006) (Appendix B). The threshold for lethality was set at the LC_{01}. The rat, mouse, and rabbit data all yielded similar time-scaling 'n' values and AEGL-3 values (Appendix B). Large variances in dog and monkey data precluded calculation of 95% confidence intervals. The rat data set was chosen for derivation of AEGL-3 values because it yielded values most consistent with the available human data. The rat data indicated a time-scaling value of 0.704 ($C^{0.704}$ x t = k). The 4-hour AEGL-3 value was adopted as the 8-hour AEGL-3 value because time scaling yielded an 8-hour value inconsistent with the AEGL-2 values that were derived from a rather robust human data set. This is likely a result of the methodology (time-scaling to 8-hrs with an exponent 'n' of 0.704).

Inter- and intraspecies uncertainty factors of 3 each are applied (total 10) and are considered sufficient because clinical signs are likely caused by a direct chemical effect on the tissues. This type of portal-of-entry effect is not likely to vary greatly between species or among individuals. The interspecies UF of 3 is supported by calculated LCT_{50} values of 88,480 mg min/m^3 for rats; 67,200 mg min/m^3 for guinea pigs; 54,090 mg min/m^3 for rabbits; and 50,010 mg min/m^3 for mice (Ballantyne and Swantson, 1978), values all well within a factor of two. The intraspecies UF of 3 is supported by the fact that responses of volunteers with jaundice, hepatitis, or peptic ulcer or those that were 50-60 years old were similar to those of "normal" volunteers when exposed to highly irritating concentration of CS for short durations (Punte et al., 1963; Gutentag et al., 1960). AEGL-3 values are summarized in Table 14 and calculations are in Appendix A.

TABLE 14. AEGL-3 Values for Tear Gas

10-min	30-min	1-h	4-h	8-h
140 mg/m^3	29 mg/m^3	11 mg/m^3	1.5 mg/m^3	1.5 mg/m^3

The AEGL-3 values are considered protective. No mortality was noted in rats exposed to 1802 mg/m^3 for 10-min (Ballantyne and Swantson, 1978), in rabbits at 1434 mg/m^3 for 10 min (Ballantyne and Swantson, 1978), or in mice and rabbits at 4250 mg/m^3 for 10-min (Ballantyne and Calloway, 1972). Dividing these concentrations by a total UF of 10, yields values ranging from 140-425 mg/m^3, suggesting that the derived 10-min AEGL-3 is appropriate. No mortality was noted in guinea pigs exposed to 44.7 mg/m^3 for 5-hr or mice exposed to 40 mg/m^3 for 5-hr (Ballantyne and Calloway, 1972). Applying a total UF of 10 to these concentrations, yields a value of approximately 4.0 mg/m^3 for 5-hours. One of ten rats died when exposed to 37 mg/m^3 for 5-hr Ballantyne and Calloway, 1972). Dividing 37 mg/m^3 by 2 to obtain an approximate threshold for lethality, yields 18.5 mg/m^3; application of a total UF of 10, yields a value of 1.9 mg/m^3 for 5-hr. The values derived from the 5-hr data show that the AEGL-3 values are protective.

8. SUMMARY OF AEGLs

8.1. AEGL Values and Toxicity Endpoints

AEGL-1and AEGL-2 values are based on irritation in humans, and AEGL-3 values are based on an estimated threshold for lethality in rats. AEGL values for tear gas are summarized in Table 15.

TABLE 15. Summary of AEGL Values

Classification	Exposure Duration				
	10-min	30-min	1-h	4-h	8-h
AEGL-1 (Nondisabling)	0.050 mg/m^3	0.050 mg/m^3	0.050 mg/m^3	0.050 mg/m^3	0.050 mg/m^3
AEGL-2 (Disabling)	0.50 mg/m^3	0.50 mg/m^3	0.50 mg/m^3	0.50 mg/m^3	0.50 mg/m^3
AEGL-3 (Lethal)	140 mg/m^3	29 mg/m^3	11 mg/m^3	1.5 mg/m^3	1.5 mg/m^3

8.2. Comparison with Other Standards and Guidelines

Extant standards and guidelines for tear gas are presented in Table 16.

TABLE 16. Standards and Guidelines for Tear Gas

Guideline	Exposure Duration				
	10-min	30-min	1-h	4-h	8-h
AEGL-1	0.050 mg/m^3	0.050 mg/m^3	0.050 mg/m^3	0.050 mg/m^3	0.050 mg/m^3
AEGL-2	0.50 mg/m^3	0.50 mg/m^3	0.50 mg/m^3	0.50 mg/m^3	0.50 mg/m^3
AEGL-3	140 mg/m^3	29 mg/m^3	11 mg/m^3	1.5 mg/m^3	1.5 mg/m^3
ERPG-1 (AIHA)[a]			0.005 mg/m^3		
ERPG-2 (AIHA)[a]			0.1 mg/m^3		
ERPG-3 (AIHA)[a]			25 mg/m^3		
IDLH (NIOSH)[b]		2 mg/m^3			
REL-TWA (NIOSH)[c]					0.4 mg/m^3
PEL-TWA (OSHA)[d]					0.4 mg/m^3
TLV-STEL (ACGIH)[e]	0.005 ppm (0.4 mg/m^3)				
MAC (The Netherlands)[f]					0.4 mg/m^3

[a]ERPG (Emergency Response Planning Guidelines, American Industrial Hygiene Association (AIHA 2008)
The ERPG-1 is the maximum airborne concentration below which it is believed nearly all individuals could be exposed for up to one hour without experiencing other than mild, transient adverse health effects or without perceiving a clearly defined objectionable odor. The ERPG-1 for tear gas is based on one of ten individuals reporting a burning or itching sensation at 0.0004 mg/m^3 and a calculated EC50 of 0.004 mg/m^3.

The ERPG-2 is the maximum airborne concentration below which it is believed nearly all individuals could be exposed for up to one hour without experiencing or developing irreversible or other serious health effects or

symptoms that could impair an individual's ability to take protection action. The ERPG-2 for tear gas is based on human irritation data.
The ERPG-3 is the maximum airborne concentration below which it is believed nearly all individuals could be exposed for up to one hour without experiencing or developing life-threatening health effects. The ERPG-3 for tear gas is based on animal lethality data (approximate one-hr LC_{50}).

[b]Immediately Dangerous to Life and Health (IDLH) is defined by the NIOSH/OSHA Standard Completions Program only for the purpose of respirator selection and represents a maximum concentration from which, in the event of respiratory failure, one could escape within 30 minutes without experiencing any escape-impairing or irreversible health effects (NIOSH, 2005). (Basis: Acute inhalation toxicity in humans, Punte et al., 1963).

[c]NIOSH REL-TWA (National Institute of Occupational Safety and Health, Recommended Exposure Limits - Time Weighted Average) (NIOSH 2005) is defined analogous to the ACGIH-TLV-TWA.

[d]OSHA PEL-TWA (Occupational Health and Safety Administration, Permissible Exposure Limits - Time Weighted Average) (OSHA 1996) is defined analogous to the ACGIH-TLV-TWA, but is for exposures of no more than 10 hours/day, 40 hours/week.

[e]ACGIH TLV-STEL (American Conference of Governmental Industrial Hygienists, Threshold Limit Value - Short Term Exposure Limit) (ACGIH 2008) is for a 15-minute exposure, ceiling.

[f]MAC (Maximaal Aanvaarde Concentratie [Maximal Accepted Concentration]). SDU Uitgevers (under the auspices of the Ministry of Social Affairs and Employment), The Hague, The Netherlands 2000, is defined analogous to the ACGIH-TLV-TWA.

9. REFERENCES

ACGIH (American Conference of Government and Industrial Hygienists). 1991. Documentation of the Threshold Limit Values and Biological Exposure Indices: o-Chlorobenzylidene malononitrile. Sixth ed., ACGIH, Cincinnati, OH.

