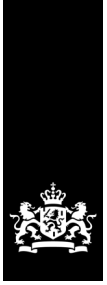


National Institute for Public Health
and the Environment
Ministry of Health, Welfare and Sport

Probit function technical support document

Date: 18 May 2020
Document id: 2020518-Phosgene-interim_v2_def
Status: interim
Author: drs. W ter Burg, RIVM
dr. ir. M. Ruijten, CrisisTox Consult for RIVM

substance name	CAS number
Phosgene	**75-44-5**

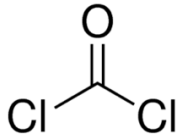
This document describes the derivation of a probit function for application in a quantitative risk analysis (QRA). The probit function has been derived according to the methodology described in RIVM report 2015-0102.

This document has been checked for completeness by the Netherlands' National Institute of Public Health and the Environment (RIVM). The contents of this document, including the probit function, has been approved by the Dutch Expert Panel on Probit Functions on scientific grounds. External parties have had the opportunity to comment on the derivation of the proposed probit function. The status of this document has now been raised to "interim", pending a decision on its formal implementation.

The decision on actual implementation depends on the results of a further consequence analysis.

Detailed information on the procedures for the derivation, evaluation and formalization of probit functions is available at
http://www.rivm.nl/en/Topics/P/Probit_functions

Technical support document phosgene



1. Substance identification

CAS-number:	75-44-5
IUPAC name:	phosgene
Synonyms:	Carbonic dichloride, carbonic oxychloride
Molecular formula:	$COCl_2$
Molecular weight:	98.92 g/mol
Physical state:	gas (at 20°C and 101.3 kPa)
Boiling point:	8.2°C (at 101.3 kPa)
Vapour pressure:	122 kPa (at 20°C)
Saturated vapor conc:	N/A (at 20°C)
Conversion factor:	1 mg/m^3 = 0.24 ppm (at 20°C and 101.3 kPa)
	1 ppm = 4.11 mg/m^3 (at 20°C and 101.3 kPa)
Labelling:	H314; H330

2. Mechanism of action and toxicological effects following acute exposure[1]

Acute effects: The main target organ and tissue for inhalation exposure to phosgene are the lower regions of the respiratory tract. Metabolic acidosis is expected because of impaired gas exchange due to pulmonary edema and the resultant hypoxemia/hypercapnia. Health endpoints are mild irritation of the upper respiratory tract showing little warning for pulmonary damage, likely due to acetylation, in the lower respiratory tract. Symptoms of high exposure are cough, chest tightness, dyspnea, tachycardia and tachypnea, and pulmonary edema. Lethality results from pulmonary edema.

Long-term effects: Delayed effects of phosgene after a single exposure may occur after several hours up to 24 hours, showing pulmonary damage in the lower regions of the respiratory tract. Chronic exposure results in the same effects as observed after single exposure.

3. Human toxicity data

No informative reports on health effects in humans following acute inhalation exposure were identified, although there are many case reports and information from the application of phosgene as chemical warfare agent. Based on the available information, estimates of the lethal concentrations for humans have been made. The AEGL on phosgene (final, 2002) describes the following:

Based on observations during World War I, the 2 min LC_{50} value for humans was estimated to be 790 ppm (3247 mg/m^3) (Chasis 1944; as cited by AEGL, 2002).

Many case reports describe symptomatology and post-mortem results from human phosgene poisonings; however, exposure concentrations were not reported. A 23-y-old man (healthy non-smoker) was exposed to phosgene at an estimated concentration of at least 5-10 ppm (20.6 – 41.1 mg/m^3) for 5 to 10 seconds (Bradley and Unger 1982). The exposure concentration is based on the symptoms reported and therefore are considered unreliable. The patient began coughing upon exposure to phosgene and experienced dyspnea and chest tightness within 30 min. Four hours after exposure, he was hospitalized with hypotension, tachycardia, tachypnea, cyanosis, and pulmonary edema. The patient was intubated and administered dopamine and methylprednisolone. From the second to the sixth day of hospitalization, he developed mediastinal and subcutaneous emphysema, bilateral

[1] AEGL 2002 final.

pneumohydrothoraces, elevated white blood cell counts, fever, and right-sided hemiparesis. Death occurred after the patient developed ventricular fibrillation.

Hegler (1928; as cited by AEGL, 2002) reported the effects of a phosgene accident that occurred in Hamburg, Germany, on May 20, 1928. Eleven metric tons of "pure phosgene" were released from a storage tank on a warm, dry, slightly windy day. Within a few hours, people as far as six miles from the release site began reporting to hospitals. Three hundred people reported to hospitals within a few days of the accident. Effects ranged from mild or moderate illness to death; 10 people were reported to have died. In general, exposed persons exhibited symptoms consistent with other reported phosgene poisonings (headache, dizziness, nausea and vomiting, irritant cough, and sickening-sweet taste, followed by a latency period and then pulmonary symptoms). Autopsies on 6 of the 10 fatalities showed pulmonary effects in all cases. Fatty degeneration of the kidneys, liver, and heart were observed in a few cases and were thought to be secondary to the pulmonary damage. In an atypical case, damage in the gray matter of the brain and spinal cord, hyperaemia, and signs of bleeding in the white matter were observed at autopsy. That patient died 11.5 days post-exposure from a blood clot lodged in the lung. It was uncertain if the extra-pulmonary effects were attributable to phosgene.

Diller and Zante (1982) performed an extensive literature review concerning human toxicity data following phosgene exposure. These included actual human data but also information from animal data extrapolated to humans. Diller and Zante based their final conclusions on lethality of phosgene exposure to humans on human data from Bickenbach (1947), but these data are not used for derivation of a probit function for ethical reasons. Regarding the remaining data, Diller and Zante concluded that a majority of these data were anecdotal or rough estimates and did not report reliable exposure concentrations and/or durations. However, despite these uncertainties and drawbacks these data indicate that overall a dose of 100 200 ppm x min (similar to approximately 14 -27 mg/m3 for a 30-min exposure) can be considered lethal to humans.

Kaerkes (1992) studied 376 occurrences of phosgene accidents in the workplace of a large chemical company between 1978 and 1988, describing symptomatology, diagnostic and therapeutical interventions, use of PPE and exposure. The exposure for 123 cases was determined with an indicator badge that provided an assessment of the cumulative dose (ppm × min). The sensitivity of the phosgene dose assessment was low, since the only outcomes were < 50, 50-150, 150-300 and > 300 in ppm × min. The thesis does not provide information on the validity and precision of the indicator readings against a proven analytical method. The study appears to focus on benefits of the availability of an exposure indication (as compared to relying only on signs and symptoms) to determine appropriate diagnostic and therapeutic interventions and duration of medical treatment. It is noted, that these indicator badges are not intended to provide an accurate exposure estimation. Further, it is stated that workers might have used respiratory protection and that therefore, the badges did not indicate the effective inhaled dose. No further details are provided on the use of respiratory protection equipment (e.g., number of workers in each exposure group using respiratory protection, level of protection, whether or not the outcome of the badges needed adjustment, etc.). This might have led to an overestimation of exposure and thus to an underestimation of health risks. Further it can be stated that health risk estimates based on a relative homogeneous, healthy worker population may provide an underestimation of health risks for a more heterogeneous general population for which the probit function is derived.
Collins *et al* (2011) reported on the USA industry-wide phosgene surveillance. A total of 338 exposure cases were identified from 2004-2009. The observations on the environmental exposure were gathered with an indicator badge similar to the one

described above and appear to be substantially lower (69% < 10 ppm×min) than the exposure levels reported by Kaerkes.
The studies bij Kaerkes (1992) and Collins *et al* (2011) provide valuable qualitative information of the human response to phosgene inhalation, but limited quantitative information to assess the concentration-time-response relationship of exposure.

4. Animal acute toxicity data

During the literature search the following technical support documents and databases were consulted:

1. AEGL final TSD, ERPG document and EU RAR and reference database for phosgene, covering references before and including 1995.
2. An additional search covering publications from 1980 onwards was performed in HSDB, MEDline/PubMed, Toxcenter, IUCLID, ECHA, RTECS, IRIS and ToxNet with the following search terms:
   - Substance name and synonyms
   - CAS number
   - lethal*, mortal*, fatal*
   - LC_{50}, LC
   - probit
3. Unpublished data were sought through networks of toxicological scientists.

Animal lethal toxicity data considering acute exposure are described in Appendix 1. A total of 16 studies were identified -with 17 datasets for 7 species- with data on lethality following acute inhalation exposure. Three datasets were assigned status A for deriving the human probit function, no datasets were assigned status B and 14 were assessed to be unfit (status C) for human probit function derivation.

Sensory irritation

No studies on sensory irritation were found.

5. Probit functions from individual studies

All available acute lethality data on phosgene are displayed in Figure 1.

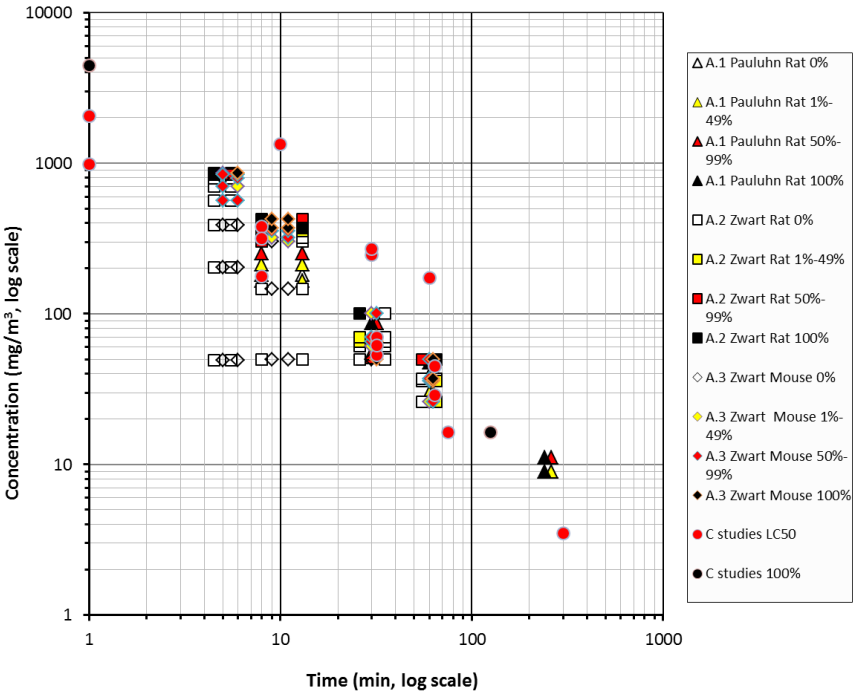


Figure 1 *All available acute lethality data for phosgene.*

The data that were selected for initial analysis of the animal probit function are presented in Table 2 and Figure 2.

