



Probit function technical support document

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substance name	CAS number
<b>Phosgene</b>	<b>75-44-5</b>

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.

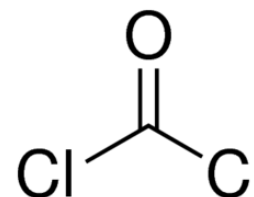
This document has been checked for completeness by the Netherlands' National Institute of Public Health and the Environment (RIVM). The contents of this document, including the probit function, has been approved by the Dutch Expert Panel on Probit Functions on scientific grounds. External parties have 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](http://www.rivm.nl/en/Topics/P/Probit_functions)

# 1 Technical support document phosgene

## 1. Substance identification



CAS-number:	75-44-5
IUPAC name:	phosgene
Synonyms:	Carbonic dichloride, carbonic oxychloride
Molecular formula:	COCl <sub>2</sub>
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:	157 kPa (at 20°C)
Saturated vapor conc:	N/A (at 20°C)
Conversion factor:	1 mg/m <sup>3</sup> = 0.24 ppm (at 20°C and 101.3 kPa)
	1 ppm = 4.11 mg/m <sup>3</sup> (at 20°C and 101.3 kPa)
Labelling:	H314; H330

## 2. Mechanism of action and toxicological effects following acute exposure<sup>1</sup>

**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<sub>50</sub> value for humans was estimated to be 790 ppm (3247 mg/m<sup>3</sup>) (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-year-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<sup>3</sup>) 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

<sup>1</sup> AEGL 2002 final.

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

3  
4 Hegler (1928; as cited by AEGL, 2002) reported the effects of a phosgene accident  
5 that occurred in Hamburg, Germany, on May 20, 1928. Eleven metric tons of "pure  
6 phosgene" were released from a storage tank on a warm, dry, slightly windy day.  
7 Within a few hours, people as far as six miles from the release site began reporting to  
8 hospitals. Three hundred people reported to hospitals within a few days of the  
9 accident. Effects ranged from mild or moderate illness to death; 10 people were  
10 reported to have died. In general, exposed persons exhibited symptoms consistent  
11 with other reported phosgene poisonings (headache, dizziness, nausea and vomiting,  
12 irritant cough, and sickening-sweet taste, followed by a latency period and then  
13 pulmonary symptoms). Autopsies on 6 of the 10 fatalities showed pulmonary effects  
14 in all cases. Fatty degeneration of the kidneys, liver, and heart were observed in a  
15 few cases and were thought to be secondary to the pulmonary damage. In an atypical  
16 case, damage in the gray matter of the brain and spinal cord, hyperaemia, and signs  
17 of bleeding in the white matter were observed at autopsy. That patient died 11.5 days  
18 post-exposure from a blood clot lodged in the lung. It was uncertain if the extra-  
19 pulmonary effects were attributable to phosgene.

20  
21 Diller and Zante (1982) performed an extensive literature review concerning human  
22 phosgene exposure and found that a majority of data were anecdotal or rough  
23 estimates and did not report reliable exposure concentrations and/or durations.  
24 Information from this review is presented in Table 1. The lowest LCT value reported  
25 to produce lethality was 100 ppm×min (13.7 mg/m<sup>3</sup> at a 30-min exposure).

26  
27 **Table 1:** Lethal effects of phosgene exposure in healthy humans (Diller 1982).

Effect – lethal concentration x time product	Cumulative phosgene exposure*
Onset of pulmonary damage	> 30 ppm × min, conc. > 0.15 ppm
Clinical pulmonary edema	> 150 ppm × min
LCT <sub>minimal</sub>	Appr. 300 ppm x min
LCT <sub>50</sub>	Appr. 500 ppm x min
LCT <sub>100</sub>	Appr. 1300 ppm x min

28 \* 300 ppm = 1233 mg/m<sup>3</sup>, 500 ppm = 2055 mg/m<sup>3</sup>, 1300 ppm = 5343 mg/m<sup>3</sup>.

29  
30 In occupational setting, data of workers with a phosgene indicator badge revealed  
31 that an exposure below 50 ppm x min (corresponding to 0.4 mg/m<sup>3</sup> for 8h exposure  
32 period) did not result in signs or symptoms of phosgene toxicity in 78 of 88  
33 individuals; however, three cases of temporary pulmonary edema were observed  
34 (AEGL, 2002).