ACGIH (American Conference of Government and Industrial Hygienists). 2008. TLVs and BEIs Based on the Documentation of the Threshold Limit Values for Chemical Substances and Physical Agents and Biological Exposure Indices: o-Chlorobenzylidene malononitrile. ACGIH, Cincinnati, OH.

AIHA (American Industrial Hygiene Association). 2008. Emergency Response Planning Guidelines for o-chlorobenzylidene malononitrile. AIHA, Fairfax, VA.

Anderson, P.J., Lau, G.S., Taylor, W.R., Critchley, J.A. 1996. Acute effects of the potent lacrimator o-chlorobenzylidene malononitrile (CS) tear gas. Hum. Exp. Toxicol. 15: 461-465.

Ballantyne, B. 1977. Riot control agents. In: Medical Annual. Eds.: R.B. Scott and J. Fraser. John Wright & Sons, Ltd., Bristol, Great Britain. pp. 7-41.

Ballantyne, B., Beswick, F.W. 1972. On the possible relationship between diarrhea and o-chlorobenzylidene malononitrile (CS). Med. Sci. Law. 12: 121-128.

Ballantyne, B., Callaway, S. 1972. Inhalation toxicology and pathology of animals exposed to o-chlorobenzylidene malononitrile. Med. Sci. Law 12: 43-65.

Ballantyne, B., Swanston, D.W. 1978. The comparative acute mammalian toxicity of 1-chloroacetophenone (CN) and 2-chlorobenzylidene malononitrile (CS). Arch Toxicol; 40: 75-95.

Bauchinger, M., Schmid, E. 1992. Clastogenicity of 2-chlorobenzylidene malonitrile (CS) in V79 Chinese Hamster cells. Mutat. Res. 282:231-234.

Beswick, F.W., Holland, P., Kemp, K.H. 1972. Acute effects of exposure to orthochlorobenzilidene malononitrile (CS) and the development of tolerance. Br. J. Indust. Med. 29: 298-306.

Blain, P.G. 2003. Tear gases and irritant incapacitants. 1-chloroacetophenone, 2-chlorobenzylidene malononitrile and dibenz[b,f]-1,4-oxazepine. Toxicol. Rev. 22: 103-210.

Breakell, A., Bodiwal, G.G. 1998. CS gas exposure in a crowded night club: the consequences for an accident and emergency department. J Accid. Emerg. Med. 15: 56-57.

Cole, T.J., Cotes, J.E., Johnson, G.R., de V. Martin, H., Reed, J.W., Saunders, M.J. 1975. Comparison of effects of ammonia and CS aerosol upon exercise ventilation and cardiac frequency in healthy men. J. Physiol. 252: 28P-29P.

Cole, T.J., Cotes, J.E., Johnson, G.R., de V. Martin, H., Reed, J.W., Saunders, M.J. 1977. Ventilation, cardiac frequency and pattern of breathing during exercise in men exposed to o-chlorobenzylidene malononitrile (CS) and ammonia gas in low concentrations. Q. J. Exp. Physiol. 62: 341-351.

Colgrave, H.F., Creasey, J.M. 1975. Ultrastructure of rat lungs following exposure to o-chlorobenzylidene malononitrile (CS). Med. Sci. Law. 15:187-197.

Corson, B.B., Stoughton, R.W. 1928. Reaction of α,β-unsaturated dinitriles. J. Am. Chem. Soc. 50: 2825-2837.

Cotes, J.E., Evans, L.R., Johnson, GR, de V. Martin, H., Reed, J.W. 1972. The effect of CS aerosol upon exercise ventilation and cardiac frequency in healthy men. J. Physiol. 222: 77P-78P.

Craig, F.N., Blevin, W.V., Cummings, E.G. 1960. Breathing patterns during human exposure to CS. US Army Chemical Corps Research and Develop Command, Report No. CWLR 2399, June 1960, Maryland.

Cucinell, S.A., Swentzel, K.C., Biskup, R., Snodgrass, H., Lovre, S., Stark, W., Feinsilver, L., and Vocci, R. 1971. Biochemical interactions and metabolic fate of riot-control agents. Fed. Proc. 30: 86-91.

Däniken, A., Friederich, U., Lutz, W.K., Schlatter, C. 1981. Tests for mutagenicity in Salmonella and covalent binding to DNA and protein in the rat of the riot control agent o-chlorobenzylidene malononitrile (CS). Arch. Toxicol. 49: 15-27.

Euripidou, E., MacLehose, R., Fletcher, A. 2004. An investigation into the short term and medium term health impacts of personal incapacitant sprays. A follow up of patients reported to the National Poisons Information Service (London). Emerg. Med. J. 21: 548-252.

Gaskins, J.R., Hehir, R.M., McCaulley, D.F., Ligon, E.W. 1972. Lacrimating agents (CS and CN) in rats and rabbits: Acute effects on mouth, eyes, and skin. Arch. Environ. Health. 24: 449-452.

Gray, P.J. 2000. Is CS spray dangerous? Formulation affects toxicity. B.M.J. 321: 46-47.

Gutentag, P.J., Hart, J., Owens, E.J., Punte, C.L. 1960. The evaluation of CS aerosol as a riot control agent in man. Technical Report CWLR 2365, April 1960, U.S. Army Chemical Warfare Laboratories, Army Chemical Center.

Haber, F.R. 1924. Zur geschichte des gaskrieges [On the history of the gas war]. In: Fuenf Vortraege aus den Jahren 1920-23 [Five lectures from the years 1920-1923]. Berlin, Germany: Verlag von Julius Springer; pp. 76-92.

Himsworth, H. 1969. Report of the Enquiry into the Medical and Toxicological Aspects of CS (Orthochlorobenzylidene Malononitrile). Part I. Enquiry into the Medical Situation Following the Use of CS in Londonderry on 13th and 14th August, 1969. Cmnd 4173, H.M.S.O., London, 1969.

HSDB (Hazardous Substances Data Base). 2008. Chlorobenzylidene malononitrile. National Library of Medicine's TOXNET System (http://toxnet.nlm.nih.gov). Retrieved on-line 2/28/2008.

Hu, H., Christiani, D. 1992. Reactive airways dysfunction after exposure to tear gas. Lancet. 339: 1535.

Hu, H., Fine, J., Epstein, P., Kelsey, K., Reynolds, P., and Walker, B. 1989. Tear gas-harassing agent or toxic chemical weapon? J. Am. Med. Assoc. 262: 660-663.

Kane, L.E., Barrow, C.S., Alarie, Y. 1979. A short-term test to predict acceptable levels of exposure to airborne sensory irritants. Am. Ind. Hyg. Assoc. J. 40: 207-229.

Karagama, Y.G., Newton, J.R., Newbegin, C.J. 2003. Short-term and long-term physical effects of exposure to CS spray. J. R. Soc. Med. 96: 172-174.

Leadbeater L. 1973. The absorption of ortho-chlorobenzylidenemalononitrile (CS) by the respiratory tract. Toxicol Appl. Pharmacol. 25: 101-110.

Marrs, T.C., Clifford, E., Colgrave, H.F. 1983a. Late inhalation toxicology and pathology produced by exposure to a single dose of 2-chlorobenzylidene malononitrile (CS) in rats and hamsters. Med. Sci. Law 23: 257-265.

Marrs, T.C., Colgrave, H.F., Cross, N.L., Gazzard, M.F., Brown, R.F. 1983b. A repeated dose study of the toxicity of inhaled 2-chlorobenzylidene malononitrile (CS) aerosol in three species of laboratory animal. Arch. Toxicol. 52: 183-198.

McDonald, E.C., Mahon, R.T. 2002. U.S. Marine Corps Amphibious Reconnaissance (Recon) students requiring hospitalization with pulmonary edema after strenuous exercise following exposure to o-chloro-benzylidenemalonitrile. Mil. Med. 167: iii-iv.

