(2-Chlorobenzylidene)malononitrile

(CAS No: 2698-41-1)

Health-based Reassessment of Administrative Occupational Exposure Limits

Committee on Updating of Occupational Exposure Limits,
a committee of the Health Council of the Netherlands

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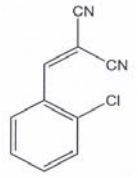
1 Introduction

The present document contains the assessment of the health hazard of (2-chlorobenzylidene)malononitrile (CS) by the Committee on Updating of Occupational Exposure Limits, a committee of the Health Council of the Netherlands. The first draft of this document was prepared by N. Smits, M.Sc. (Environmental and Occupational Health Group, Wageningen University and Research Centre, Wageningen, the Netherlands)*.

The evaluation of the toxicity of CS has been based on the review by the American Conference of Governmental Industrial Hygienists (ACGIH) (ACG99). Where relevant, the original publications were reviewed and evaluated as will be indicated in the text. In addition, in December 1999, literature was searched in the databases Medline, Toxline, and Chemical Abstracts, starting from 1966, 1985, and 1950, respectively, and using the following key words: chlorobenzylidenemalononitrile, chlorobenzylidene malononitrile, and 2698-41-1. The final literature search was carried out in Toxline and Medline in October 2003.

In October 2003, the President of the Health Council released a draft of the document for public review. Comments were received by the following individuals and organisations: TML Scheffers (DSM Chemelot, Geleen, the Netherlands). These comments were taken into account in deciding on the final version of the document.

2 Identity

name	:	(2-chlorobenzylidene)malononitrile
synonyms	:	(*o*- chlorobenzal)malononitrile; *o*-chlorobenzylidene malononitrile; 2-chlorobenzylidene malononitrile; ß,ß-dicyano-*o*-chlorostyrene; [(2-chlorophenyl)methylene)]propanedinitrile; CS
molecular formula	:	$C_{10}H_5ClN_2$
structural formula	:	
CAS number	:	2698-41-1

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3 Physical and chemical properties

molecular weight	:	188.62
boiling point	:	310-315°C
melting point	:	95-96°C
flash point	:	not available
vapour pressure	:	at 20°C: 0.005 Pa
solubility in water	:	not soluble (4 mg/100 mL)
log $P_{octanol/water}$	:	2.76 (estimated)
conversion factors	:	not applicable

Data from ACG99, NLM03, http://esc.syrres.com.

CS is a white crystalline solid with a pepper-like odour. It is rapidly hydrolysed following contact with water (half-life at pH 7.4 and 25°C: approximately 14 minutes) (ACG99).

4 Uses

CS came into widespread use as a tear gas in the 1960s and, because of its greater effectiveness and lower potential for toxicity, largely replaced the tear gas chloroacetophenone (CN) as the agent of choice for military and police crowd control missions. Lower concentrations of CS are required to achieve an equivalent response. It can be disseminated as a fine dust via an aerosol or, when mixed with a pyrotechnic compound, as a smoke or fog of minute particles from a grenade or canister. Sprays currently used by UK police forces contain a solution of CS in methyl isobutyl ketone. Methylene chloride and acetone are or have been used as well (ACG99, Bal77, Wor99).

5 Biotransformation and kinetics

Human data

Human volunteers exposed by inhalation to CS aerosols at concentrations of 0.03 to 9.0 mg·min/m^3, had no elevated thiocyanate levels in their urines (Swe70). One out of 6 human volunteers exposed to CS aerosol at a concentration of 90 mg·min/m^3 had trace amounts of the metabolite 2-chlorobenzylmalononitrile (see below) in his urine (Lea73).

Animal data

Following inhalation exposure of mice to CS aerosol (3500 mg/m^3, 6 min), thiocyanate was excreted in the urine within 24 hours at concentrations that were about 7 times higher compared to background levels. After an intraperitoneal injection of 146 µmoles/kg bw (27.5 mg/kg bw; 0.5 LD_{50}), about 50% of the CS dose was excreted as thiocyanate in the urine, more than 90% in the first 24 hours. It was suggested that CS is biotransformed into thiocyanate via malononitrile and subsequent release of cyanide. Cyanide concentrations in blood, following intraperitoneal administration, peaked between 4 and 16 minutes and the half-life of elimination was about 16 min. The time course of toxic symptoms was related to that of blood cyanide concentrations (Fra73). Following inhalation exposure of cats to CS aerosol (750 mg/m^3 for 60 min), CS and two of its metabolites, 2-chlorobenzylmalononitrile and 2-chlorobenzaldehyde, were detected in the blood. The half-life of CS in blood of 5.5 sec. was found after intra-arterial injection (Lea73). CS reacts with plasma proteins and glutathione *in vivo* (Cuc71). The latter was demonstrated by reduced sulphydryl concentrations in the plasma of dogs after intravenous doses of CS (Cuc71) and by excretion of the metabolite 2-chlorobenzylmercapturic acid in the urine of rats (4% of dose) following intraperitoneal administration of CS (Rie83). The fate of ^{3}H-ring-labelled, ^{14}C-cyanide labelled, and (^{14}C=C)-side-chain-labelled CS was studied in Porton rats by intravenous injections of doses of 0.08 to 80 µmol/kg bw (0.015-15 mg/kg bw) or giving oral (gavage) doses of 80 to 159 µmol/kg bw (15-30 mg/kg bw). The recovery of radioactivity in urine up to 96 hours after dosing was 44-74 and 55-100% after intravenous and oral administration, respectively. Following oral dosing, the principal urinary metabolites were 2-chlorohippuric acid (49% of dose), 1-*O*- (2-chlorobenzyl) glucuronic acid (9.8%), 2-chlorobenzyl cysteine (8.2%), and 2-chlorobenzoic acid (8.2%). Minor metabolites identified were 2-chlorophenyl acetyl glycine, 2-chlorobenzyl alcohol, and 2-chlorophenyl-2-cyanopropionate. It was also found that the proportion of urinary thiocyanate excretion increased with increasing oral CS doses (29.9 % of dose at 212 µmol/kg bw or 40 mg/kg bw) (Bre87). The principal pathways of the CS metabolism are shown in Figure 1 (see Annex I).