#### 35 36 **4. Animal acute toxicity data**

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

- 39 1. AEGL final TSD, ERPG document and EU RAR and reference database for  
40 phosgene, covering references before and including 1995.
- 41 2. An additional search covering publications from 1980 onwards was performed in  
42 HSDB, MEDline/PubMed, Toxcenter, IUCLID, ECHA, RTECS, IRIS and ToxNet with  
43 the following search terms:
  - 44 • Substance name and synonyms
  - 45 • CAS number
  - 46 • lethal\*
  - 47 • mortal\*
  - 48 • fatal\*
  - 49 • LC<sub>50</sub>, LC
  - 50 • probit

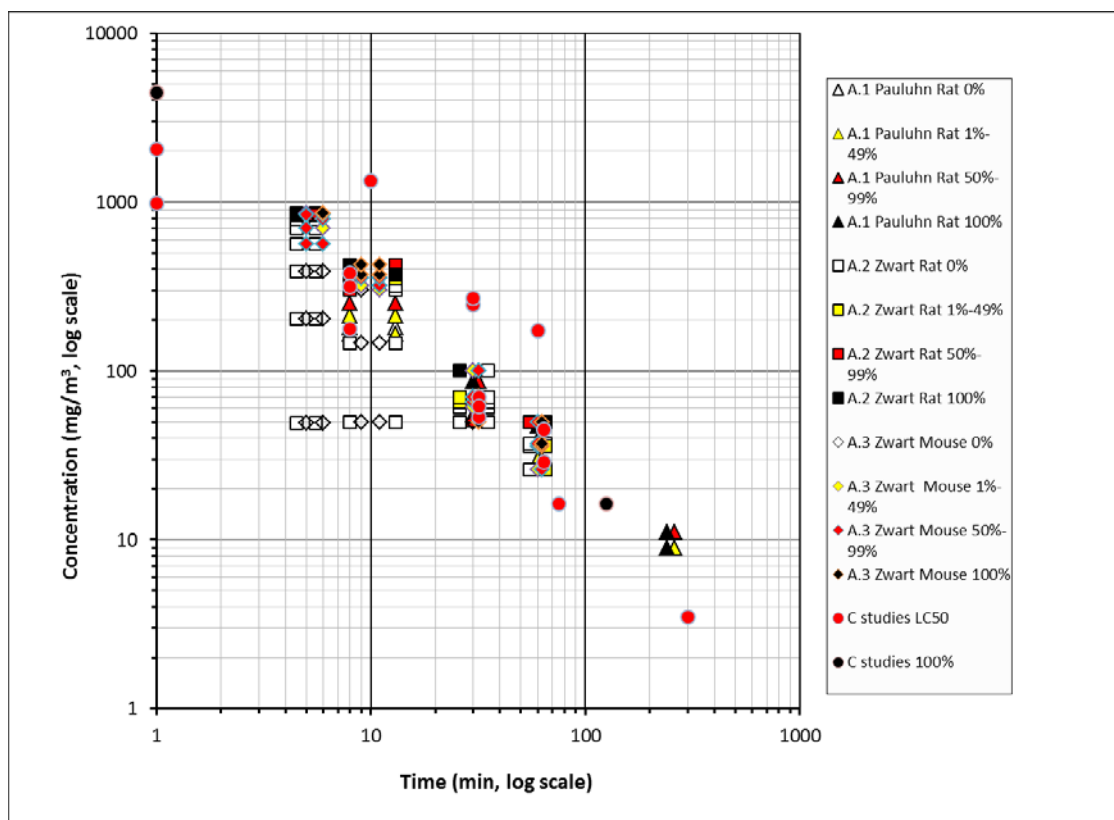
3. Unpublished data were sought through networks of toxicological scientists. Animal lethal toxicity data considering acute exposure are described in Appendix 1. A total of 16 studies were identified -with 17 datasets for 7 species- with data on lethality following acute inhalation exposure. Three datasets 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.

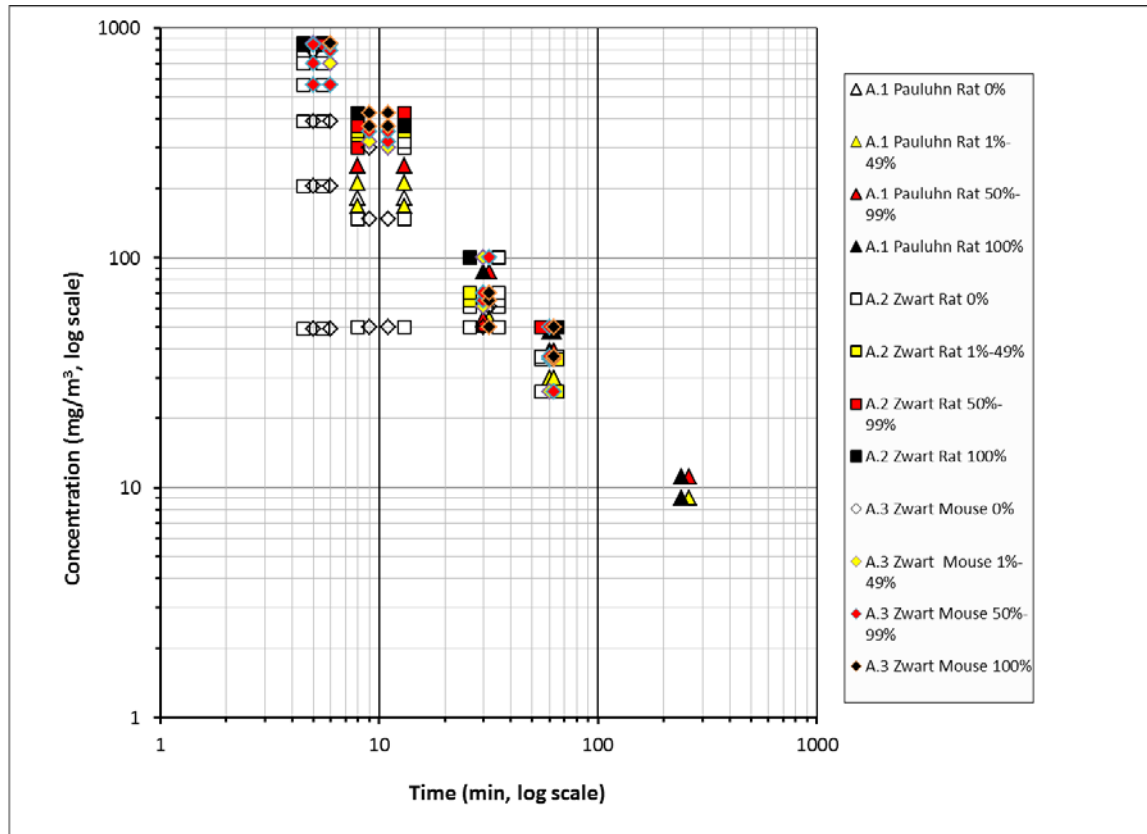
Probit functions have been calculated and reported in Appendix 1 for each of the reported studies. The results of the calculations are presented in Table 2.