McElhatton, P.R., Sidhu, S., Thomas, S.H. 2004. Exposure to CS Gas in Pregnancy. J. Toxicol. Clin. Toxicol. 42: 547.

McGregor, D.B., Brown, A., Cattanach, P., Edwards, I., McBride, D., Caspary, W.J. 1988. Responses of the L5178Y tk^{+}/tk^{-} mouse lymphoma cell forward mutation assay II: 18 coded chemicals. Environ Mol Mutagen 11: 91-118.

McNamara, B.P., Owens, E.J., Weimer, J.T., Ballard, T.A., Vocci, F.J. 1969. Toxicology of riot control chemicals CS, CN, and DM. Edgewood Arsenal Technical Report, EATR-4309 (Nov. 1969), Dept of the Army, Edgewood Arsenal Medical Research Laboratory, Edgewood Arsenal, MD.

Meshram, G.P., Malini, R.P., Rao, K.M. 1992. Mutagenicity evaluation of riot control agent o-chlorobenzylidene malononitrile (CS) in the Ames Salmonella/microsome test. J. Appl. Toxicol. 12:377-384.

Ministry of Social Affairs and Employment (SDU Uitgevers). 2000. Nationale MAC (Maximum Allowable Concentration) List, 2000. The Hague, The Netherlands.

NIOSH (National Institute of Occupational Safety and Health). 1996. o-Chlorobenylidene malononitrile. Retrieved online from www.cdc.gov/niosh/idlh/2698411.html on 4/1/2007.

NIOSH (National Institute for Occupational Safety and Health). 2005. NIOSH Pocket Guide to Chemical Hazards. Retrieved online from www.cdc.gov/niosh/npg/npgd0122.html on 4/1/2007.

NRC (National Research Council). 2001. Standing operating procedures for developing acute exposure guideline levels for hazardous chemicals. Committee on Toxicology, Board on Toxicology and Environmental Health Hazards, Commission on Life Sciences, National Research Council. National Academy Press, Washington, DC.

NTP (National Toxicology Program). 1990. Toxicology and carcinogenesis studies of CS2 (94% o-chlorobenzalmalononitrile in F344/N rats and B6C3F1 mice (inhalation studies). NTP working group. National Toxicology Program Technical Report Series Vol: 377. 211 p.

O'Neil et al. 2001. The Merck Index. Merck & Co., Inc. Whitehouse Station, N.J. o-Chlorobenzylidenemalononitrile. p. 367.

OSHA. 1996. Limits for Air Contaminants. CFR, Title 29, Part 1910.1000, Table Z-1, retrieved online from www.osha.gov on 4/2/2007.

Owens, E.J., Punte, C.L. 1963. Human respiratory and ocular irritation studies utilizing o-chlorobenzilidene malononitrile aerosols. Ind. Hyg. J. 24:262-264.

Paradowski, M. 1979. Metabolism of toxic doses of o-chlorobenzylidene malononitrile in rabbits. Pol. J. Pharmacol. Pharm. 31: 563-572.

Park, S., and Giammona, S.T. 1972. Toxic effects of tear gas on an infant following prolonged exposure. Am. J. Dis. Child. 123(3): 245-246.

Punte, C.L., Owens, E.J., Gutentag, P.J. 1963. Exposures to ortho-chlorobenzylidene malononitrile: Controlled human exposures. Arch Environ Health 6: 72-80.

Punte, C.L., Weimer, J.T., Ballard, T.A., Wilding, J.L. 1962. Toxicologic studies on o-chlorobenzylidene malononitrile. Toxicol. Appl. Pharmacol. 4: 656-662.

Rengstorff, R.H. 1969. The effects of the riot control agent CS on visual acuity. Milit. Med. 134: 219-221.

Rietveld, E.C., Delbressine, L.P., Waegemaekers, T.H., Seutter-Berlage, F. 1983. 2-Chlorobenzylmercapturic acid, a metabolite of the riot control agent 2-chlorobenzylidene malononitrile (CS) in the rat. Arch. Toxicol. 54: 139-144.

Rietveld, E.C., Hendrikx, M.M.P., Seutter-Berlage, F. 1986. Glutathione conjugation of chlorobenzylidene malononitriles in vitro and the biotransformation to mercapturic acids in rats. Arch. Toxicol. 59: 228-234.

Rinehart, W. E., Hatch, T. 1964. Concentration-time product (CT) as an expression of dose in sublethal exposures to phosgene. Ind. Hyg. J. 25: 545-553.

Roth, V.S., Franzblau, A. 1996. RADS after exposure to a riot-control agent: a case report. J. Occup. Environ. Med. 38: 863-865.

Salassidis, K., Schmid, E., Bauchinger, M. 1991. Mitotic spindle damage induced by 2-chlorobenzylidene malonitrile (CS) in V79 Chinese hamster cells examined by differential staining of the spindle apparatus and chromosomes. Mutat. Res. 262: 263-266.

Schmid, E., Bauchinger, M. 1991. Analysis of the aneuploidy inducing capacity of 2-chlorobenzylidene malonitrile (CS) and metabolites in V79 Chinese hamster cells. Mutagenesis. 6: 303-305.

Striker, G.E., Streett, C.S., Ford, D.F., Herman, L.H., Helland, D.R. 1967. U.S. Clearinghouse

Fed. Sci. Tech. Inform., AD-808732.

ten Berge, W.F., Zwart, A., Appelman, L.M. 1986. Concentration-time mortality response relationship of irritant and systemically acting vapours and gases. J. Hazard. Mat. 13: 301-309.

ten Berge. W.F. 2006. Online Excel Program: http://home.wxs.nl/~wtberge/doseresp.html.

Thomas, R.J., Smith, P.A., Rascona, D.A., Louthan, J.D., Gumpert, B. 2002. Acute pulmonary effects from o-chlorobenzylidenemalonitrile "tear gas": a unique exposure outcome unmasked by strenuous exercise after a military training event. Mil. Med. 167:136-139.

Upshall, D.G. 1973. Effects of o-chlorobenzylidene malononitrile(CS) and the stress of aerosol inhalation upon rat and rabbit embryonic development. Toxicol. Appl. Pharmacol. 24: 45-59.

U.S. Army, Marine Corps, Navy, Air Force. 2005. Military compounds and their properties. Chapter 3, In: Potential Military Chemical/Biological Agents and Compounds. FM 3-11.9, MCRP 3-37.1B, NTRP, 3-11.32, AFTTP(I) 3.255; January 2005.

WHO (World Health Organization). 1970. Health Aspects of Chemical and Biological Weapons. Report of a World Health Organization Group of Consultants. Geneva: WHO.

Wild, D., Eckhardt, K., Harnasch, D., King, M.T. 1983. Genotoxicity study of CS (ortho-chlorobenzylidenemalononitrile) in Salmonella, Drosophila, and mice. Failure to detect mutagenic effects. Arch. Toxicol. 54: 167-170.

Ziegler-Skylakakis, K., Summer, K.H., Andrae, U. 1989. Mutagenicity and cytotoxicity of 2-chlorobenzylidene malonitrile (CS) and metabolites in V79 Chinese hamster cells. Arch. Toxicol. 63: 314-319.

APPENDIX A: Derivation of Tear Gas AEGLs

Derivation of AEGL-1 Values

Key Study: Punte et al., 1963

Toxicity endpoint:

Human exposure to 1.5 mg/m^3 for 90 minutes. All four subjects could tolerate the exposure, but experienced eye and nose irritation and headache.

Time scaling:

Not applied. The critical effect (irritation) is a function of direct contact with the tear gas and is not likely to increase with duration of exposure at this level of severity (NRC, 2001).