6 Effects and mechanism of action

Human data

Most studies on human health effects of CS are from CS application in riot control. When detonated outside, a CS grenade generates a cloud 6 to 9 meters in diameter; at the centre, a concentration of 2,000 to 5,000 mg/m^3 can be produced, with concentrations rapidly tapering off at the periphery (Wei69). Marked harassment occurred at exposures to 4 mg/m^3, with an almost immediate onset of effects on the eyes, the skin, and the respiratory tract, such as a burning sensation in the eye, excess lachrymation, blepharospasm, conjunctivitis, and photophobia, stinging or burning sensations of the skin, particularly in moist areas and often accompanied by erythema**,** and sneezing, coughing, soreness and tightness of the chest, difficulty with breathing, and voluntary holding of the breath. CS caused primary and allergic contact dermatitis (confirmed by patch testing) and skin burns, and factors influencing their severity included duration and degree of exposure, heat, humidity, and the material used as solvent. Other sighs and symptoms included increased salivation, irritation of the throat, nausea and occasionally vomiting and apprehension. In a few cases, CS induced symptoms of lung injury indicative of reactive airways dysfunction syndrome (RADS). Usually, recovery is complete within 15 to 30 minutes after the end of an exposure, although a few signs (like erythema of the lid margins and photophobia) may persist slightly longer. Delayed reactions, appearing hours to days after exposure, are extremely unlikely after brief outdoor or other low exposures. However, they may occur at unusually high doses and medically important injury may result from conditions such as excessive application of the agent, delivery in enclosed spaces, prolonged exposure (no way to flee), a high minute ventilation (during a fight), and (for skin reactions) high temperature and relative humidity. Despite reports on alleged fatal cases, mortality in humans following CS application has not been authenticated (Bal77, Hil00, Ola01). Histories of asthma, chronic obstructive pulmonary disease (COPD), or cardiac disease may exacerbate effects (Wor99).

Estimates of the human LCt_{50} (i.e., the concentration x time, lethal to 50% of an exposed population) range between 25,000 to 150,000 mg·min/m^3; estimates of the minimal irritant concentration and the ICt_{50} (i.e., the concentration x time, incapacitating/irritating to 50% of an exposed population) were 0.004 and 5 mg·min/m^3, respectively (Ola01).

Human volunteers (n=7) were exposed to CS aerosols dispersed from a 10% solution in methylene chloride or from the pure molten compound. The mass median aerodynamic diameters (MMAD) were 1.0 and 0.5 μm, respectively. Responses were measured in tolerance time, which was defined as the time a subject could no longer remain in an atmosphere containing CS. There were no differences in response between subjects exposed to aerosols obtained from the solution or from the molten compound. When 4 men were exposed to a concentration of 1.5 mg/m^3 for 90 minutes, 4 subjects developed slight eye irritation, 1 slight eye irritation, and 3 headache, which lasted for 24 hours in 2 of them. When after a 40-minute exposure to 1.5 mg/m^3, concentration was raised - without informing the subjects - to 11 mg/m^3 within about 10 minutes, all 4 volunteers left the exposure room within 2 minutes because of respiratory irritation. The first and last men left the room at estimated concentrations of ca. 4 and 7 mg/m^3, respectively. Exposure to 6 mg/m^3, attained in 10 minutes, induced eye, nose, throat, and skin irritation, sneezing, and chest burning, the latter being the cause of leaving the chamber by 3 out of 4 subjects within 18 to 29 minutes. In contrast, 3 out of 4 sustained a 60-minute exposure to 6.6 mg/m^3 gradually attained in 30 minutes, having the usual signs but to a lesser degree. The fourth person left the room after 2 minutes, at an estimated concentration of ca. 1 mg/m^3, because of a violent cough, voluntarily re-entered the chamber after a few minutes (estimated level: ca. 2 mg/m^3) until the end of the experiment. When 5 individuals were exposed to concentrations of 4 to 5 mg/m^3, the time to complete a set of simple mathematical problems was significantly affected, but accuracy was not impaired. When the 7 men received 10 exposures to CS concentrations ranging from 1-13 mg/m^3 over a 20-day period, no clinical abnormalities were observed, except for one person who had an abnormal thymol turbidity value, indicating an effect on the liver. The predominated symptom experienced by a subject in the initial exposure remained the dominant symptom upon repeated exposures. No volunteer developed a tolerance to CS (Pun63). Bestwick et al. studied the effects of acute exposure to CS aerosols on 35 healthy male volunteers by investigating eye irritation, pain and discomfort in the upper respiratory tract and chest, haematology and blood chemistry parameters, cardiology (electrocardiogram), and respiratory function (peak flow, tidal volume, vital capacity). There were 10 separate trials in which 2 to 6 volunteers were exposed for 1 hour to relatively low and constant concentrations of ca. 0.8 mg/m^3 (3 trials) or to concentrations progressively rising over the exposure period to final values of ca. 2 mg/m^3. Apart from changes that could be ascribed to emotional stress and discomfort of the experiment, no abnormalities were observed in the haematology, blood chemistry, cardiology, and respiratory