All A studies were selected for derivation of the animal probit function for phosgene.

Probit functions have been calculated and reported in Appendix 1 for each of the reported studies. The results of the calculations are presented in Table 2.

Table 1 *Data selected for initial analysis of the animal probit function of phosgene.*

Study ID	Species	Probit (C in mg/m^3, t in min)	LC_{50}, 30 minutes (mg/m^3) 95% C.I.	n-value 95% C.I.
A.1	Rat	-17.6 + 2.84 ×lnC + 3.16 ×lnt	64.1 (51.0-73.2)	0.898 (0.766-1.03)
A.2	Rat	-36.8 + 4.76 ×lnC + 5.91 ×lnt	95.2 (87.6-107)	0.806 (0.762-0.851)
A.3	Mouse	-12.9 + 2.05 ×lnC + 2.78 ×lnt	62.1 (47.5-73.2)	0.739 (0.658-0.820)

The data of the three A studies with rats and mice are presented graphically below.

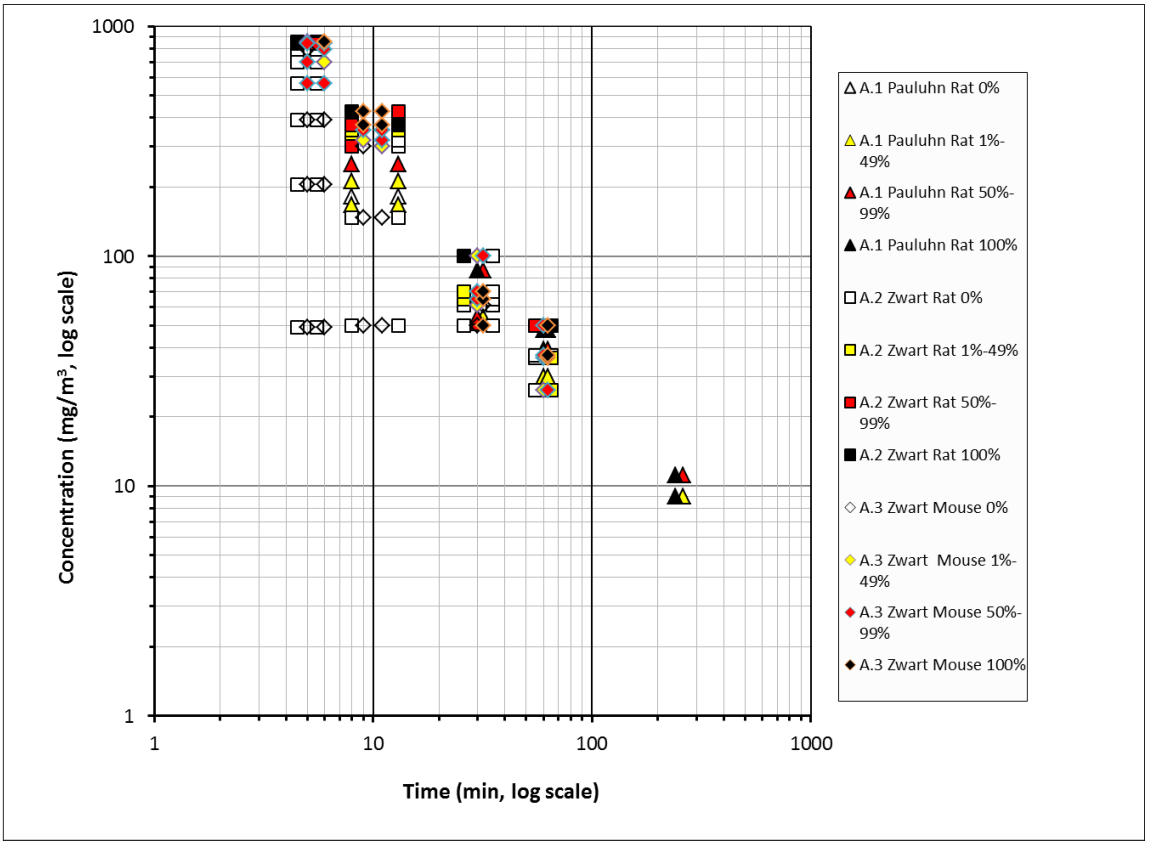


Figure 2 *Data selected for the initial analysis for the derivation of the animal probit function of phosgene.*

Based on criteria outlined in the guideline the data from studies A.1, A.2 and A.3 were selected for the final dataset for the derivation of the animal probit function. The three studies with A quality were selected to derive the animal probit function as they were well performed and provided many C x t combinations in both the rat and the mouse. Figure 3 provides an overview of LC_{50}-time relationships for all studies in the final analysis. The data that were selected for final analysis of the animal probit function are presented in Table 3 and Figure 4.

The final data eligible for calculating the animal probit function contains three datasets from two studies and includes data from two animal species.

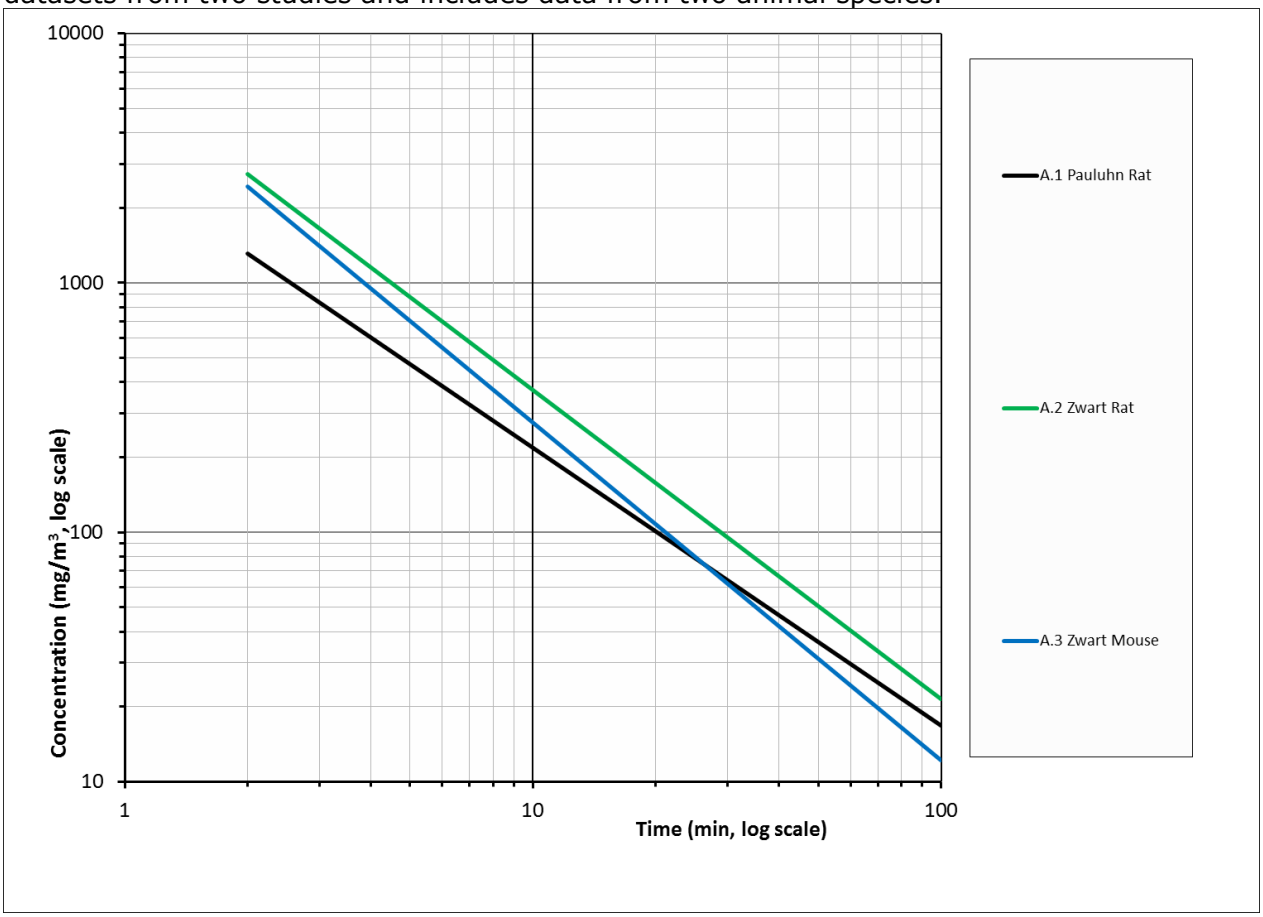


Figure 3 *LC_{50} values of A.1, A.2, and A.3 datasets for phosgene, over time where available.*

Table 2 *Data selected for the derivation of the animal probit function of phosgene (identical to table 2).*

Study ID	Species	Probit (C in mg/m³, t in min)	LC_{50}, 30 minutes (mg/m³) 95% C.I.	n-value 95% C.I.
A.1	Rat	-17.6 + 2.84 ×lnC + 3.16 ×lnt	64.1 (51.0-73.2)	0.898 (0.766-1.03)
A.2	Rat	-36.8 + 4.76 ×lnC + 5.91 ×lnt	95.2 (87.6-107)	0.806 (0.762-0.851)
A.3	Mouse	-12.9 + 2.05 ×lnC + 2.78 ×lnt	62.1 (47.5-73.2)	0.739 (0.658-0.820)

The data of the selected datasets are presented graphically below.