Uncertainty factors: Total uncertainty factor: 3
Interspecies: 1, human data
Intraspecies: 3, contact irritation is a portal of entry effect and is not expected to vary widely between individuals. The intraspecies UF of 3 is also supported by the fact that responses of volunteers with jaundice, hepatitis, or peptic ulcer or those that were 50-60 years old were similar to those of "normal" volunteers when exposed to a highly irritating concentration of CS for short durations (Punte et al., 1963; Gutentag et al., 1960).

Modifying factor: 10: Reduction of point-of-departure from LOAEL to NOAEL for AEGL-1 effects

Calculations:

10-minute, 30-minute, 1-hour, 4-hour and 8-hour AEGL-1:

1.5 mg/m^3 ÷ 10 ÷ 3 = 0.050 mg/m^3

Derivation of AEGL-2 Values

Key Study: Punte et al., 1963

Toxicity endpoint:

Human exposure to 1.5 mg/m^3 for 90 minutes. All four subjects could tolerate the exposure, but experienced eye and nose irritation and headache.

Time scaling:

Not applied. The critical effect (irritation) is a function of direct contact with the tear gas and is not likely to increase with duration of exposure at this level of severity (NRC, 2001).

Uncertainty factors: Total uncertainty factor: 3
Interspecies: 1, human data
Intraspecies: 3, contact irritation is a portal of entry effect and is not expected to vary widely between individuals. The intraspecies UF of 3 is also supported by the fact that responses of volunteers with jaundice, hepatitis, or peptic ulcer or those that were 50-60 years old were similar to those of "normal" volunteers when exposed to a highly irritating concentration of CS for short durations (Punte et al., 1963; Gutentag et al., 1960).

Modifying factor: None applied

Calculations:

10-minute, 30-minute, 1-hour, 4-hour and 8-hour AEGL-2:

1.5 mg/m^3 ÷ 3 = 0.50 mg/m^3

Derivation of AEGL-3 Values

Key Studies: McNamara et al.. (1969); Ballantyne & Calloway (1972); Ballantyne & Swantson (1978)

Toxicity endpoint: Threshold for lethality in rats (L_{01}) calculated using probit-analysis dose-response program of ten Berge (2006).

Time scaling: $C^n \times t = k$ where n = 0.704 based on rat lethality data. The 4-hour AEGL-3 value was adopted as the 8-hour AEGL-3 value because time scaling yielded an 8-hour value inconsistent with the AEGL-2 values that were derived from a rather robust human data set. This is likely a result of the methodology (time-scaling to 8-hrs with an exponent 'n' of 0.704).

Uncertainty factors: Total uncertainty factor: 10

Interspecies: 3 – effects are likely caused by a direct chemical effect on the tissues. This type of portal-of-entry effect is not likely to vary greatly between species. Supported by calculated LCT_{50} values of 88,480 mg min/m^3 for rats; 67,200 mg min/m^3 for guinea pigs; 54,090 mg min/m^3 for rabbits; and 50,010 mg min/m^3 for mice (Ballantyne and Swantson, 1978), values all well within a factor of two.

Intraspecies: 3- effects are likely caused by a direct chemical effect on the tissues. This type of portal-of entry effect is not likely to vary greatly among individuals. Supported by the fact that responses of volunteers with jaundice, hepatitis, or peptic ulcer or those that were 50-60 years old were similar to those of "normal" volunteers when exposed to highly irritating concentration of CS for short durations (Punte et al., 1963; Gutentag et al., 1960).

Data for calculations		
Concentration (mg/m^3)	**Exposure duration (min)**	**Mortality incidence**
560	25	1/10
543	35	2/10
489	45	3/10
454	55	5/10
500	60	2/10
500	80	6/10
500	90	8/10
750	30	0/8
150	120	0/8
3950	5	0/10
4760	5	0/10
4250	10	1/10
4330	10	1/10
4150	15	0/10
5167	15	7/10
4000	20	9/10
4300	20	8/10
1802	10	0/20
1806	45	8/20
1911	45	9/20
2629	60	20/21
2699	60	20/20
37	300	1/10

Program output		
Exposure Duration	**LC_{01} point estimate**	**AEGL-3 Value (UF = 10)**
10 minutes	1385 mg/m^3	140 mg/m^3
30 minutes	291 mg/m^3	29 mg/m^3
1 hour	109 mg/m^3	11 mg/m^3
4 hours	15 mg/m^3	1.5 mg/m^3
8 hours	5.6 mg/m^3	1.5 mg/m^3*

n= 0.704 mg/m^3

***** The 4-hour AEGL-3 value was adopted as the 8-hour AEGL-3 value because time scaling yielded an 8-hour value inconsistent with the AEGL-2 values that were derived from a rather robust human data set. This is likely a result of the methodology (time-scaling to 8-hrs with an exponent 'n' of 0.704).

APPENDIX B: Time Scaling Calculations

The relationship between dose and time for any given chemical is a function of the physical and chemical properties of the substance and the unique toxicological and pharmacological properties of the individual substance. Historically, the relationship according to Haber (1924), commonly called Haber's Law or Haber's Rule (i.e., $C \times t = k$, where C = exposure concentration, t = exposure duration, and k = a constant) has been used to relate exposure concentration and duration to effect (Rinehart and Hatch, 1964). This concept states that exposure concentration and exposure duration may be reciprocally adjusted to maintain a cumulative exposure constant (k) and that this cumulative exposure constant will always reflect a specific quantitative and qualitative response. This inverse relationship of concentration and time may be valid when the toxic response to a chemical is equally dependent upon the concentration and the exposure duration. However, an assessment by ten Berge et al. (1986) of LC_{50} data for certain chemicals revealed chemical-specific relationships between exposure concentration and exposure duration that were often exponential. This relationship can be expressed by the equation $C^n \times t = k$, where n represents a chemical specific, and even a toxic endpoint specific, exponent. The relationship described by this equation is basically the form of a linear regression analysis of the log-log transformation of a plot of C vs t. ten Berge et al. (1986) examined the airborne concentration (C) and short-term exposure duration (t) relationship relative to death for approximately 20 chemicals and found that the empirically derived value of n ranged from 0.8 to 3.5 among this group of chemicals. Hence, the value of the exponent (n) in the equation $C^n \times t = k$ quantitatively defines the relationship between exposure concentration and exposure duration for a given chemical and for a specific health effect endpoint. Haber's Rule is the special case where $n = 1$. As the value of n increases, the plot of concentration vs time yields a progressive decrease in the slope of the curve.

An n of 0.704 mg/m^3 for tear gas was obtained following analysis of lethality data in rats (McNamara et al., 1969; Ballantyne & Calloway, 1972; Ballantyne & Swantson, 1978) using the software of ten Berge. This exposure-time relationship for lethality was considered appropriate for AEGL-3 development. The 4-hour AEGL-3 value was adopted as the 8-hour AEGL-3 value because time scaling yielded an 8-hour value inconsistent with the AEGL-2 values that were derived from a rather robust human data set. This is likely a result of the methodology (time-scaling to 8-hrs with an exponent 'n' of 0.704).