function tests. Two men left the inhalation chamber untimely: one at 8 minutes with severe stinging of the eyes, irritation of the throat, cough, dyspnoea, salivation, and complaining of nausea, the other at 55 minutes to vomit. All subjects experienced effects of exposure to CS instantaneously as unpleasant, but most of them found them tolerable after 4 to 5minutes even at about 4-foldly rising concentrations. Most prominent symptoms, observed in 50% of the volunteers, were watering and stinging eyes, running and stinging nose, stinging face, salivation, dry and irritating throat, and cough. In addition, nausea and burning and tight chest were frequently reported. Generally, severity of symptoms did not increase over the exposure period. However, in 2 trials, 4 subjects were exposed unprotectedly for the full hour to increasing concentrations and the other 4 wore respirators until 5 minutes from the end when concentrations were ca. 2 mg/m^3 while roles changed in the other trial. Seven volunteers tolerated exposure unprotectedly (the 8th leaving to vomit; see above), while when having worn a respirator, only one man was able to stay for longer than one minute and 5 left within 30 seconds. Generally, symptoms disappeared readily after removing from exposure (Bes72).

In a dermal human volunteer study, cutaneous irritant reactions produced by CS and CN were compared following application of dry or moistened test substance on 4 cm diameter for 1 hour. Application of amounts of dry CS of 20 and 25 mg caused faint erythema and transient irritation commencing up to 30 minutes following application in 3/3 and 3/7 volunteers, respectively, while no effects were observed in any of the volunteers (n=6/group) treated with doses ranging from 2 to 10 mg. Applying moistened CS at amounts of 10, 20, or 30 mg, faint to mild erythema and irritation was seen in about 50% or more of the volunteers (n=4 or 7/group), while amounts as little as 0.5 mg of dry or moistened CN produced erythema and vesication in more than 50% of the subjects (Hol72). Concentrations of 14,000 mg/m^3 or more for one hour under simulated, tropical conditions produced extreme irritation, erythema, and vesication of the skin. The dermal effects noted were related to climatic conditions, race, and skin qualities (Wei69).

In a chemical plant manufacturing CS, 25 out of 28 workers gave a history of dermatitis involving the arms and neck. Erythema was more likely present if the symptomatic site was under partial occlusion such as boots and the collar-gas mask area at the posterior part of the neck. Two of the 25 workers showed skin reactions consistent with allergic sensitisation to CS (by positive patch test reactions when tested with a 1:1000 dilution of CS in olive oil). CS concentrations in 3 sampling areas were 12 mg/m^3 during filling of plastic bags

in an open process, 1 mg/m^3 in the storage room, and 0.64 mg/m^3 during milling. In general, 50% of men would develop some degree of erythema at 3,5±1,5 mg/m^3 at moist and warm climatological conditions (Shm73).

Thomas et al. reported the hospitalisation of 9 U.S. marines, participating in a military training event, with a transient pulmonary syndrome (cough and shortness of breath in 9, haemoptosis in 5, hypoxia in 4). Symptoms began to appear 36 to 84 hours after heavy ('voluntary') exposure to CS during which the marines had been submitted to strenous physical exercise including a 1.5-mile run and several 1000-1500-meter pool or open-ocean swims. All signs and symptoms resolved within 3 days of admission, and lung function was normal when tested one week after exposure (Tho02). Although Thomas et al. ascribed the effects to CS, the committee is in agreement with McDonald and Mahon (McD02) of the opinion that they were more likely caused by the swimming. On one hand, CS exposure leads to acute, transient effects, mostly of the eyes, but sometimes of the respiratory tract. Delayed onset and/or persistent pulmonary symptoms are highly unusual, and despite the fact that about 200,000 marines have been exposed to CS since 1996 under field conditions, no such cases were reported. On the other hand, the symptoms appeared in all marines immediately after their swims and are similar to those associated with water aspiration or swimming-induced pulmonary oedema (McD02).