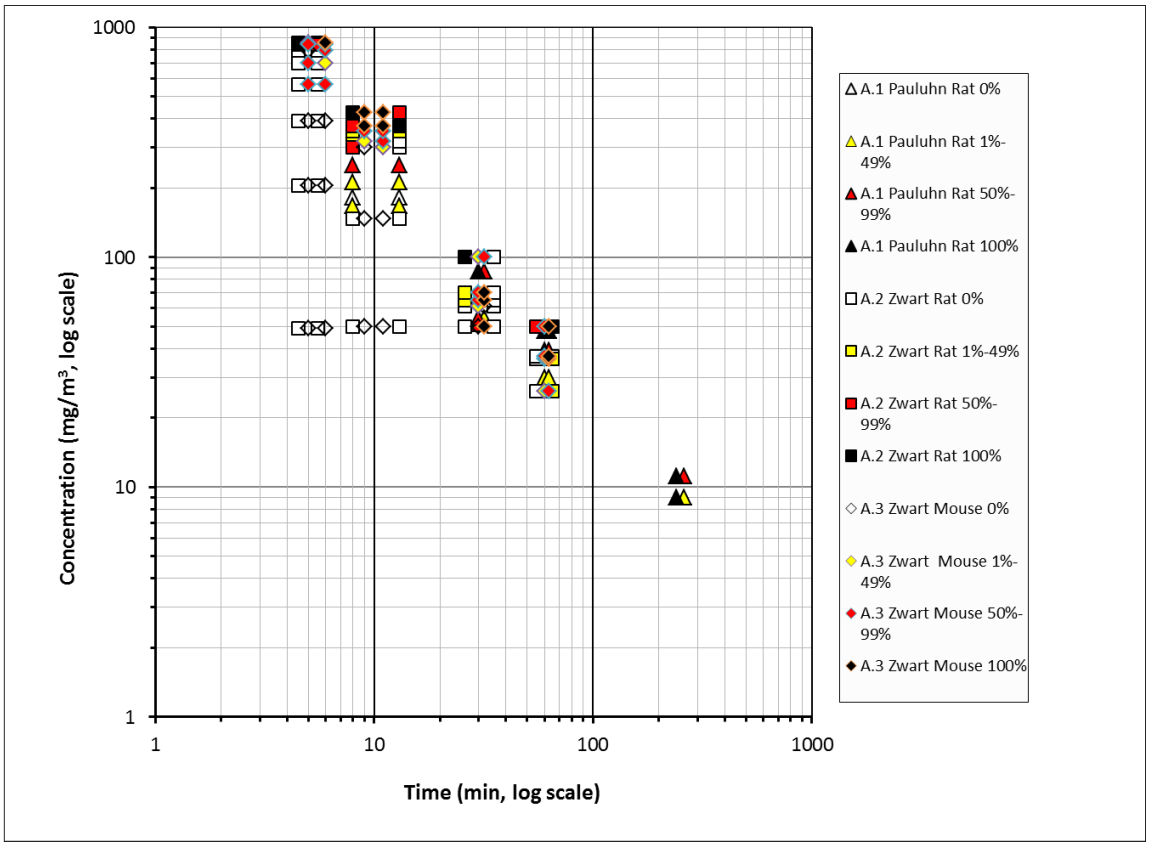


Figure 4 *Final data selected for derivation of the animal probit function of phosgene (identical to figure 2).*

6. Derivation of the human probit function

To derive the human probit function the results from Pauluhn et al. (2006a; A.1) and Zwart et al. (1990; A.2 and A.3) have been used to derive a point of departure as outlined above.

The species-specific n-value was 0.852 for the rat (arithmetic mean of A.1 and A.2) and 0.739 for the mouse. The mean n-value across species is the arithmetic mean of the species-specific mean n-values (without weight) and was calculated to be 0.796.

Second, the LC_{50}-values of all A-studies were calculated for a common exposure duration of 30 minutes.

The species-specific geometric mean LC_{50}-values were calculated from the 30-min LC_{50} values of studies A.1, A.2 for the rat and A.3 for the mouse. The species-specific LC_{50}-values were 78.1 and 62.1 mg/m^3 for the rat and mouse, respectively. Finally, the geometric mean overall LC_{50}-value was calculated as:

$$\overline{LC_{50}} = \left[\prod_{j=1}^{s} \left(\prod_{i=1}^{m} LC_{50,i} \right)^{1/m} \right]^{(1/s)}$$

With $\overline{LC_{50}}$ = geometric mean LC_{50}-value across species
$LC_{50,i}$ = LC_{50}-value of study i.
m = number of observations on LC_{50}-values within a species (i=1…m).
s = number of species for which LC_{50}-values are pooled (j= 1…s).

The choice of an appropriate interspecies factor for phosgene requires some discussion. Pauluhn (2006b) discussed whether the dog is a better model for human phosgene inhalation toxicity than rodent species. He concluded that "dogs are considered more human-like and a better model for humans", whereas "Small rodents are associated with the higher ventilation rate and with rodent-specific sensory bronchopulmonary defense reflexes (i.e. reflex bradypnoea)." Pauluhn (2006b) compared the bronchoalveolar lavage (BAL) fluid proteins that are indicative for pulmonary injury after phosgene intoxication both in rats (Pauluhn, 2006c) and dogs (Pauluhn, 2006b), in addition to other parameters including lung weights, arterial blood gases and lung histopathology. Focusing on the BAL liquid proteins -Pauluhn considers this parameter the most sensitive predictor for pulmonary injury- Pauluhn showed that rats had 10-fold higher levels at similar C x t products (appr. 1050 mg/m^3 x min, which is the LCt_{01} in rats in Pauluhn, 2006a). It should be noted, however, that Pauluhn hypothesized that the 10-fold higher protein exudation into the BAL at this high dose could be a reflection of the rat (or rodent) specific defence mechanism, as the major part of the lungs is "lavaged and proteinaceous secretions from airways may contribute markedly to the total protein and inflammatory cells detected in BAL." Hence, absolute differences between BAL fluid proteins may not provide information on susceptibility differences between species. The comparison between rats and dogs as to what dosage produces 150% BAL proteins compared to background levels showed a 3-fold lower C x t product in rats (117 mg/m^3 x min) compared to dogs (375 mg/m^3 x min) (Pauluhn, 2006b, see Figure 8 therein (see also appendix II), which the author considered to be a better indication for differences in susceptibility. "The occurrence of pulmonary responses suggestive of mild edema and inflammation at 495 mg/m^3 ×min in dogs compares favourably with similar observations in humans at >600 mg/m^3 ×min by the National Research Council, 2002, and Diller & Zante, 1982" (as cited by Pauluhn, 2006b). However, as Diller and Zante stated themselves, the concentration levels reported in their literature review are unreliable (cf. above). Pauluhn's studies provide no information that allows a direct comparison of lethality rates at different concentration-time combinations between rodents and dogs.

There is no reliable lethality study in dogs. In Table 4, ranges of 30-min LC_{50} values are presented for several species including rats and dogs, mostly derived from C-studies unless indicated otherwise. LC_{50} values suggest that LC_{50} values for dogs are a factor of 3 to 4 higher than those of rats.

Table 4 *Ranges of 30-min LC_{50} values for several species.*

Species	LC_{50}, 30 minutes (mg/m^3)
Mouse	21 – 62*
Rat	64* – 95*
Guinea pig	74 – 580
Rabbit	82 – 452 (20-min LC_{50}); 411 – 555 (30-min LC_{50})
Dog	247 – 288

* Values derived from A studies.

Based on the information above, it appears that dogs are less susceptible than rats and mice to adverse respiratory system effects of phosgene inhalation exposure. The anatomy of human (and dog) lungs is fundamentally different from rodent lungs, with humans having a 'leaf tree' structure and rodents having a 'pine-tree' structure. Since no reliable dog inhalation lethality study is available, the rat and mouse data have been used as point of departure for probit function derivation as calculated above. It

should be noted that the most susceptible species have been used to derive a PoD, while dogs, having the same anatomy as humans, are thought to be a better (lung) model for humans and are considerably less susceptible than rats.

Application of an overall assessment factor of 3 (determined by the default interspecies factor of 3) would result in a calculated 30-min and 60-min human LC_1 of 7 and 3 mg/m^3, respectively (probit function and resultant LC-values not shown). Although the human data as provided by Diller and Zante (1982) and Kaerkes (1992) have their limitations (see section 3), these data can nevertheless be used to support the choice of a reduced interspecies factor. On the one hand, data of workers with a phosgene indicator badge revealed that an exposure below 50 ppm x min (corresponding to ca 8 mg/m^3 for 30 min assuming a linear relationship between concentration and duration) indicated no signs or symptoms of phosgene toxicity in the majority (maximally 73) of 88 individuals (Kaerkes, 1992). On the other hand, the information for human exposure to phosgene provided by Diller and Zante (1982) indicated that exposure to a phosgene dose of 100-200 ppm x min (ca. 14 – 27 mg/m^3 for 30 min) may be lethal to humans.

The Point of Departure for the human probit function is a 30-minute geometric mean animal LC_{50} value of 69.6 mg/m^3 and an arithmetic mean n-value of 0.796. As mentioned, the rat is considered to be more susceptible to phosgene than humans. However, starting from the rodent data and applying an interspecies factor of 1 would lead to a 30-min LC_{01} of 22 mg/m^3, which appears to be too high as compared with the overall data presented by Diller and Zante (1982). In addition, application of the default factor of 3 appears to result in a relatively low 30-min LC_{01} of 7 mg/m^3, as compared to the information provided by Kaerkes (1992). Therefore, an interspecies factor of 2 is applied for the derivation of the probit function.

The human equivalent LC_{50} was calculated by applying the following assessment factors:

Table 5 *Rationale for the applied assessment factors.*

Assessment factor for:	Factor	Rationale
Animal to human extrapolation:	2	See above for the explanation for lowering the default factor from 3 to 2.
Nominal concentration	1	Analytical concentrations were determined in all three A-study datasets
Adequacy of database:	1	Three A-study datasets are available.

The estimated human equivalent 30-minute LC_{50} value is 69.6 / 2 = 34.8 **mg/m^3**.