1
2
3

Tear Gas ten Berge program results- lethality 1%

Species	Exponent 'n'	LC_{01} Point Estimate					Reference(s)
		10-min	30-min	1-hr	4-hr	8-hr	
Rat	0.704 (0.543-0.865)	1385 (477-2500)	290 (97-496)	109 (32-196)	15 (3.1-35)	5.6 (-0.93-15)	McNamara et al., 1969 Ballantyne and Calloway, 1972 Ballantyne and Swantson, 1978
Mouse	0.701 (0.509-0.892)	998 (208-1899)	208 (36-404)	77 (11-166)	11 (-0.86-3.2)	4.0 (-0.23-15)	McNamara et al., 1969 Ballantyne and Calloway, 1972 Ballantyne and Swantson, 1978
Rabbit	0.658 (0.467-0.849)	656 (227-1136)	124 (28-249)	43 (7.0-103)	5.2 (0.40-19)	1.8 (0.094- 8.6)	McNamara et al., 1969 Ballantyne and Calloway, 1972 Ballantyne and Swantson, 1978
Guinea pig	0.559 (0.018-1.099)	3.65 (0-100)	0.51 (0-25)	0.15 (0-12)	0.012 (0-3.3)	0.0036 (0-1.8)	McNamara et al., 1969 Ballantyne and Calloway, 1972 Ballantyne and Swantson, 1978
Dog	0.356 (-1.464-0.751)	349*	7604*	53150*	2597000*	18150000*	McNamara et al., 1969
Monkey	0.187 (-0.281-0.656)	26*	0.075*	0.0018*	0.0000011*	0.000000028*	McNamara et al., 1969 Striker et al., 1967
Monkey	2.123 (-21-25)	11*	6.6*	4.7*	2.5*	1.8*	McNamara et al., 1969

4 *Large variances precluded estimating 95% confidence limits.

Filename: Tear gas rat for Log Probit Model
Date: 02 October 2008 Time: 09:11:09

Seq.nr	conc mg/m3	minutes	exposed	responded
1	560	25	10	1
2	543	35	10	2
3	489	45	10	3
4	454	55	10	5
5	500	60	10	2
6	500	80	10	6
7	500	90	10	8
8	750	30	8	0
9	150	120	8	0
10	3950	5	10	0
11	4760	5	10	0
12	4250	10	10	1
13	4330	10	10	1
14	4150	15	10	0
15	5176	15	10	7
16	4000	20	10	9
17	4300	20	10	8
18	1802	10	20	0
19	1806	45	20	8
20	1911	45	20	9
21	2629	60	21	20
22	2699	60	20	20
23	37	300	10	1

Used Probit Equation Y = B0 + B1*X1 + B2*X2
X1 = conc mg/m3, ln-transformed
X2 = minutes, ln-transformed

ChiSquare = 50.11
Degrees of freedom = 20
Probability Model = 2.13E-04

Ln(Likelihood) = -47.54

B 0 = -9.6233E+00 Student t = -3.9484
B 1 = 1.1705E+00 Student t = 5.6748
B 2 = 1.6634E+00 Student t = 5.6382

variance B 0 0 = 5.9402E+00
covariance B 0 1 = -4.8485E-01
covariance B 0 2 = -6.7032E-01
variance B 1 1 = 4.2542E-02

covariance B 1 2 = 4.9302E-02
variance B 2 2 = 8.7045E-02

Estimation ratio between regression coefficients of ln(conc) and ln(minutes)
Point estimate = 0.704
Lower limit (95% CL) = 0.543
Upper limit (95% CL) = 0.865

Estimation of conc mg/m3 at response of 1 %
minutes = 10
Point estimate conc mg/m3 = 1.385E+03 for response of 1 %
Lower limit (95% CL) conc mg/m3 = 4.772E+02 for response of 1 %
Upper limit (95% CL) conc mg/m3 = 2.500E+03 for response of 1 %

Estimation of conc mg/m3 at response of 1 %
minutes = 30
Point estimate conc mg/m3 = 2.906E+02 for response of 1 %
Lower limit (95% CL) conc mg/m3 = 9.659E+01 for response of 1 %
Upper limit (95% CL) conc mg/m3 = 4.963E+02 for response of 1 %

Estimation of conc mg/m3 at response of 1 %
minutes = 60
Point estimate conc mg/m3 = 1.085E+02 for response of 1 %
Lower limit (95% CL) conc mg/m3 = 3.223E+01 for response of 1 %
Upper limit (95% CL) conc mg/m3 = 1.958E+02 for response of 1 %

Estimation of conc mg/m3 at response of 1 %
minutes = 120
Point estimate conc mg/m3 = 4.052E+01 for response of 1 %
Lower limit (95% CL) conc mg/m3 = 1.021E+01 for response of 1 %
Upper limit (95% CL) conc mg/m3 = 8.137E+01 for response of 1 %

Estimation of conc mg/m3 at response of 1 %
minutes = 240
Point estimate conc mg/m3 = 1.513E+01 for response of 1 %
Lower limit (95% CL) conc mg/m3 = 3.122E+00 for response of 1 %
Upper limit (95% CL) conc mg/m3 = 3.501E+01 for response of 1 %

Estimation of conc mg/m3 at response of 1 %
minutes = 480
Point estimate conc mg/m3 = 5.649E+00 for response of 1 %
Lower limit (95% CL) conc mg/m3 = 9.345E-01 for response of 1 %
Upper limit (95% CL) conc mg/m3 = 1.540E+01 for response of 1 %

APPENDIX C: Derivation Summary for Tear Gas

AEGL-1 VALUES FOR TEAR GAS				
10-min	**30-min**	**1-h**	**4-h**	**8-hour**
0.050 mg/m^3	0.050 mg/m^3	0.050 mg/m^3	0.050 mg/m^3	0.050 mg/m^3

Key Reference: Punte, C.L., Owens, E.J., Gutentag, P.J. 1963. Exposures to ortho-chlorobenzylidene malononitrile: Controlled human exposures. Arch Environ Health 6: 72-80.

Test Species/Strain/Number: Human/4

Exposure Route/Concentration/Duration: Inhalation/ 1.5 mg/m^3 for 90 minutes

Effects:
Clinical signs of irritation. Exposure tolerated for full 90-minutes. One subject developed nasal irritation within 2 minutes, three subjects developed headache (at 45, 50, and 83 minutes), and all four experienced ocular irritation (at 20, 24, 70, and 75 minutes).

Endpoint/Concentration/Rationale: 90-minute exposure to 1.5 mg/m^3 resulted in nasal and ocular irritation and headache.

Uncertainty Factors/Rationale:
Total uncertainty factor: 3
Interspecies: 1, human data.
Intraspecies: 3, contact irritation is a portal of entry effect and is not expected to vary widely between individuals. Also supported by the fact that responses of volunteers with jaundice, hepatitis, or peptic ulcer or those that were 50-60 years old were similar to those of "normal" volunteers when exposed to a highly irritating concentration of CS for short durations (Punte et al., 1963; Gutentag et al., 1960).

Modifying Factor: 10- Reduction of point-of-departure from LOAEL to NOAEL for AEGL-1 effects

Animal to Human Dosimetric Adjustment: Not applicable

Time Scaling: Not applied. The critical effect (irritation) is a function of direct contact with the tear gas and is not likely to increase with duration of exposure at this level of severity (NRC, 2001).

Data Adequacy: No data meeting the definition of AEGL-1 were available, necessitating MF application to estimate a NOAEL for AEGL-1 effects.

AEGL-2 VALUES FOR TEAR GAS				
10-minute	**30-minute**	**1-hour**	**4-h**	**8-h**
0.50 mg/m^3	0.50 mg/m^3	0.50 mg/m^3	0.50 mg/m^3	0.50 mg/m^3

Key Reference: Punte, C.L., Owens, E.J., Gutentag, P.J. 1963. Exposures to ortho-chlorobenzylidene malononitrile: Controlled human exposures. Arch Environ Health 6: 72-80.

Test Species/Strain/Number: Human/4

Exposure Route/Concentration/Duration: Inhalation/ 1.5 mg/m^3 for 90 minutes

Effects:
Clinical signs of irritation. Exposure tolerated for full 90-minutes. One subject developed nasal irritation within 2 minutes, three subjects developed headache (at 45, 50, and 83 minutes), and all four experienced ocular irritation (at 20, 24, 70, and 75 minutes).

Endpoint/Concentration/Rationale: 90-minute exposure to 1.5 mg/m^3 resulted in nasal and ocular irritation and headache.