All, but 2 out of 34 young adults who had been exposed to CS spray in a confined space during a confrontation with the police (10 directly on the face, the others indirectly) still reported ocular symptoms one hour after the event. Other major symptoms concerned the respiratory tract (in 23) and the throat. At one month, ocular, throat, and respiratory symptoms were still present in 18, 20, and 14 individuals, respectively. At 10 months, 5 persons still complained of respiratory symptoms, but no abnormalities were seen at physical examination or lung function tests (Kar03).

Reproduction toxicity

In an in-depth inquiry into the adverse health and toxicological effects of CS following its use in Londonderry, Northern Ireland, in 1969, no increase in the incidence of spontaneous abortions, stillbirths, or congenital abnormalities was found, comparing a 9-month period of heavy tear gas exposure with a previous 9-month period (Ola01).

The committee did not find data from other studies.

Animal data

Irritation and sensitisation

Following 6-hour unoccluded application of 12.5% solutions of CS in acetone or corn oil, CS was found to be moderately irritating to the skin of rabbits (n=6/group) and slightly irritating to the skin of rats (n=6/group) and the guinea pigs (n=6/group). Within 7 to 14 days, all effects had resolved (Bal78). Following application of 0.5 mL of solutions of CS in trioctyl phosphate to the intact clipped back skin of rabbits for 30 minutes, primary irritation scores (representing mean values from 2 rabbits treated at each of 6 timed intervals between 1 and 30 minutes after exposure) of 2.3 (treated skin not washed) for a 1% solution and of 1.0 (unwashed) or 1.3 (skin washed with water or washed with water and soap) for 4% solutions were calculated. Scores of 5.0 or more were stated to be required to meet the definition for a skin irritant (Gas72).

Rothberg studied the skin-sensitising potential of CS in guinea pigs. Serial solutions (in polyethylene glycol) of 0.000001 to 1% were injected intradermally into guinea pigs (5 groups; n=2/group), 3 times a week, for 4 weeks. After a 3- to 4-week rest period, all animals were both intradermally and topically challenged with a 0.1% solution. The challenge doses caused erythema in 8/10 animals; the remaining 2 died during the sensitisation period. Another group of 6 animals was treated topically with a 1% solution of CS in acetone, 3 times a week, for 3 weeks. After a 2-week rest period, animals were challenged with a topically applied 0.1% solution of CS in acetone/olive oil (1:1), which caused a positive response in all animals. No evidence of skin damage was seen in the vehicle-treated controls (Rot70).

Topical administration (0.2 mL of 1% or 0.5% acetone solution) or intradermal administration (of 0.5 mL containing 10-25 g CS) and topical (0.1 mL of 0.1 to 1% solutions in acetone) or intradermal (0.1 mL of a solution in saline containing 1-10 µg/mL) challenge caused contact sensitisation or delayed hypersensitivity, especially when routes of induction and challenge were identical (Chu72).

Instillation of 0.05 of a 10% or of a 50% solution of CS in methylene dichloride into the eyes of rabbits resulted almost immediately in severe conjunctivitis persisting for 30 to 60 minutes. There also was erythema of the eyelids, lasting for a day or 2. No permanent eye damage was observed (Pun62).

Instillation of 1-4% solutions of CS in 1,1,1-trichloroethane into the eyes of rabbits resulted in transient conjunctival redness while solutions of 5 or 10% caused chemosis and moderate conjunctivitis, respectively, all eyes being normal

within 7 days. Corneal opacity or iritis was not observed (Gas72). Instillation of 0.1 mL of 0.5-10% solutions of CS in polyethylene glycol 300 into the eye of rabbits (n=10/group) caused lachrymation, blepharitis, and chemosis and hyperaemia of conjunctivae and nictating membranes at all concentrations, and sloughing, iritis, and keratitis at concentrations of 1%. Generally, severity and durations of the effects increased with the concentration. All eyes were normal within 14 days. Instillation as a solid at amounts of 0.5-5 mg caused similar but less severe effects, roughly resolving within 4 to 7 days (Bal74).

With respect to the respiratory tract, the sensory irritation in the upper part was studied by determining the concentration associated with a 50% decrease in the respiratory rate (RD_{50}). In male Swiss-Webster mice, exposed to aerosols for 1 or 3 minutes, RD_{50} values of 8.6 and 3.2 mg/m^3 (ca. 1.1 and 0.4 ppm) were obtained, respectively (Ala72).

Acute toxicity

Acute lethal toxicity data (LCt_{50}s/ LD_{50}s) in experimental animals are summarised in Table 1.