The experimentally determined n-value was **0.796** (see above). Assuming a regression coefficient (b×n) of 2 for the slope of the curve, the b-value can be calculated as 2 / n = **2.514**.

The human probit function is then calculated on the human equivalent 30 min LC_{50} using the above parameters to solve the following equation to obtain the a-value (the intercept): $5 = a + 2.51 \times \ln (34.8^{0.796} \times 30)$ resulting in the a-value of **-10.652**.

$Pr = -10.7 + 2.51 \times \ln (C^{0.80} \times t)$ with C in mg/m^3 and t in min.

The derived human probit function has a scientifically sound basis. The probit function is based on two studies and three datasets in the rat and mouse with A quality,

containing in total 60 C x t combinations with 10 animals (5 per sex) per C x t combination.

The calculated human 60 min $LC_{0.1}$ (Pr = 1.91) calculated with this probit equation is 3.2 mg/m^3 and the calculated human 60 min LC_1 (Pr = 2.67) is 4.7 mg/m^3.

Table 6 *LC-vales calculated with the derived probit function compared with existing acute inhalation exposure guidelines.*

Estimated level	30 min (mg/m^3)	60 min (mg/m^3)
0.1% lethality, this probit	7.7	3.2
1% lethality, this probit	11.2	4.7
AEGL-3[2] (2002, final)	6.2	3.1
ERPG-3[2] (2020)		4.1
LBW (2016)	9.4	4.2

Compared with equivalent (inter)national guideline levels as presented in the table above, the lethal levels derived with this probit function are similar.

[2] AEGL and ERPG values were converted from ppm to mg/m^3 with the conversion factor calculated in section 1. Therefore, the AEGL and ERPG values in mg/m^3 can deviate slightly from those reported in the AEGL and ERPG TSDs.

Appendix 1 Animal experimental research

Study ID: A.1

Author, year: Pauluhn, 2006a

Substance: phosgene
Species, strain, sex: Rat, Wistar Hsd Cpb:WU (SPF), males and females
Number/sex/concentration group: 5/sex/concentration
Age and weight: young adults, 184 to 209 g (males), 160 to 180 g (females)
Observation period: 2 weeks

Evaluation of study quality

Criteria	***Comment***
Study carried out according to GLP	*yes*
Study carried out according to OECD 403 guideline(s)	*No statement of compliance with OECD guideline 403 provided.*
Stability of test compound in test atmosphere	*Stable*
Use of vehicle (other than air)	*Synthetic air or conditioned dry air*
Whole body / nose-only (incl. head/nose-only) exposure	*Nose-only*
Type of restrainer	*No information*
Pressure distribution	*The pressure difference between the inner inhalation chamber and exposure zone was 0.02 cm H_2O. A positive balance between air volume supplied and extracted ensured that no passive influx into exposure chamber occurred.*
Homogeneity of test atmosphere in breathing zone of animals	*Phosgene in synthetic air was diluted prior to forcing the airflow through the inner concentric cylinder of the chamber toward the rats' breathing zone. Each segment of the exposure chamber, 'ports', had an internal volume of 3.8 L.*
Number of air changes per hour	*30 L/min to maintain an airflow rate of 0.75 L/min/animal*
Equilibration time (t95)	*Not relevant*
Start of exposure relative to equilibration	*The respective target concentration was achieved by dilution cascades prior to entering the directed-flow nose-only chamber. The test atmosphere was then forced through openings in the inner concentric cylinder of the chamber, directly toward the rats' breathing zone.*

Actual concentration measurement	*Concentrations were determined analytically using OSHA method 61: sampling from the vicinity of the breathing zone, analyses using gas chromatography with a run time of 28 mins. Essentially covering the entire exposure period.* *Simultaneously, real-time monitoring took place using IR spectroscopy. A CM4 toxic gas paper tape monitor was used at concentration in the range and lower than 1 ppm (4.1 mg/m^3).*
Particle size distribution measurement in breathing zone of the animals in case of aerosol exposure	*N/A*
Assessment of Reliability	***A*** *Studies is well performed and considered several C x t combinations.*

Results

Species	Concentration (mg/m^3)		Exposure duration (min)	Lethality	
	Measured	Adjusted		Male	Female
				Dead/tested	
Rat	0	N/A	60	0/5	0/5
	166.5		10	2/5	1/5
	181.6		10	0/5	0/5
	212.0		10	1/5	1/5
	250.9		10	3/5	3/5
	51.3		30	3/5	1/5
	54.5		30	3/5	1/5
	67.7		30	5/5	5/5
	86.9		30	5/5	4/5
	29.9		60	2/5	2/5
	39.4		60	5/5	4/5
	47.6		60	5/5	5/5
	9.0		240	5/5	1/5
	11.1		240	5/5	4/5

Probit function

The probit function and associated LC-values have been calculated using the DoseResp program (Wil ten Berge, December 2016) as

$Pr = a + b \times \ln C + c \times \ln t + d \times S$

with C for concentration in mg/m^3, t for time in minutes and S for sex (0 = female, 1 = male).

Probit function	*Species*	*a*	*b*	*C*	*d*	*n-value*
Sex as covariate	*Rat*	*-19.3*	*3.01*	*3.36*	*0.67*	*0.894 (0.776 – 1.011)*
Sexes combined	*Rat*	*-17.6*	*2.84*	*3.16*		*0.898 (0.766 -1.030)*

The LC_{50} values for both sexes did not differ by more than a factor 2. This does not support that sex differences exist in the lethal response. For this reason the data from both sexes were pooled and analyzed to derive the animal probit function.

Duration (minutes)	*LC_{50} (mg/m^3) 95%-C.I.* ***Male***	*LC_{50} (mg/m^3) 95%-C.I.* ***Female***	*LC_{50} (mg/m^3) 95%-C.I.* ***Sexes combined***
10	196 (157 – 253)	245 (200 – 376)	218 (180 – 318)
30	57.3 (41.5 – 67.1)	71.5 (60.0 – 87.9)	64.1 (51.0 – 73.2)
60	26.4 (16.4 – 31.8)	32.9 (25.2 – 39.2)	29.6 (19.3 – 34.5)

A graphical overview of the data is presented below. Each concentration-time combination represents one point in the plot.

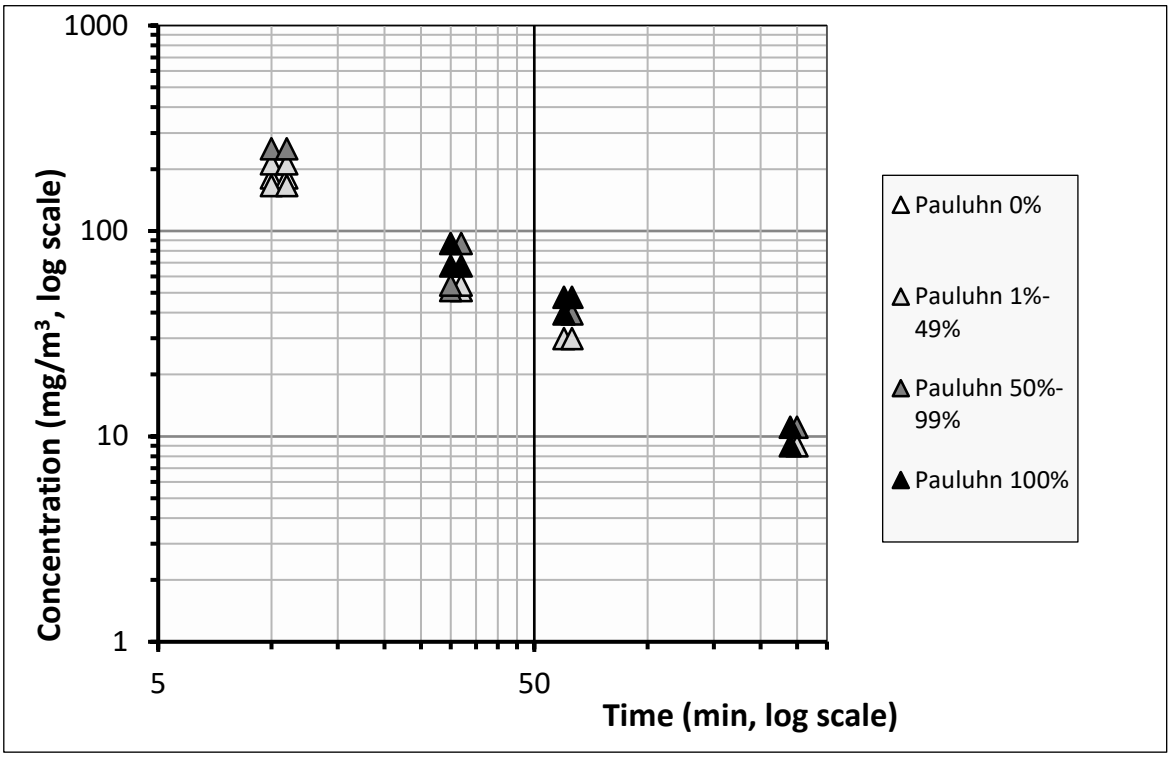


Figure: C x t data Pauluhn et al (2006) study. The study included 4 exposure durations. At each point, male data are indicated on the left and female data on the right.