Uncertainty Factors/Rationale:
Total uncertainty factor: 3
Interspecies: 1, human data.
Intraspecies: 3, contact irritation is a portal of entry effect and is not expected to vary widely between individuals. Also supported by the fact that responses of volunteers with jaundice, hepatitis, or peptic ulcer or those that were 50-60 years old were similar to those of "normal" volunteers when exposed to a highly irritating concentration of CS for short durations (Punte et al., 1963; Gutentag et al., 1960).

Modifying Factor: None applied

Animal to Human Dosimetric Adjustment: Not applicable

Time Scaling: Not applied. The critical effect (irritation) is a function of direct contact with the tear gas and is not likely to increase with duration of exposure at this level of severity (NRC, 2001).

Data Adequacy: AEGL-2 values are supported by the data of Beswick et al. (1972). When a total of 35 subjects were exposed for 60 minutes to CS concentrations ranging from 0.31-2.3 mg/m^3, one subject left at 5 minutes due to vomiting but returned for the duration of the exposure, and another vomited at 55 minutes of exposure (vomiting in both cases ascribed to swallowing large amounts or saliva). One subject voluntarily left the exposure at 8 minutes due to irritation; this subject was exposed in the range of 0.56-0.86 mg/m^3, and the AEGL-2 values are below this exposure range. Although clinical signs of irritation were noted, five subjects exposed to a constant 0.78 mg/m^3 CS for 60 minutes all remained in the chamber for the entire exposure. Again, the AEGL-2 values are below this exposure concentration.

AEGL-3 VALUES FOR TEAR GAS				
10-min	**30-min**	**1-h**	**4-h**	**8-h**
140 mg/m^3	29 mg/m^3	11 mg/m^3	1.5 mg/m^3	1.5 mg/m^3

Key References: McNamara, B.P., Owens, E.J., Weimer, J.T., Ballard, T.A., Vocci, F.J. 1969. Toxicology of riot control chemicals CS, CN, and DM. Edgewood Arsenal Technical Report, EATR-4309 (Nov. 1969), Dept of the Army, Edgewood Arsenal Medical Research Laboratory, Edgewood Arsenal, MD.

Ballantyne, B., Callaway, S. 1972. Inhalation toxicology and pathology of animals exposed to o-chlorobenzylidene malononitrile. Med. Sci. Law 12: 43-65.

Ballantyne, B., Swanston, D.W. 1978. The comparative acute mammalian toxicity of 1-chloroacetophenone (CN) and 2-chlorobenzylidene malononitrile (CS). Arch Toxicol; 40: 75-95.

Test Species/Strain/Number: Rat/Various /8, 10, 20, or 21 per group

Exposure Route/Concentration/Duration: Inhalation/Rats exposed to varying concentrations of tear gas for varying durations. Exposure durations ranged from 5 to 300 minutes and concentrations ranged from 37 to 5176 mg/m^3.

Effects: Mortality: Concentrations, durations, incidence as in Appendix A: Derivation of AEGL-3 values.

Endpoint/Concentration/Rationale: Threshold for lethality in rats (LC01) calculated using probit-analysis dose-response program of ten Berge (2006).

Uncertainty Factors/Rationale:

Total uncertainty factor: 10

Interspecies: 3, effects are likely caused by a direct chemical effect on the tissues. This type of portal-of-entry effect is not likely to vary greatly between species. Supported by calculated LCT_{50} values of 88,480 mg min/m^3 for rats; 67,200 mg min/m^3 for guinea pigs; 54,090 mg min/m^3 for rabbits; and 50,010 mg min/m^3 for mice (Ballantyne and Swantson, 1978), values all well within a factor of two.

Intraspecies: 3, effects are likely caused by a direct chemical effect on the tissues. This type of portal-of entry effect is not likely to vary greatly among individuals. Supported by the fact that responses of volunteers with jaundice, hepatitis, or peptic ulcer or those that were 50-60 years old were similar to those of "normal" volunteers when exposed to highly irritating concentration of CS for short durations (Punte et al., 1963; Gutentag et al., 1960).

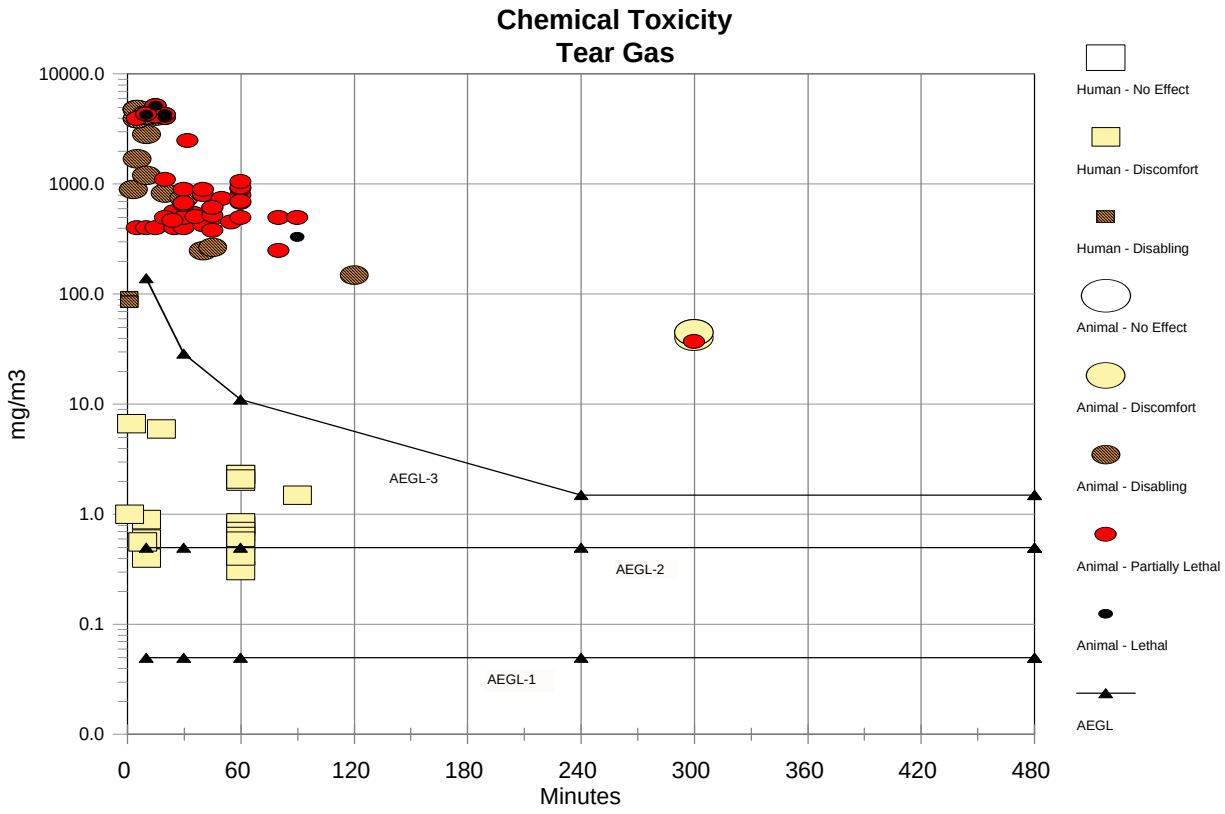
Modifying Factor: None applied

Animal to Human Dosimetric Adjustment: Not applicable

Time Scaling: $C^n \times t = k$, where n = 0.704 based on rat lethality data. The 4-hour AEGL-3 value was adopted as the 8-hour AEGL-3 value because time scaling yielded an 8-hour value inconsistent with the AEGL-2 values that were derived from a rather robust human data set. This is likely a result of the methodology (time-scaling to 8-hrs with an exponent 'n' of 0.704).