Table 1 Summary of acute lethal toxicity studies in experimental animals.

exposure route	species (strain, sex)	LCt_{50}[a] or LD_{50}	reference
inhalation	mouse	74,050 mg·min/m^3 [b]	Bal78
	rat	68,400 mg·min/m^3 [b]	Bal78
	rabbit	65,600 mg·min/m^3 [b]	Bal78
	rat (Porton-Wistar; male)	88,480 mg·min/m^3 [c]	Bal78
	rat	32,500 mg·min/m^3 [c]	Pun62
	mouse (Porton albino; male)	50,010 mg·min/m^3 [c]	Bal78
	mouse	43,500 mg·min/m^3 [c]	Pun62
	rabbit (New Zealand; female)	54,090 mg·min/m^3 [c]	Bal78
	rabbit	17,300 mg·min/m^3 [c]	Pun62
	guinea pig (Dunkin Hartley; female)	67,200 mg·min/m^3 [c]	Bal78
	guinea pig	8,300 mg·min/m^3 [c]	Pun62
oral	rat (Osborne-Mendel; male, female)	178-358 mg/kg bw [d]	Gas72
	rat (Porton-Wistar; male)	1366 mg/kg bw	Bal78
	rat (Porton-Wistar; female)	1284 mg/kg bw	Bal78
	mouse	282 mg/kg bw	NIO03
	rabbit (New Zealand; male)	231 mg/kg bw	Bal78
	rabbit (New Zealand; female)	143 mg/kg bw	Bal78
	guinea pig (Dunkin Hartley; female)	212 mg/kg bw	Bal78
intravenous	rat (Porton-Wistar; female)	28 mg/kg bw	Bal78
	mouse (Porton albino; male)	48 mg/kg bw	Bal78
	rabbit (New Zealand; male)	31 mg/kg bw	Bal78

	rabbit (New Zealand; female)	28 mg/kg bw	Bal78
	rabbit	8 mg/kg bw	Pun62
intraperitoneal	rat (Porton-Wistar; male)	48 mg/kg bw	Bal78
	mouse	32 mg/kg bw	NIO03
	guinea pig (Dunkin Hartley; female)	73 mg/kg bw	Bal78

[a] Inhalation exposure dose (function of concentration and exposure duration) causing mortality in 50% of the animals.
[b] Pyrotechnically generated smoke.
[c] Aerosol.
[d] Depending on vehicle.

Animals dying from acute inhalation exposure showed congested and oedematous lungs with multiple haemorrhages. Histological examination showed moderate to marked congestion of alveolar capillaries and intra-pulmonary veins. In addition, congestion of the liver, kidney, spleen, and small intestine was demonstrated (Bal78). Ballantyne and Swanston concluded that, within the limits of the experimental procedures, CS was similarly toxic in rats, mice, rabbits, and guinea pigs when exposed briefly to high concentrations of aerosols generated from the pure compound or of grenade smokes (Bal78).

Exposure to unreported aerosol concentrations of CS, with 90% of the particles having an aerodynamic equivalent diameter between 1.5 and 2 µm, for up to 20 minutes affected lung physiology (i.e., decreases in minute ventilation) and induced histological lesions in the trachea (cytoplasmic vacuoles in epithelial cells) and the lungs (emphysema) of male Wistar rats (Deb99).

Short-term toxicity

In 14-day inhalation studies, preceding 13-week and 2-year studies, F344/N rats (n=10/sex/group) and B6C3F$_1$ mice (n=10/sex/group) were exposed to CS aerosols, comprising of a mixture of 94% CS, 5%Cab-O-Sil colloidal silica, and 1% hexamethyldisilazane, at concentrations of 0, 1, 3, 10, 30, and 100 mg/m^3, 6 hours/day, 5 days/week. All rats exposed to 30 and 100 mg/m^3 and all mice exposed to 10, 30 and 100 mg/m^3 died before the end of the study. Compound-related clinical signs included erythema, blepharospasm, listlessness, nasal discharge, and mouth breathing (NTP90).

In the subsequent 13-week study, F344/N rats (n=10/sex/group) and B6C3F$_1$ mice (n=10/sex/group) were exposed to similar CS aerosols at concentrations of 0, 0.4, 0.75, 1.5, 3, and 6 mg/m^3 (6 hours/day, 5 days/week). One rat exposed to 6 mg/m^3 died before the end of the study. Mean body weights of rats exposed to 1.5 mg/m^3 or more were reduced by 17-44% (males) or 10-24% (females)

compared to controls. The absolute and relative thymus weights were reduced at 6 mg/m^3. Treatment-related nasal lesions in rats included focal erosion with regenerative hyperplasia and squamous metaplasia of the respiratory epithelium and suppurative inflammation. All mice exposed to 6 mg/m^3 and one of each sex exposed to 3 mg/m^3 died before the end of the study. Treatment-related lesions in mice also included squamous metaplasia of the nasal epithelium and inflammation (NTP90).

Wistar rats (n=50/group), Porton mice (n=75/group), and Dunkin-Hartley guinea pigs (n=50/group) were exposed to CS aerosols concentrations of 0, 3, and 30 mg/m^3, one hour/day, 5 days/week, until they had undergone 120, 55, and 120 exposures, respectively. Initially, groups of animals were also exposed to a concentration of 233 mg/m^3, but due to high mortality of mice and guinea pigs, exposure to this level was stopped after 3-5 days. At the end of the exposure periods, the animals were observed daily for 6 months. No statistically significant differences in mortality were seen between the control and the groups exposed to 3 or 30 mg/m^3. Weight gain was reduced in a dose-related fashion in mice, but not in rats or guinea pigs. Histological examination showed laryngitis and tracheitis in mice exposed to 30 mg/m^3, but not in the other species. No treatment-related increased incidences of neoplastic lesions at any site in any species were observed (Mar83).