Study ID: A.2

Author, year: Zwart, 1990

Substance: phosgene
Species, strain, sex: SPF-bred Wistar-derived rats, males and females
Number/sex/conc.: 5
Age and weight: Age unknown, males weighed between 119-181 g and females 103-137 g.
Observation period: 14 days

Evaluation of study quality

Criteria	***Comment***
Study carried out according to GLP	*Yes.*
Study carried out according to OECD 403 guideline(s)	*No statement of compliance with OECD guideline 403 provided.*
Stability of test compound in test atmosphere	*Not specified*
Use of vehicle (other than air)	*No*
Whole body / nose-only (incl. head/nose-only) exposure	*Whole body*
Type of restrainer	*N/A*
Pressure distribution	*Not specified*
Homogeneity of test atmosphere in breathing zone of animals	*An adjustable flow of test substance was mixed with airflow.*
Number of air changes per hour	*Approximately 100-150 air changes/hour* *(Content exposure cylinder (length: 0.9 m, r: 0.075 m) 15.9 l; air flow 25-40 l/min)*
Equilibration time (t95)	*1.2-1.9 min*
Start of exposure relative to equilibration	*Not specified. However, the shortest exposure duration in this study (i.e. 5 min) corresponds to 3 x t95 (i.e. 3.6-5.7 min).*
Actual concentration measurement	*Concentrations were measured continuously, by IR analysis and gas chromatography.*
Particle size distribution measurement in breathing zone of the animals in case of aerosol exposure	*N/A*
Assessment of Reliability	***A*** *Well performed study. Multiple concentration levels and durations were tested.*

Results

Species	Concentration (mg/m^3)		Exposure duration (min)	Lethality	
	Measured	Adjusted		Male	Female
				Dead/tested	
Rat	49		5	0/5	0/5
	204		5	0/5	0/5
	390		5	0/5	0/5
	563		5	0/5	0/5
	698		5	0/5	0/5
	791		5	0/5	0/5
	838		5	5/5	4/5
	856		5	0/5	0/5
	50		10	0/5	0/5
	147		10	0/5	0/5
	301		10	3/5	0/5
	320		10	1/5	0/5
	353		10	2/5	2/5
	370		10	4/5	5/5
	424		10	5/5	4/5
	50		30	0/5	0/5
	61		30	0/5	0/5
	65		30	1/5	0/5
	70		30	2/5	0/5
	100		30	5/5	0/5
	26		60	0/5	1/5
	36		60	0/5	2/5
	37		60	0/5	0/5
	50		60	4/5	5/5

Probit function

The probit function and associated LC-values have been calculated using the DoseResp program (Wil ten Berge, December 2016) as

$Pr = a + b \times lnC + c \times lnt + d \times S$

with C for concentration in mg/m^3, t for time in minutes and S for sex (0 = female, 1 = male).

Probit function	*Species*	*a*	*b*	*C*	*d*	*n-value*
Sex as covariate	*Rat*	*-37.8*	*4.86*	*6.03*	*0.40*	*0.806 (0.761 – 0.851)*
Sexes combined	*Rat*	*-36.8*	*4.76*	*5.91*		*0.806 (0.762 – 0.851)*

The LC_{50} values for both sexes did not differ by more than a factor 2. This does not support that sex differences exist in the lethal response. For this reason, the data from both sexes were pooled and analysed to derive the animal probit function.

Duration (minutes)	*LC_{50} (mg/m^3) 95%-C.I.* ***Male***	*LC_{50} (mg/m^3) 95%-C.I.* ***Female***	*LC_{50} (mg/m^3) 95%-C.I.* ***Combined***
10	357 (325 – 403)	388 (351 – 450)	372 (347 – 414)
30	91.3 (82.0 – 104)	99.2 (89.0 – 116)	95.2 (87.6 – 107)
60	38.7 (33.5 – 45.4)	42.0 (36.5 – 50.4)	40.3 (35.7 – 46.8)

A graphical overview of the data is presented below. Each concentration-time combination represents one point in the plot.

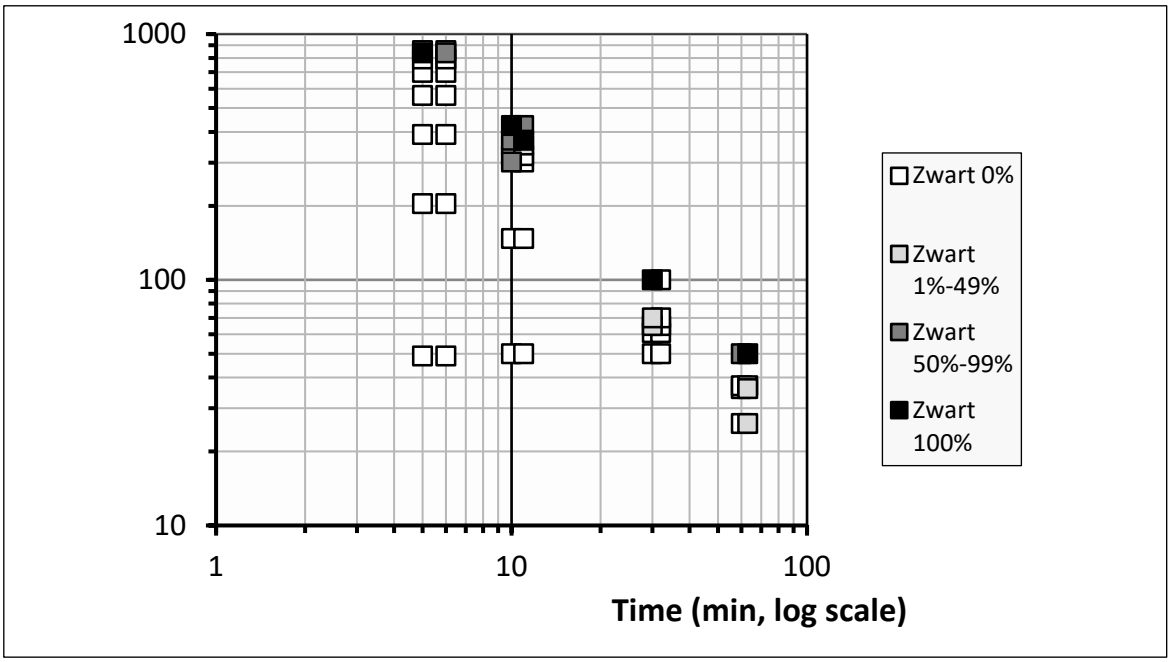


Figure: C x t data Zwart et al (2006) study. The study included 4 exposure durations. At each point, male data are indicated on the left and female data on the right.

Study ID: A.3

Author, year: Zwart, 1990

Study details of study A.3 are the same as the study details of A.2 (based on personal communication with TNO Zeist, The Netherlands, where the study was performed). The mouse study A.3 was performed simultaneously with the rat study A.2.

Substance: phosgene
Species, strain, sex: Swiss mice, males and females
Number/sex/conc.: 5
Age and weight: unknown. (Animals arrived at an age of 7-8 weeks with body weights 23-34 grams and acclimatized for at least 5 days after arrival. The time span between the end of the acclimatization period and the start of the experiments is however not indicated).
Observation period: 14 days

Evaluation of study quality

Criteria	***Comment***
Study carried out according to GLP	*Yes.*
Study carried out according to OECD 403 guideline(s)	*No statement of compliance with OECD guideline 403 provided.*
Stability of test compound in test atmosphere	*Not specified*
Use of vehicle (other than air)	*No*
Whole body / nose-only (incl. head/nose-only) exposure	*Whole body*
Type of restrainer	*N/A*
Pressure distribution	*Not specified*
Homogeneity of test atmosphere in breathing zone of animals	*An adjustable flow of test substance was mixed with airflow.*
Number of air changes per hour	*Approximately 100-150 air changes/hour* *(Content exposure cylinder (length: 0.9 m, r: 0.075 m) 15.9 l; air flow 25-40 l/min)*
Equilibration time (t95)	*1.2-1.9 min*
Start of exposure relative to equilibration	*Not specified. However, the shortest exposure duration in this study (i.e. 5 min) corresponds to 3 x t95 (i.e. 3.6-5.7 min).*
Actual concentration measurement	*Concentrations were measured continuously, by IR analysis and gas chromatography.*
Particle size distribution measurement in breathing zone of the animals in case of aerosol exposure	*N/A*
Assessment of Reliability	***A*** *Well-performed study. Multiple concentration levels and durations were tested.*

Results

Species	Concentration (mg/m^3)		Exposure duration (min)	Lethality	
	Measured	Adjusted		Male	Female
				Dead/ tested	
Mouse	49	N/A	5	0/5	0/5
	204		5	0/5	0/5
	390		5	0/5	0/5
	563		5	3/5	4/5
	698		5	3/5	2/5
	791		5	0/5	4/5
	838		5	4/5	4/5
	856		5	1/5	5/5
	50		10	0/5	0/5
	147		10	0/5	0/5
	301		10	0/5	2/5
	320		10	1/5	3/5
	353		10	4/5	3/5
	370		10	5/5	5/5
	424		10	5/5	5/5
	50		30	0/5	5/5
	61		30	1/5	0/5
	65		30	4/5	5/5
	70		30	4/5	5/5
	100		30	2/5	3/5
	26		60	2/5	4/5
	36		60	3/5	5/5
	37		60	3/5	5/5
	50		60	4/5	5/5

Probit function

The probit function and associated LC-values have been calculated using the DoseResp program (Wil ten Berge, December 2016) as

$Pr = a + b \times lnC + c \times lnt + d \times S$

with C for concentration in mg/m^3, t for time in minutes and S for sex (0 = female, 1 = male).

Probit function	*Species*	*A*	*b*	*C*	*d*	*n-value*
Sex as covariate	*Mouse*	*-13.8*	*2.20*	*2.97*	*-0.76*	*0.739 (0.665 – 0.812)*
Sexes combined	*Mouse*	*-12.9*	*2.05*	*2.78*		*0.739 (0.658 – 0.820)*

The LC_{50} values for both sexes did not differ by more than a factor 2. This does not support that sex differences exist in the lethal response. For this reason the data from both sexes were pooled and analyzed to derive the animal probit function.

Duration (minutes)	*LC_{50} (mg/m^3) 95%-C.I.* ***Male***	*LC_{50} (mg/m^3) 95%-C.I.* ***Female***	*LC_{50} (mg/m^3) 95%-C.I.* ***Combined***
10	326 (273 – 401)	231 (178 – 276)	275 (229 – 317)
30	73.6 (59.3 – 89.5)	52.2 (36.9 – 64.0)	62.1 (47.5 – 73.2)
60	28.8 (21.3 – 36.8)	20.4 (13.2 – 26.5)	24.3 (16.6 – 30.8)

A graphical overview of the data is presented below. Each concentration-time combination represents one point in the plot.