Data Adequacy: The AEGL-3 values are considered protective. No mortality was noted in rats exposed to 1802 mg/m^3 for 10-min (Ballantyne and Swantson, 1978), in rabbits at 1434 mg/m^3 for 10 min (Ballantyne and Swantson, 1978), or in mice and rabbits at 4250 mg/m^3 for 10-min (Ballantyne and Calloway, 1972). Dividing these concentrations by a total UF of 10, yields values ranging from 140-425 mg/m^3, suggesting that the derived 10-min AEGL-3 is appropriate. No mortality was noted in guinea pigs exposed to 44.7 mg/m^3 for 5-hr or mice exposed to 40 mg/m^3 for 5-hr (Ballantyne and Calloway, 1972). Applying a total UF of 10 to these concentrations, yields a value of approximately 4.0 mg/m^3 for 5-hours. One of ten rats died when exposed to 37 mg/m^3 for 5-hr Ballantyne and Calloway, 1972). Dividing 37 mg/m^3 by 2 to obtain an approximate threshold for lethality, yields 18.5 mg/m^3; application of a total UF of 10, yields a value of 1.9 mg/m^3 for 5-hr. The values derived from the 5-hr data show that the AEGL-3 values are protective.

APPENDIX D: CATEGORY PLOT FOR TEAR GAS



1

Source	Species	Sex	# Exposures	mg/m^3	Minutes	Category	Comments
NAC/AEGL-1				0.050	10	AEGL	
NAC/AEGL-1				0.050	30	AEGL	
NAC/AEGL-1				0.050	60	AEGL	
NAC/AEGL-1				0.050	240	AEGL	
NAC/AEGL-1				0.050	480	AEGL	
NAC/AEGL-2				0.5 0	10	AEGL	
NAC/AEGL-2				0.5 0	30	AEGL	
NAC/AEGL-2				0.5 0	60	AEGL	
NAC/AEGL-2				0.5 0	240	AEGL	
NAC/AEGL-2				0.5 0	480	AEGL	
NAC/AEGL-3				140	10	AEGL	
NAC/AEGL-3				29	30	AEGL	
NAC/AEGL-3				11	60	AEGL	
NAC/AEGL-3				1.5	240	AEGL	
NAC/AEGL-3				1.5	480	AEGL	
	human		1	94	1	2	Intolerable airway and ocular irritation (Owens and Punte, 1963)
	human		1	85	1	2	Intolerable airway and ocular irritation (Owens and Punte, 1963)
	human		1	1.5	90	1	nasal & ocular irritation, headache (Punte et al, 1963)
	human		1	6.7	2	1	Intolerable irritation; escape possible (Punte et al, 1963)
	human		1	6	18	1	Intolerable irritation; escape possible (Punte et al, 1963)
	human		1	0.4	10	1	Intense eye irritation (Rengsdorf, 1969)
	human		1	0.6	10	1	Intense eye irritation (Rengsdorf, 1969)
	human		1	0.9	10	1	Intense eye irritation (Rengsdorf, 1969)
	human		1	1	1	1	Intense eye irritation (Rengsdorf, 1969)
	human		1	0.78	60	1	eye, nose, throat irritation, nausea, chest discomfort, headaces (Beswick et al., 1972)
	human		1	0.56	8	1	eye, nose, throat irritation, nausea, chest discomfort, headaces (Beswick et al., 1972)
	human		1	0.31	60	1	eye, nose, throat irritation, nausea, chest discomfort, headaces (Beswick et al., 1972)
	human		1	0.8	60	1	eye, nose, throat irritation, nausea, chest discomfort, headaces (Beswick et al., 1972)
	human		1	0.84	60	1	eye, nose, throat irritation, nausea, chest discomfort, headaces (Beswick et al., 1972)
	human		1	2.3	60	1	eye, nose, throat irritation, nausea, chest discomfort, headaces (Beswick et al., 1972)
	human		1	0.7	60	1	eye, nose, throati rritation, nausea, chest discomfort, headaces (Beswick et al., 1972)
	human		1	2	60	1	eye, nose, throat irritation, nausea, chest discomfort, headaces (Beswick et al., 1972)
	human		1	0.63	60	1	eye, nose, throat irritation, nausea, chest discomfort, headaces (Beswick et al., 1972)
	human		1	2.3	60	1	eye, nose, throat irritation, nausea, chest discomfort, headaces (Beswick et al., 1972)
	human		1	0.57	60	1	eye, nose, throat irritation, nausea, chest discomfort, headaces (Beswick et al., 1972)

human	1	2.1	60	1	eye, nose, throat irritation, nausea, chest discomfort, headaces (Beswick et al., 1972)
human	1	0.42	60	1	eye, nose, throat irritation, nausea, chest discomfort, headaces (Beswick et al., 1972)
monkey	1	900	3	2	Pulmonary congestion, emphysema (Striker et al, 1967)
monkey	1	1700	5	2	Pulmonary congestion, emphysema (Striker et al, 1967)
monkey	1	2850	10	2	Pulmonary congestion, emphysema, ocular/respiratory irritation (Striker et al, 1967)
monkey	1	2500	32	pl	Severe irritation,Pulmonary edema, emphysema, Mortality: 5/8 (Striker et al, 1967)
mouse	1	40	300	1	Rhinorrhea and lacrimation (Ballantyne and Calloway, 1972)
rat	1	37	300	pl	Rhinorrhea and lacrimation; Mortality 1/10 (Ballantyne and Calloway, 1972)
GP	1	45	300	1	Sneezing (Ballantyne and Calloway, 1972)
rat	1	560	25	pl	Mortality: 1/10 (McNamara et al., 1969)
rat	1	543	35	pl	Mortality: 2/10 (McNamara et al., 1969)
rat	1	489	45	pl	Mortality: 3/10 (McNamara et al., 1969)
rat	1	454	55	pl	Mortality: 5/10 (McNamara et al., 1969)
rat	1	500	60	pl	Mortality: 2/10 (McNamara et al., 1969)
rat	1	500	80	pl	Mortality: 6/10 (McNamara et al., 1969)
rat	1	500	90	pl	Mortality: 8/10 (McNamara et al., 1969)
Mouse	1	1200	10	2	Mortality: 0/20 (McNamara et al., 1969)
mouse	1	1100	20	pl	Mortality: 7/20 (McNamara et al., 1969)
mouse	1	900	30	pl	Mortality: 2/20 (McNamara et al., 1969)
mouse	1	800	40	pl	Mortality: 5/20 (McNamara et al., 1969)
mouse	1	740	50	pl	Mortality: 5/20 (McNamara et al., 1969)
mouse	1	683	60	pl	Mortality: 14/20 (McNamara et al., 1969)
GP	1	400	5	pl	Mortality: 1/10 (McNamara et al., 1969)
GP	1	400	10	pl	Mortality: 2/10 (McNamara et al., 1969)
GP	1	400	15	pl	Mortality: 4/10 (McNamara et al., 1969)
GP	1	500	20	pl	Mortality: 3/10 (McNamara et al., 1969)
GP	1	400	25	pl	Mortality: 7/10 (McNamara et al., 1969)
GP	1	400	30	pl	Mortality: 7/10 (McNamara et al., 1969)
GP	1	425	40	pl	Mortality: 8/10 (McNamara et al., 1969)
Rabbit	1	500	30	pl	Mortality: 1/4 (McNamara et al., 1969)
Rabbit	1	250	40	2	Mortality: 0/4 (McNamara et al., 1969)
Rabbit	1	267	45	2	Mortality: 0/4 (McNamara et al., 1969)
Rabbit	1	250	80	pl	Mortality: 3/4 (McNamara et al., 1969)
Rabbit	1	333	90	3	Mortality: 4/4 (McNamara et al., 1969)
Dog	1	833	20	2	Mortality: o/4 (McNamara et al., 1969)
Dog	1	649	30	pl	Mortality: 1/4 (McNamara et al., 1969)
Dog	1	508	36	pl	Mortality: 2/4 (McNamara et al., 1969)
Dog	1	899	40	pl	Mortality: 2/4 (McNamara et al., 1969)
Dog	1	520	45	pl	Mortality: 2/4 (McNamara et al., 1969)
Dog	1	612	45	pl	Mortality: 2/4 (McNamara et al., 1969)
Dog	1	797	60	pl	Mortality: 3/4 (McNamara et al., 1969)
Dog	1	909	60	pl	Mortality: 2/4 (McNamara et al., 1969)
Monkey	1	469	24	pl	Mortality: 1/4 (McNamara et al., 1969)
Monkey	1	673	30	pl	Mortality: 2/4 (McNamara et al., 1969)
Monkey	1	381	45	pl	Mortality: 2/4 (McNamara et al., 1969)
Monkey	1	612	45	pl	Mortality: 1/4 (McNamara et al., 1969)
Monkey	1	699	60	pl	Mortality: 1/4 (McNamara et al., 1969)
Monkey	1	941	60	pl	Mortality: 3/4 (McNamara et al., 1969)