No mortality was observed in male rats (n=56) during or after exposure to thermally generated CS aerosol concentrations of 1000-2000 mg/m^3, 5 minutes/day, for 5 days. At post-mortem examinations, performed at various time points after the final exposure ranging from 1 hour to 21 days, lung lesions were observed in a total of 10 rats, including minimal congestion and a few small scattered alveolar haemorrhages in 5 and bronchopneumonia in the other 5 rats. Exposure to similarly generated concentrations of 12-15 mg/m^3, 80 minutes/day, for 9 days resulted in mortality in 5/50 rats due to bronchopneumonia. Post-mortem examination of the remaining 45 rats at various time points after the final exposure showed minimal congestion or alveolar haemorrhages of the lungs in 7 and bronchopneumonia in 9 rats (Bal72).

Rats (n=30) and dogs (n=5) were exposed 5 days/week for 5 weeks to CS aerosol (mass median diameter: 1.5 µm) concentrations of ca. 720 mg/m^3 for 5 minutes and 680 mg/m^3 for 1 minute, respectively. Dogs reacted vigorously during each exposure. The only obvious sign observed was salivation, which lasted for ca. 1 minute after exposure. Treatment did not affect haematology and blood chemistry parameters during the 5-week period. No other data were presented. Rats showed vigorous reactions as well. Visible signs included bloody noses. During the exposure period, 6 rats died without having post-mortem gross

pathological changes. The exposed rats lost weight by about 1% compared with gain weight in controls by about 20%. at post-mortem examinations of animals sacrificed during and after the 5-week period, no effects on relative heart, kidney, lung, liver, and spleen weights or macroscopic changes were seen (Pun62).

The effect of CS on the immune system was studied in mice given daily intraperitoneal injections of 8 or 16 mg/kg bw CS in olive oil for 10 days. The humoral immune response to sheep red blood cells was suppressed at both doses. Increased serum protein and corticosterone levels were also measured. Nagarkatti et al. suggested that CS may directly act on the immune system at low doses, while at higher doses, in addition to this direct effect, increased corticosterone levels may also contribute to immunosuppression (Nag81).

Long-term toxicity and carcinogenicity

In the 2-year NTP toxicity and carcinogenicity studies in rats and mice, F344/N rats (n=50/sex/group) were exposed to CS aerosol concentrations of 0, 0.075, 0.25 or 0.75 mg/m^3, 6 hours/day, 5 days/week. The CS aerosol was of the same composition as in the 2- and 13-week NTP studies (see 'Short-term toxicity'). No increased mortality or clinical signs of intoxication were observed in any of treated groups compared to the controls. Final mean body weights of rats exposed to 0.75 mg/m^3 were about 10-12% lower than those of controls. There were no compound-related increases in the incidences of neoplasms at any site. Non-neoplastic lesions were present in the nasal passage only. In animals exposed to 0.75 mg/m^3, they consisted of statistically significant increases in the incidences of hyperplasia and squamous metaplasia of the respiratory epithelium and degeneration of the olfactory epithelium with ciliated columnar and/or squamous metaplasia, and of proliferation of the periosteum of the turbinate bones; in females, there was an increased incidence of chronic focal inflammation and histiocytic cellular infiltrates in the lungs. At 0.25 mg/m^3, increased incidences of olfactory epithelial metaplasia in males and of respiratory epithelial metaplasia in females were observed (NTP90). From this 2-year rat inhalation study, the committee concludes that 0.075 mg/m^3 is a NOAEL for rats, based on increased incidences of metaplasia in the olfactory and respiratory epithelium in male and female rats, respectively, at the next higher concentration of 0.25 mg/m^3 (NTP90).

B6C3F$_1$ mice (n=50/sex/group) were exposed to concentrations of 0, 0.75 or 1.5 mg/m^3. No increased mortality or clinical signs of intoxication were observed in any of treated groups compared to the controls. Final mean body weights of

mice were 12-17% lower than those of controls. There were no compound-related increases in the incidences of neoplasms at any site. In female mice, there were pronounced dose-related decreases in the incidences of adenomas of the pituitary pars distalis and of malignant lymphomas. As with rats, non-neoplastic lesions were present in the nasal passage only and included statistically significant increases in the incidences of suppurative inflammation with hyperplasia and squamous metaplasia of the respiratory epithelium in male animals exposed to 0.75 and 1.5 mg/m^3 and in females exposed to 1.5 mg/m^3 (NTP90). From this 2-year mouse inhalation study, the committee could not establish a NOAEL since respiratory epithelial lesions were observed in male rats at 0.75 mg/m^3, the lowest concentration tested.

Mutagenicity and genotoxicity

Mutagenicity assays comprised tests for the detection of gene mutations in bacteria, yeast, and mammalian cells (*in vitro*) and for cytogenicity (*in vitro* and *in vivo*) and other genotoxicity assays *(in vitro* and *in vivo*).