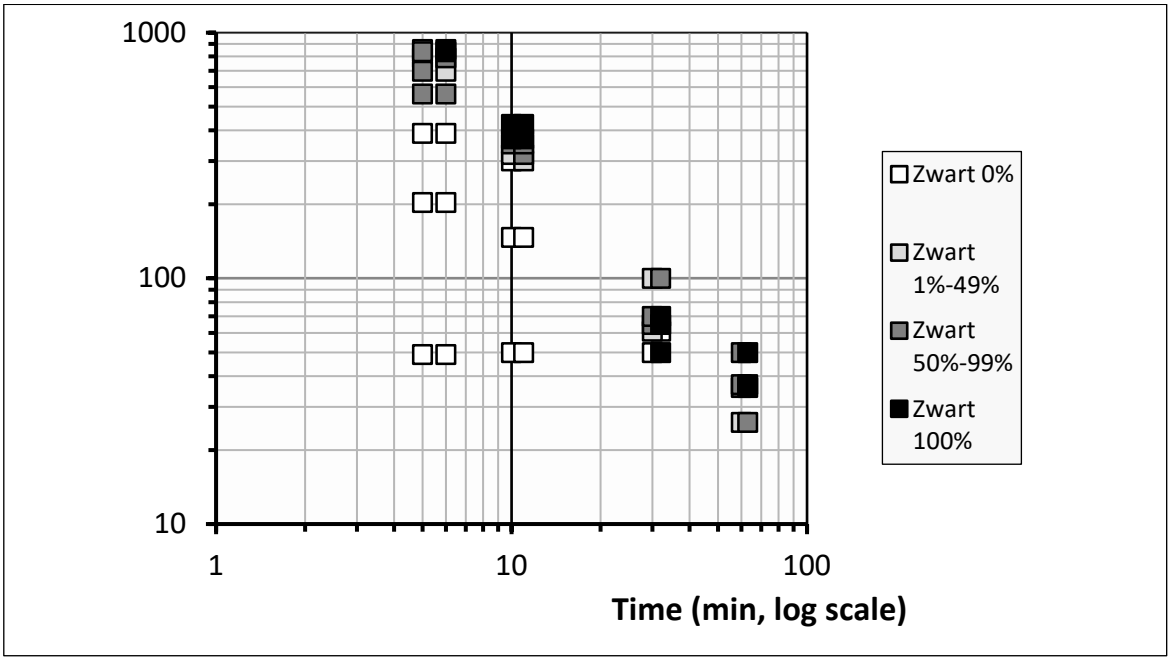


Figure: C x t data Zwart et al (2006) study. The study included 4 exposure durations. At each point, male data are indicated on the left and female data on the right.

Study ID: C studies

Acute lung injury models

The public literature contains a number of recent publications on acute lung injury models (mostly in the mouse) to study the lung injury after acute intoxication with phosgene. These studies focus primarily on the LCt_{50} values after 24 or 48 hours of exposure to determine the diagnosis of illness and treatment possibilities, such as Plahovinsak et al. (2015). Acute lung injury studies provide little information that is relevant for deriving the animal probit function for lethality as they rarely contain raw datasets, too short observation periods and only one exposure concentration. The applied dose is often based on previous results from acute inhalation studies to assure lethality occurs.

Non-human primates

Chasis (1944; as cited in AEGL 2002) reported a 1-min LC_{50} of 240 ppm (986 mg/m^3) for a group of monkeys. The strain, gender, and number of animals were not reported. A 1-min LC_{50} of 500 ppm (2055 mg/m^3) was reported for 19 male and 18 female Rhesus monkeys (Weston and Karel 1947; as cited in AEGL 2002). Moor and Gates (1946; as cited in AEGL 2002) found that all monkeys died when exposed to phosgene at a concentration of 1,087 ppm (4468 mg/m^3) for 1 min. No other experimental details were available for either study.

Sheep

Two Dorset crossbred wethers/group were exposed to 5620, 10,000, 17,800, or 31,600 mg x min/m^3 phosgene gas for 10 minutes and observed for 24 hours (Keeler et al., 1990a). Sheep were exposed through a cone sealed over the nose and mouth after the gas passed through a 5-L anesthesia bag and one-way valve. The 10-minute LCt_{50} was estimated to be 13,300 mg x min/m^3 (1330 mg/m^3) using Thompson and Weil analysis. All sheep had shallow breathing with some breath holding, as well as periods of dyspnea during and after exposure. All concentrations produced pulmonary edema in the sheep grossly and microscopically. Gross findings in the decedents included the trachea filled with a mixture of stringy mucous and frothy material and moist, heavy lungs. Histopathology findings included edema filled alveoli, perivascular spaces, and interlobular septa. The sheep that survived 24 hours had less prominent gross lesions with mild to moderate alveolar edema.

Keeler et al. (1990b; as cited in Glass et al, 2009) exposed an additional five Dorset crossbred wethers for 10 minutes to 2.0–2.5 g/m^3 phosgene and two sheep to air only after they had undergone surgery to cannulate the caudal mediastinal lymph node to monitor pulmonary lymph flow. The sheep were also instrumented with a carotid arterial catheter, pulmonary artery catheter, and left atrial catheter to monitor systemic and pulmonary hemodynamics. The animals were allowed a 5- or 6-day recovery from the surgery prior to exposure to phosgene and then were sacrificed 4 hours post exposure. The sheep demonstrated a 2- to 3-fold increase in pulmonary lymph flow after exposure at every time point, compared to controls. No change was observed in the lymphoplasma protein ratio and a slight increase was observed in the pulmonary microvascular pressure. Histopathological examination of the lungs showed mild to moderate edema most prominent in the ventral aspects of the lung.

Rats

A total of 118 male Wistar rats were exposed to phosgene at 0.5 to 4.0 ppm (2.1 to 16.4 mg/m^3) for 5 min to 8 h (Rinehart 1962; Rinehart and Hatch 1964). The exposures were varied to give CT products between 12 and 360 ppm x min (49.3 and 1480 mg/m^3 x min) and were carried out in 1,700-L wooden exposure chambers operating at a constant ventilation rate of 1,000 L/min. The chamber surfaces were lacquered, and thus, potential loss of phosgene by reaction with the wooden surface was minimized. Details on the exact concentrations and exposure durations were not

provided. This system provided for air "turnover" every 2 min and a 99% equilibrium time of 8 min. Air samples were taken frequently during exposures, and adjustments were made when necessary to maintain constant phosgene concentrations. An $L(CT)_0$ of 180 ppm x min (740 mg/m^3 x min), a 75-min LC_{50} of 4 ppm, and a 125-min LC_{100} of 4 ppm were determined. The authors concluded that different combinations of concentration and time exposure giving equal products of C × T constitute equally effective doses.

Gross et al. (1965; as cited in Glass et al. 2009) extended the study of Rinehart and Hatch (1964) and exposed an additional 117 Wistar rats to concentrations of phosgene ranging from 0.5 to 4.0 ppm (2.1 to 16.4 mg/m^3) for 5 minutes to 8 hours, with 18 unexposed control rats. Most of the animals were sacrificed 4 days post exposure; however, there was a group of 15 rats exposed to 1.7 ppm (7.0 mg/m^3) phosgene for 120 minutes and sacrificed in groups of three at 4, 8, 24, and 48 hours or 1 week post exposure. Another group was exposed to 2.2 ppm (9.0 mg/m^3) for 80 minutes and sacrificed 3 months post exposure; these rats developed chronic pneumonitis. Pneumonitis was defined as slight mural thickening of respiratory bronchioles with involvement of adjacent alveoli; moderate alveolar involvement in a peribronchiolar zone; and severe mural thickening of the respiratory bronchiole accompanied by obliteration of adjoining alveoli. The lowest concentration producing moderate pneumonitis was 0.8 ppm (3.3 mg/m^3) for 1 hour (48 ppm x min; 197 mg/m^3 x min) and severe chronic pneumonitis occurred at 1.5 ppm (6.2 mg/m^3) for 40 minutes (60 ppm x min; 247 mg/m^3 x min). In the group exposed to 1.7 ppm for 120 minutes, severe pneumonitis was observed in 2/3 rats 48 hours post exposure, with most other time points indicating slight or moderate changes; in rats sacrificed 1 week after exposure, 2/3 rats had slight and 1/3 had moderate pneumonitis.

Box and Cullumbine (1947; as cited in AEGL, 2002) investigated phosgene-induced lethality in rats after the rats had experienced an exposure to phosgene at a nonlethal concentration. Rats were divided into two groups (12 per group). Half of each group was exposed to 19.2 ppm (78.9 mg/m^3) phosgene for 10 min and the other half served as a control group. Five days later, the pre-exposed and control rats were exposed to phosgene at 55.2, 60, 75.6, and 105.6 ppm (227, 247, 311 and 434 mg/m^3) for 10 min. The rats were then observed for the next 48 h for deaths. The pretreated rats had a reduced percentage of mortality (33%) compared with the control animals (74%). Thus, partial protection from phosgene-induced lethality was obtained by the phosgene pretreatment.

Hobson et al (2019) exposed groups of 2-7 rats nose-only to analytically determined exposure levels ranging from 95-191 mg/m^3 for 10 minutes. The initial exposure trials with 191-316 mg/m^3 for 10 minutes produced 100% lethality within 24 hours. The purpose of the study was to screen novel therapeutics against relevant short-term high concentration phosgene exposures consistent with real-world human accidental exposure. The animals were sacrificed after 24 hours for histopathological evaluation of the lungs. Due to the brief post-exposure observation period, this study does not qualify to be used in the derivation of a probit function.

Mouse

Cameron et al. (1942; as cited in AEGL, 2002) exposed 20 mice to phosgene at an average concentration of 0.86 ppm (3.5 mg/m^3) for 5 h. Twelve mice were dead the next morning. Several other acute lethality studies of phosgene in mice have been reported. However, these studies do not contain experimental details such as strain or gender of mouse, number of animals exposed, or analytical methodology.

Aggarwal et al (2019) exposed groups of 5 mice whole-body to concentrations ranging of 41 or 82 mg/m^3 (10 or 20 ppm) for 10 minutes, including the build-up time of the concentration; it is not stated whether the concentration was allowed to fall to

zero before removal of the animals. All animals were sacrificed after 24 hours following exposure to study damage to the pulmonary blood-gas barrier and red blood cells. Due to the brief post-exposure observation period, this study does not qualify to be used in the derivation of a probit function.