Monkey		1	1057	60	pl	Mortality: 2/4 (McNamara et al., 1969)
Rat	m	1	750	30	2	Mortality: 0/8 (Ballantyne and Calloway, 1972))
Rat		1	150	120	2	Mortality: 0/8 (Ballantyne and Calloway, 1972))
Rat		1	3950	5	2	Mortality: 0/10 (Ballantyne and Calloway, 1972)
Rat		1	4760	5	2	Mortality: 0/10 (Ballantyne and Calloway, 1972)
Rat		1	4250	10	pl	Mortality: 1/10 (Ballantyne and Calloway, 1972)
Rat		1	4330	10	pl	Mortality: 1/10 (Ballantyne and Calloway, 1972)
Rat		1	4150	15	2	Mortality: 0/10 (Ballantyne and Calloway, 1972)
Rat		1	5167	15	pl	Mortality: 7/10 (Ballantyne and Calloway, 1972)
Rat		1	4000	20	pl	Mortality: 9/10 (Ballantyne and Calloway, 1972)
Rat		1	4300	20	pl	Mortality: 8/10 (Ballantyne and Calloway, 1972)
Mouse		1	3950	5	pl	Mortality: 1/10 (Ballantyne and Calloway, 1972)
Mouse		1	4760	5	2	Mortality: 0/10 (Ballantyne and Calloway, 1972)
Mouse		1	4250	10	2	Mortality: 0/10 (Ballantyne and Calloway, 1972)
Mouse		1	4330	10	pl	Mortality: 4/10 (Ballantyne and Calloway, 1972)
Mouse		1	4150	15	pl	Mortality: 3/10 (Ballantyne and Calloway, 1972)
Mouse		1	5167	15	pl	Mortality: 3/10 (Ballantyne and Calloway, 1972)
Mouse		1	4000	20	pl	Mortality: 8/10 (Ballantyne and Calloway, 1972)
Mouse		1	4300	20	pl	Mortality: 6/10 (Ballantyne and Calloway, 1972)
GP		1	3950	5	pl	Mortality: 1/5 (Ballantyne and Calloway, 1972)
GP		1	4760	5	2	Mortality: 0/5 (Ballantyne and Calloway, 1972)
GP		1	4250	10	3	Mortality: 5/5 (Ballantyne and Calloway, 1972)
GP		1	4330	10	pl	Mortality: 3/5 (Ballantyne and Calloway, 1972)
GP		1	4150	15	pl	Mortality: 3/5 (Ballantyne and Calloway, 1972)
GP		1	5167	15	3	Mortality: 5/5 (Ballantyne and Calloway, 1972)
GP		1	4000	20	3	Mortality: 5/5 (Ballantyne and Calloway, 1972)
GP		1	4300	20.00	3	Mortality: 5/5 (Ballantyne and Calloway, 1972)
Rabbit		1	3950	5	2	Mortality: 0/5 (Ballantyne and Calloway, 1972)
Rabbit		1	4760	5	2	Mortality: 0/5 (Ballantyne and Calloway, 1972)
Rabbit		1	4250	10	2	Mortality: 0/5 (Ballantyne and Calloway, 1972)
Rabbit		1	4330	10	pl	Mortality: 2/5 (Ballantyne and Calloway, 1972)
Rabbit		1	4150	15	pl	Mortality: 2/5 (Ballantyne and Calloway, 1972)
Rabbit		1	5167	15	pl	Mortality: 2/5 (Ballantyne and Calloway, 1972)
Rabbit		1	4000	20	pl	Mortality: 4/5 (Ballantyne and Calloway, 1972)
Rabbit		1	4300	20	3	Mortality: 5/5 (Ballantyne and Calloway, 1972)
Rat	m	1	1802	10	2	Mortality: 0/20 (Ballantyne and Swantson, 1978)
Rat	m	1	1806	45	pl	Mortality: 8/20 (Ballantyne and Swantson, 1978)
Rat	m	1	1911	45	pl	Mortality: 9/20 (Ballantyne and Swantson, 1978)
Rat	m	1	2629	60	pl	Mortality: 20/21 (Ballantyne and Swantson, 1978)
Rat	m	1	2699	60	3	Mortality: 20/20 (Ballantyne and Swantson, 1978)
Mouse	m	1	1432	15	pl	Mortality: 1/40 (Ballantyne and Swantson, 1978)
Mouse	m	1	2753	20	pl	Mortality: 17/40 (Ballantyne and Swantson, 1978)
Mouse	m	1	2333	30	pl	Mortality: 10/19 (Ballantyne and Swantson, 1978)
Mouse	m	1	2400	30	pl	Mortality: 17/40 (Ballantyne and Swantson, 1978)
Mouse	m	1	2550	30	pl	Mortality: 24/36 (Ballantyne and Swantson, 1978)
GP	f	1	2326	10	pl	Mortality: 2/20 (Ballantyne and Swantson, 1978)
GP	f	1	2380	15	pl	Mortality: 2/10 (Ballantyne and Swantson, 1978)
GP	f	1	1685	25	pl	Mortality: 10/20 (Ballantyne and Swantson, 1978)
GP	f	1	2310	20	pl	Mortality: 8/20 (Ballantyne and Swantson, 1978)
GP	f	1	1649	30	pl	Mortality: 11/20 (Ballantyne and Swantson, 1978)
GP	f	1	1302	45	pl	Mortality: 9/11 (Ballantyne and Swantson, 1978)
GP	f	1	2041	30	pl	Mortality: 13/20 (Ballantyne and Swantson, 1978)

GP	f	1	2373	30	pl	Mortality: 10/19 (Ballantyne and Swantson, 1978)
Rabbit	f	1	846	5	2	Mortality: 0/10 (Ballantyne and Swantson, 1978)
Rabbit	f	1	836	10	2	Mortality: 0/10 (Ballantyne and Swantson, 1978)
Rabbit	f	1	1434	10	2	Mortality: 0/10 (Ballantyne and Swantson, 1978)
Rabbit	f	1	1802	10	pl	Mortality: 1/5 (Ballantyne and Swantson, 1978)
Rabbit	f	1	2188	15	pl	Mortality: 2/10 (Ballantyne and Swantson, 1978)
Rabbit	f	1	2380	15	pl	Mortality: 3/8 (Ballantyne and Swantson, 1978)
Rabbit	f	1	1407	30	pl	Mortality: 4/10 (Ballantyne and Swantson, 1978)
Rabbit	f	1	1653	30	pl	Mortality: 2/10 (Ballantyne and Swantson, 1978)
Rabbit	f	1	1309	45	pl	Mortality: 4/5 (Ballantyne and Swantson, 1978)
Rabbit	f	1	2118	45	pl	Mortality: 9/10 (Ballantyne and Swantson, 1978)
Rabbit	f	1	2133	60	pl	Mortality: 7/8 (Ballantyne and Swantson, 1978)
Rabbit	f	1	3066	60	pl	Mortality: 8/9 (Ballantyne and Swantson, 1978)

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