- *In vitro* tests:
  - Gene mutation assays. Tests for reverse mutations in several strains of *S. typhimurium* (TA98, TA100, TA1535, TA1537, TA1538) were negative at concentrations ranging from 10 to 2000 µg/plate both with and without metabolic activation. Equivocal results were obtained with TA100 (Dän81). The absence of gene mutations in *S. typhimurium* was confirmed in later studies with strains TA97a, TA98, TA100, TA102, and TA104 at doses ranging from 12.5 to 800 µg/plate (Mes92, Zei87) or with strain TA100 at doses up to 3500 µg/plate (Rie83, Wil83) with or without S9 activation.
    In contrast, CS induced forward mutations in cultured mouse lymphoma L5178Y cells at concentrations of 2.5 µg/mL in the absence of S9 activation (McG88). Gene mutations were also induced in V79 Chinese hamster cells at concentrations up to 75 µM (Zie89).
  - Cytogenicity assays. CS induced micronuclei in cultured Chinese hamster V79 cells at doses up to 75 µM (Zie89). In another study, chromosomal aberrations and sister chromatid exchanges (SCE) were induced in V79 cells in a dose-dependent manner at concentrations ranging from 9.4 to 37.7 µM, without S9 activation (Bau92). This was confirmed in cultured Chinese hamster ovary (CHO) cells in the presence or absence of S9 from rat or hamster liver (NTP90).

- Other genotoxicity assays. CS did not induce DNA repair synthesis in cultured V79 Chinese hamster cells at concentrations up to 75 μM (Zie89). However, CS caused a concentration-dependent c-mitotic effect in V79 cells, indicating an interaction of the chemical with the mitotic apparatus. The percentage of mitosis with abnormal spindle morphology was increased in a dose-dependent manner at CS concentrations up to 18.8 μM (Sal91, Sch89).

- *In vivo* tests:
  CS did not cause increases in the frequencies of sex-linked recessive lethal mutations in sperm of *D. melanogaster* fed sucrose solutions containing $2.6x10^{-3}$ or $5x10^{-4}$ M CS (Wil83).
  No increases in the frequency of micronucleated polychromatic erythrocytes was observed in bone marrow cells of mice given single oral and intraperitoneal doses of 113 and 226 and of 18,9 and 37.8 mg/kg bw, respectively (Wil83).
  CS bound covalently to nuclear proteins but not to DNA in liver and kidney of rats given single intraperitoneal injections of ^{14}C-side-chain-labelled CS of 13 mg/kg bw (Dän81).
  In summary, CS has the potential to induce gene mutations as well as clastogenic and aneugenic effects in cultured mammalian cells, mostly in the absence of S9-mix. However, no gene mutations could be demonstrated in bacterial assays and no clastogenic effects were observed in the bone marrow of CS-treated mice.

  The CS metabolites malononitrile, 2-chlorobenzaldehyde, 2-chlorobenzoic acid, and 2-chlorobenzyl alcohol did not induce gene mutations in *S. typhimurium* (Rie83, NTP90); malononitrile and 2-chlorobenzaldehyde did not induce chromosomal aberrations in cultured V79 cells (Bau92).

Reproduction toxicity

Pregnant rats (Porton SPF; n=22/group) and rabbits (New Zealand White; n=12/group) were exposed to CS aerosol concentrations of 6, 20, or 60 mg/m^3, 5 minutes/day on gestational days 6-15 and 6-18, respectively. In addition, separate groups of 8 to 12 rats received intraperitoneal injections of doses of CS of 20 mg/kg on gestational days 6, 8, 9, 10, 12, and 14. There were no statistically significant differences in maternal body weight increase, placental weight, number of litters, litters size, number of live fetuses, percentage of fetal

loss, and fetal body weight between any of the treatment groups and the controls. The number of external, visceral or skeletal abnormalities was not statistically significantly different between the groups (Ups73).

7 Existing guidelines

The current administrative occupational exposure limit (MAC) for CS in the Netherlands is 0.4 mg/m^3, as a ceiling value.

Existing occupational exposure limits for CS in some European countries and in the USA are summarised in Annex II.

8 Assessment of the hazard

CS is absorbed into the body by inhalation of its aerosol and is rapidly metabolised into breakdown products, which are mainly excreted in the urine. CS is a potent sensory irritant. Human beings in contact with CS in air may experience effects on the eyes, respiratory tract, and skin. Typical signs and symptoms during exposure to aerosols include eye discomfort, excess lachrymation, blepharospasm, burning sensations in the nose and throat, rhinorrhoea, excess salivation, constricting sensations in the chest, sneezing, coughing, and stinging or burning sensations in exposed skin. Characteristic is the prompt onset of effects upon exposure and the rapid disappearance of signs and symptoms after ending exposure.