Pigs

Five young adult female white pigs/group were anesthetized and exposed to either air or 244 mg/m^3 (60 ppm) phosgene for 10 minutes (Brown et al., 2002; as cited in Glass et al. 2009). Prior to exposure, pigs were anesthetized, and arterial and venous catheters were placed. After the surgery, the pigs were allowed to equilibrate for 1 hour, then phosgene was administered by the endotracheal tube and the concentration of phosgene was monitored continuously using an infrared gas analyzer. At 30 minutes post exposure, anesthesia was deepened to allow intermittent positive pressure ventilation to occur for up to 24 hours. Cardiovascular and respiratory measurements were taken every 30 minutes and blood was obtained for arterial and mixed venous blood gas analysis at 0, 10, 30 minutes and then every hour. All control animals survived the full 24 hours; only one treated pig survived the entire 24 hours, the rest died 16–23 hours post exposure. Histopathology was performed on all pigs. Control animals had minimal passive congestion of the lungs and the treated animals had widespread pulmonary edema with bronchial epithelial necrosis. Lung wet weight/body weight was significantly ($p < 0.001$) increased in the treated pigs. From 6 hours post exposure on, arterial pH, PaO_2 and lung compliance were all significantly ($p < 0.01$) decreased with treatment, with oxygen delivery and consumption decreased from hour 15 on.

Dogs

Underhill (1920; as cited in Glass et al. 2009) exposed a total of 327 dogs (breed/age/sex not provided) to phosgene at concentrations ranging from 44 to 120 ppm (181 to 493 mg/m^3) for 30 minutes. Limited details were given as to the type of exposure chamber besides stating that it was an airtight chamber through which a mixture of gas and air flowed with frequent analysis of samples taken to check concentration. Unlike their behavior during exposure to other gasses, the dogs remained lying quietly in the chamber during the phosgene exposures. The LC_{50} (estimated concentration lethal to 50% of animals) for 30 minutes was approximately 61–70 ppm (247 – 288 mg/m^3) based on the number of dogs that died in the first 3 days. Winternitz et al. (1920; as cited in AEGL 2002) reported on the histopathology of these dogs. Of those exposed, 68% of the dogs died within the first 48 hours with most deaths occurring between 12 and 24 hours. These dogs showed little variation in histopathology, along with voluminous, heavy lungs, a frothy exudate found in the lower trachea, and right heart dilation. Systemic effects other than those in the cardiorespiratory system were not identified. Dogs that died 3–10 days after dosing were found to have more respiratory infections in the deep lung.
Pauluhn (2006c) cites a reference of Cucinell et al. 1974, where dogs were exposed for 20 minutes and a LCt_{50} of 4200 mg/m$^3\times$min was reported.

Several species

Mice, guinea pigs, and rats were exposed to various concentrations of phosgene for times ranging from 1 to 64 minutes to determine if the LC_{50} values varied over time (Boyland et al., 1946; as cited in Glass et al. 2009). The study author also presented data from dog studies. Animals were exposed in a Bruhl jar with the phosgene regulated by a flowmeter. Chamber concentrations were sampled by absorption bubblers at a measured rate. The LC_{50} values were presented in the table below. The study lacked details, but similarity of acute lethality values, especially among the small animal species, was demonstrated.

LC_{50} values (ppm (mg/m^3)) for several species (Boyland et al., 1946; as cited in Glass et al. 2009)

	Guinea pig	Rat	Mouse	Dog*
8 min	43 ppm (177 mg/m^3)	92 ppm (378 mg/m^3)	77 ppm (316 mg/m^3)	-
32 min	13 ppm (53 mg/m^3)	17 ppm (70 mg/m^3)	15 ppm (62 mg/m^3)	66 ppm (271 mg/m^3)
64 min	11 ppm (45 mg/m^3)	11 ppm (45 mg/m^3)	7 ppm (29 mg/m^3)	42 ppm (173 mg/m^3)

* Dogs were exposed for 30 and 60 minutes.

Overview of acute lethality studies in several species.

The tables have been copied from AEGL; not all mentioned studies were included in the aforementioned sections.

TABLE 1-7 Acute Lethality of Phosgene in Rats

Strain	Number/ Gender	Exposure Time (min)	Concentration (ppm)	End Point	Reference
NR	NR	10	35	LC_{20}	Shils 1943
NR	NR	10	60	LC_{40}	Shils 1943
NR	NR	1	1,625	LC_{50}	Chasis 1944
NR	44/NR	10	38-75	LC_{50}	Box and Cullumbine 1947a
NR	NR	12	30	LC_{50}	Chasis 1944
NR	NR	15	35	LC_{50}	Cameron and Foss 1941
NR	NR	20	15	LC_{50}	Kimmerle and Diller 1977
Wistar	40/NR	30	10-15	LC_{75}	Henschler and Laux 1960
NR	NR	20	25	LC_{50}	Rothlin 1941
NR	NR	12	85	LC_{60}	Shils 1943
NR	NR	10	40	LC_{70}	Kimmerle and Diller 1977
NR	32/NR	10	39-103	LC_{75}	Box and Cullumbine 1947a

(Continued)

TABLE 1-7 *Continued*

Strain	Number/ Gender	Exposure Time (min)	Concentration (ppm)	End Point	Reference
Wistar	40/NR	20	25	LC_{50}	Henschler and Laux 1960
NR	NR	3	220	LC_{100}	Winternitz et al. 1920
NR	12/NR	10	147	LC_{100}	Box and Cullumbine 1947a
NR	10/NR	13	73	LC_{100}	Schultz 1945
NR	NR	20	37	LC_{100}	Rothlin 1941
NR	NR	30	22	LC_{100}	Winternitz et al. 1920

NR, not reported.

TABLE 1-6 Acute Lethality of Phosgene in Mice

Time (min)	LC_{50} (ppm)	Reference
1	850	Chasis 1944
1	3,300	Moor and Gates 1946
5	33	Kawai 1973
10	77 (male); 61 (female)	Zwart et al. 1990
15	15	Cameron and Foss 1941
30	18 (male); 11 (female)	Zwart et al. 1990
30	5.1	Kawai 1973
60	9 (male); 5 (female)	Zwart et al. 1990

TABLE 1-8 Acute Lethality of Phosgene in Guinea Pigs

Exposure Time (min)	Concentration (ppm)	End Point	Reference
1	672	LC_{50}	Chasis, 1944
15	32	LC_{50}	Underhill, 1920
30	18	LC_{50}	Chasis, 1944
30	141	LC_{50}	Moor and Gates, 1946
9	85	LC_{99}	Coman et al., 1947
3	220	LC_{100}	Winternitz et al., 1920
30	20	LC_{100}	Winternitz et al., 1920
20	77	LC_{100}	Ong, 1972

TABLE 1-9 Acute Lethality of Phosgene in Rabbits

Exposure Time (min)	Concentration (ppm)	End Point	Reference
30	17	LC_{40}	Frosolono 1977
1	3,200	LC_{50}	Moor and Gates 1946
15	187	LC_{50}	Underhill 1920
20	110	LC_{50}	Cameron and Courtice 1946
20	20	LC_{50}	Laquer and Magnus 1921

Exposure Time (min)	Concentration (ppm)	End Point	Reference
30	100-135	LC_{70}	Halpern et al. 1950
30	93	LC_{75}	Frosolono 1976
30	82	LC_{90}	Shils 1943
35	151	LC_{99}	Coman et al. 1947
15	220	LC_{100}	Winternitz et al. 1920
30	110	LC_{100}	Winternitz et al. 1920

TABLE 1-10 Acute Lethality of Phosgene in Dogs

Strain	Number/ Gender	Exposure Time (min)	Concentration (ppm)	End Point	Reference
NR	12/NR	10	110	LC_{25}	Cameron and Courtice 1946
NR	NR	1	2,100	LC_{50}	Chasis 1944
NR	NR	10	45	LC_{50}	Kimmerle and Diller 1977
NR	24/NR	15	60-70	LC_{50}	Underhill 1920
NR	NR	20	502	LC_{50}	Chasis 1944
NR	6/NR	30	100-175	LC_{50}	Patt et al. 1946
NR	NR	30	78	LC_{55}	Postel and Swift 1945
Mongrel	18/NR	3	745-880	LC_{70}	Coman et al. 1947
NR	94/NR	20	135	LC_{70}	Freeman et al. 1945
NR	42/NR	30	98	LC_{70}	Postel and Swift 1945
Mongrel	15/M,F	30	124	LC_{90}	Schultz 1945
Mongrel	32/NR	10	39-103	LC_{75}	Box and Collumbine 1947
Mongrel	NR	3	734	LC_{99}	Coman et al. 1947
Mongrel	NR	30	90	LC_{99}	Coman et al. 1947

NR, not reported.

Appendix 2 Rat versus dog comparison by Pauluhn

Pauluhn studied the non-lethal effects of phosgene exposure on the respiratory system in both rats (Pauluhn, 2006c) and dogs enabling a comparison between the species (Pauluhn, 2006b).

In the rat study, young adult male Wistar rats of the strain Hsd Cpb:WU (SPF) were exposed to various concentrations of phosgene for 30 or 240 minutes. Only male rats were used as previous studies supported absence of gender specific toxicity (Pauluhn, 2006a). The animals were exposed using a direct flow nose-only exposure design. The table below (copied from Pauluhn 2006c) shows the concentrations applied in the study. Note that for each C x t combinations on days 1, 3, 7, 28 and 84 after exposure, six rats/group are sacrificed for BAL and lung weight determinations. Except for the 1008 mg/m^3 x min group where on days 1, 7, 14, and 28 examinations took place. Additionally on days 28 and 84 additional 6 rats (not lavaged) were used for histopathology.

TABLE 1

Concentration × time exposure matrix of rats nose-only exposed to phosgene gas

Exposure time (min)	Target concentration (mg/m^3)	Actual concentration[a] (mg/m^3)	Concentration × time (mg/m^3 × min)	Observation period (days)
30	1	0.94	28.2	84
30	2	2.02	60.6	84
30	4	3.89	116.7	84
30	8	7.35	220.5	84
30	16	15.36	460.8	84
240	Air control	0	0	84
240	0.2	0.96	47.0	84
240	0.4	0.39	92.9	84
240	0.8	0.79	188.6	84
240	1.6	1.57	376.0	84
240[b]	4.2	4.20	1008	28

[a] Actual breathing zone concentration.