Based on acute lethal toxicity studies in test animals, the committee considers the compound as toxic after inhalation and as harmful via the oral route. The systemic acute toxicity of CS in experimental species is due to the biotransformation of CS to cyanide. In thirteen-week toxicity studies in rats and mice, dose-related lesions of the nasal passage including squamous metaplasia of the nasal epithelium were observed, with a LOAEL of 0.4 mg/m^3 (lowest concentration tested) in rats and a NOAEL of 0.75 mg/m^3 in mice. Results of 2-year inhalation studies showed hyperplasia, squamous metaplasia of the respiratory epithelium, and degeneration of the olfactory epithelium with ciliated columnar and/or squamous metaplasia in rats. The committee feels that these effects could be explained from severe tissue irritation by CS. The NOAEL was 0.075 mg/m^3. No evidence was found of carcinogenic activity of CS in rats or mice. *In vitro* mutagenicity assays with CS produced negative results in bacterial test systems but positive results when using mammalian cell cultures. *In vitro* cytogenicity tests (SCE, micronucleus) were also positive, but no clastogenic effects were observed in CS-treated mice. No covalent binding with DNA could

be demonstrated in rats, following CS administration. The committee considers that in view of the negative inhalation carcinogenicity studies, the positive *in vitro* mutagenicity findings are not relevant for workers. One human and one animal study produced no evidence for teratogenicity or embryotoxicity.

The committee considers the hyperplasia and squamous metaplasia of the respiratory tract epithelium as the critical effect, and takes the NOAEL of 0.075 mg/m^3 found in the 2-year study in rats as a starting point in deriving a health-based recommended occupational exposure limit (HBROEL). For the extrapolation to a HBROEL, an overall assessment factor of 4 is established. This factor covers the following aspects: intra- and interspecies variation and the type of effect. Thus applying this factor and the preferred-value approach, a health-based occupational exposure limit of 0.02 mg/m^3 is proposed for CS.

The committee recommends a health-based occupational exposure limit for (2-chlorobenzylidene)malonitrile of 0.02 mg/m^3, as inhalable dust, as an 8-hour time-weighted average (TWA).

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Annex I

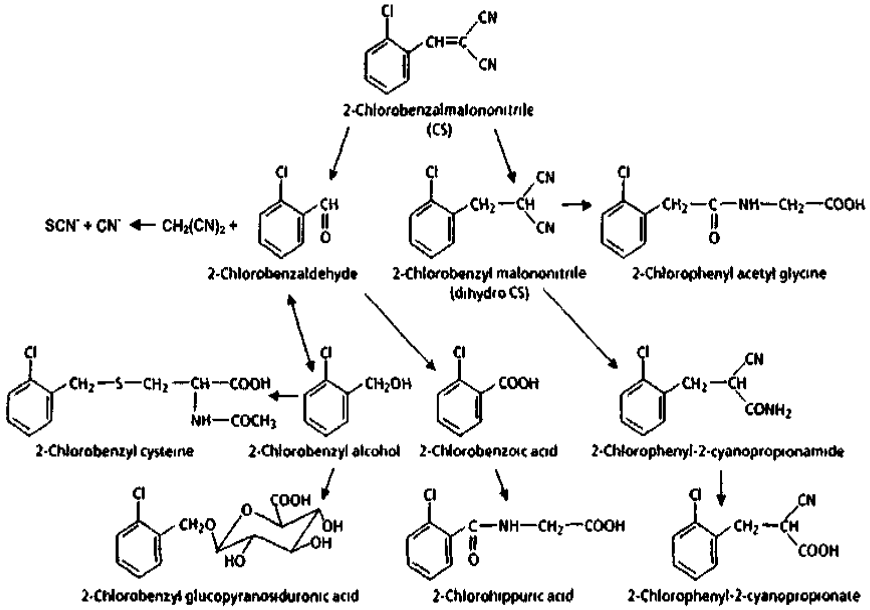

Figure 1 Principal pathways of CS metabolism (Bre87; adapted from NTP90).

Annex II

Occupational exposure limits for (2-chlorobenzylidene)malonitrile in various countries.

country - organisation	occupational exposure limit		time-weighted average	type of exposure limit	note[a]	reference[b]
	ppm	mg/m^3				
the Netherlands						
- Ministry of Social Affairs and Employment	0.05	0.4	ceiling	administrative	S	SZW03
Germany						
- AGS	-	0.4		S		TRG00
- DFG MAK-Kommission	-	-				DFG03
Great-Britain						
- HSE	-	-				HSE02
Sweden	-	-				Swe00
Denmark	0.05	0.4	ceiling	S		Arb02
USA						
- ACGIH	0.05	-	ceiling	TLV	S, A4[c]	ACG03b
- OSHA	0.05	0.4	8 h	PEL		ACG03a
- NIOSH	0.05	0.4	ceiling	REL	S	ACG03a
European Union						
- SCOEL	-	-				EC04

[a] S = skin notation; which mean that skin absorption may contribute considerably to body burden; sens = substance can cause sensitisation.

[b] Reference to the most recent official publication of occupational exposure limits.

[c] Classified in carcinogenicity category A4, i.e., not classifiable as a human carcinogen: agents which cause concern that they could be carcinogenic for humans but which cannot be assessed conclusively because of a lack of data. *In vitro* or animal studies do not provide indications of carcinogenicity which are sufficient to classify the agent into one of the other categories.