[b] Data duplicated from Pauluhn (2006).

Atmospheres were generated by diluting phosgene in synthetic air with filtered ambient air and consequently passing the gas mixture into the nose-only chamber. Sampling took place at various exposure ports and continuously measured by infrared analyser or CM4 toxic gas paper tape monitor. Characterisation of the test atmosphere was also determined by gas chromatography. As results from the rat study were used in the dog study for comparison, the rat results are presented together with the dog study results below.

In the dog study by Pauluhn (2006b), four young purebred male and female beagle dogs (strains Hsd Cpb: DOBE for 9 mg/m^3 and Hsd Jan:DOBE for 16.5 mg/m^3 and 35 mg/m^3) were exposure to phosgene head-only for 30 minutes to 9 mg/m^3, 16.5 mg/m^3 and 35 mg/m^3. The respective C ×t products were 270, 495, and 1050 mg/m^3 ×min. The objective of this "proof of principle" study was to compare the magnitude of changes of selected nonlethal endpoints (lung weights, bronchoalveolar lavage

data, arterial blood gases, and histopathology) in dogs with similar data from rats (except arterial blood gases). "Controlled flows of phosgene were discharged from a cylinder certified to contain 101 ppm phosgene in synthetic air and were dosed (by flow meters) into a continuous flow of conditioned, dry air. The respective target concentrations were attained by dilution cascades prior to entering the head-only inhalation chamber. This chamber was made from transparent plastics, its internal volume (V) was approximately V=11 L (base 20×20 cm; height 25 cm; height of pyramidal top 7 cm) and was operated in a well-ventilated room. Additionally, a cubic jacket made of transparent plastic served the purpose to prevent leakage of the test substance into the surrounding area. The stability and homogeneity of test atmospheres, as well as the time required to attain steady state, were controlled continuously using a real-time phosgene analyser. Phosgene atmospheres were passed through the openings of the inner chamber, directly toward the dogs' breathing zone. The ratio between the air supplied into and exhausted from the inhalation chamber was chosen so that approximately 75% of the air supplied was removed through the exhaust system (push and pull system). For each dog an adequate air flow of approximately 25 L×min^{-1} was provided, which minimizes the rebreathing of atmospheres. This air exchange rate provides at least five times the respiratory minute volume of normally breathing dogs."

Test atmosphere analyses were the same as reported in the rat study (Pauluhn, 2006c, and as described above).

Results: In both studies, Pauluhn showed results of lung weight to body weight ratios, total cell count, neutrophils, polymorphonuclear leukocytes, proteins, and collagen in BAL fluids (the reader is referred to the papers by Pauluhn 2006b,c). For both species very similar results were observed in terms of same treatment related trends. For example the lung weight to body weight ratios.

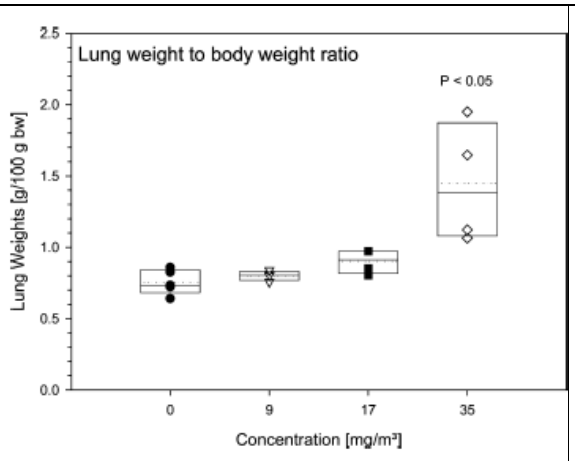


FIG. 3. Lung weight to body weight ratios of dogs head-only exposed for 30 min to 9, 16.5, or 35 mg phosgene/m^3. The boundaries of the boxes represent the 25th and 75th percentiles; medians and means are represented by solid and dotted lines, respectively. Nonexposed dogs ($n = 5$) served as control. Data points represent individual dogs.

Dogs

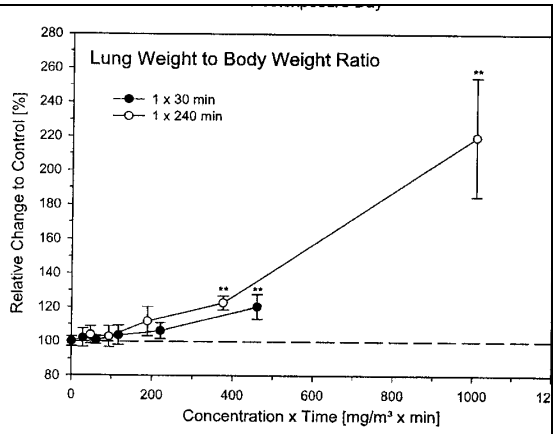


FIG. 1. Lung weight to body weight ratios of rats exposed fo either 30 or 240 min to various $C \times t$ products of phosgen gas. Upper panel: Concentration–duration of exposure depen dence and time course of effects during a postexposure perio of 4 (4.2×240 mg/m^3 × min) or 12 wk. Lower panel: Rela tionship of lung weight to body weight ratios on postexposur day 1 based on $C \times t$ products. At each serial sacrifice six rat were examined. Data represent group means ± SDs. Asterisk denote statistical significance to nose-only air-exposed control ($^*p < .05$, $^{**}p < .01$).

Rats

Respiratory minute volumes were recorded for dogs indicating that the average ventilation of dogs (0.4 L/(min × kg body weight)) is two to three times less than that of rats, although some dogs have experienced higher respiratory minute volumes. The analysis of breathing patterns obtained from measurements shortly after exposure in dogs did not reveal any evidence of apnoea periods similar to those observed during the exposure of rats to phosgene in similar concentrations (referring to Pauluhn, 2006a). Collectively, the comparison of indicators of acute lung injury in BAL total protein supports a respiratory minute volume-dependent degree of pulmonary damage (see also the 3- fold difference in Figure 8 below, copied from Pauluhn, 2006b).

Focusing on the BAL liquid proteins as -since this parameter is considered the most sensitive predictor for pulmonary injury, Pauluhn showed that rats had 10-fold higher levels at similar C x t products (approx. 1050 mg/m^3 x min, which is the LCt_{01} in rats in Pauluhn, 2006a). It should be noted, however, that Pauluhn hypothesized that the 10-fold higher protein exudation into the BAL at this high dose could be a reflection of the rat (or rodent) specific defence mechanism, as major part of the lungs is "lavaged and proteinaceous secretions from airways may contribute markedly to the total protein and inflammatory cells detected in BAL." Hence, absolute differences between BAL fluid proteins may not provide information on susceptibility differences between species. The comparison between rats and dogs as to what dose produces 150% BAL proteins compared to background levels showed a 3-fold lower C x t product in rats (117 mg/m^3 x min) compared to dogs (375 mg/m^3 x min) (Pauluhn, 2006b, see Figure 8 therein and copied in below), which according to the author is considered to be a better indication for differences in susceptibility.

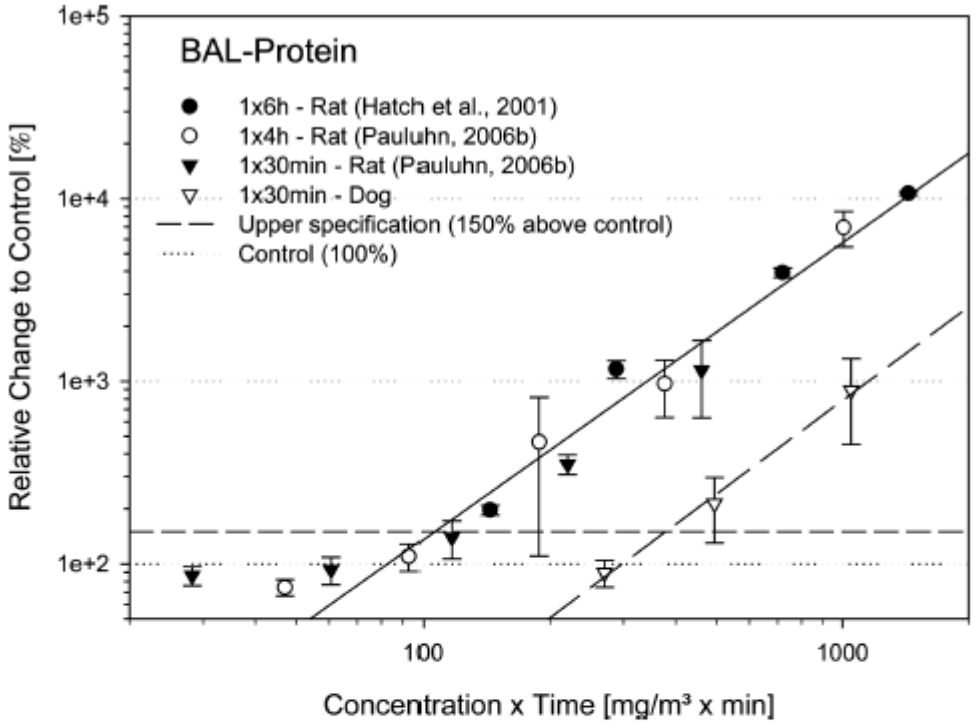


FIG. 8. Concentration × time dependence of total protein in bronchoalveolar lavage fluid protein (obtained from the lobus accessorius) in head-only exposed dogs to phosgene (dashed line: $y_{dog} = -2.2 + 1.7x$; $r^2 = .99$), whole-body exposed Fischer 344 rats (Hatch et al., 2001), or nose-only exposed Wistar rats (Pauluhn, 2006b) rats to various concentrations of phosgene bracketing exposure durations from 30 to 360 min (solid line: $y_{rat} = -1.11 + 1.62x$; $r^2 = .97$). Rats and dogs were sacrificed approximately 24 h following exposure. Data represent group means ±SDs.

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