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

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

2  
3  
4

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



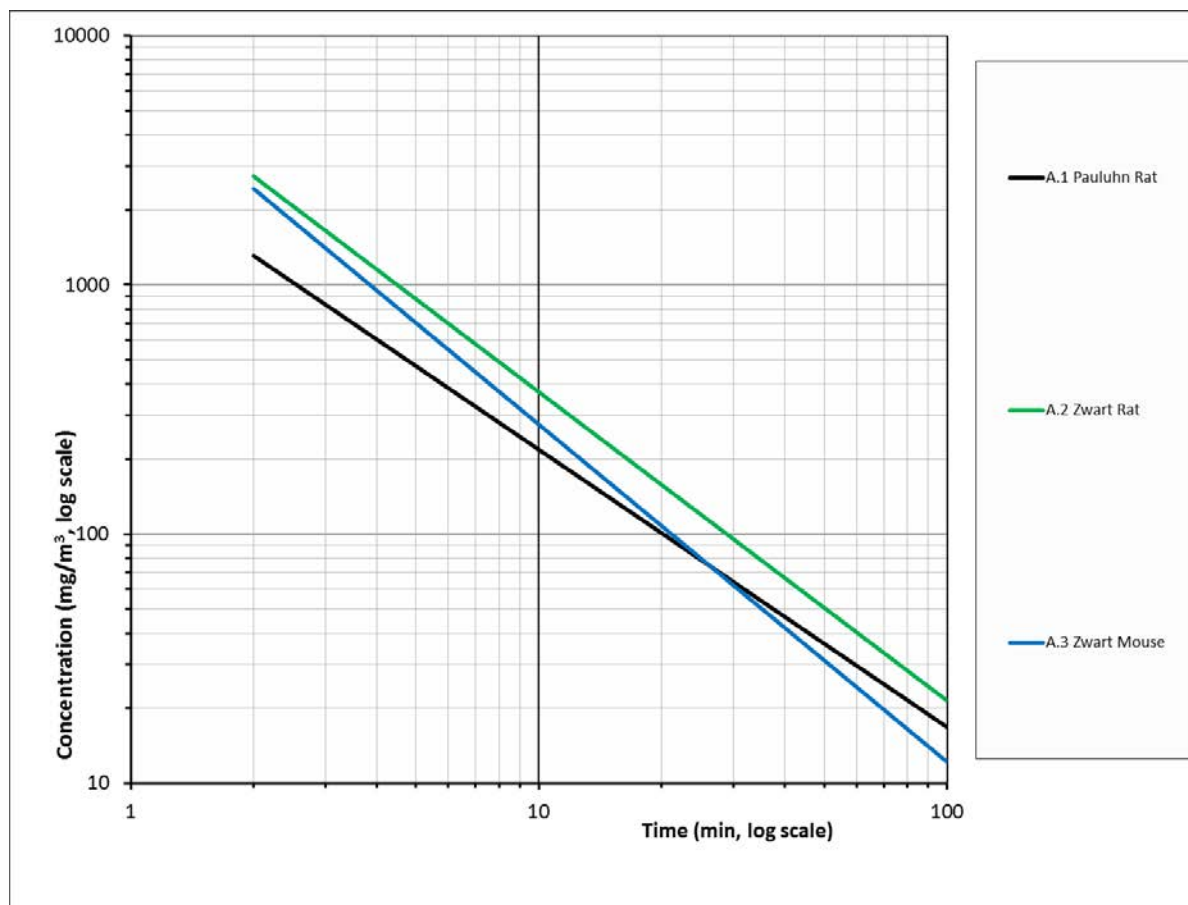
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**Figure 2** Data selected for the initial analysis for the derivation of the animal probit function of phosgene.

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

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18  
19

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



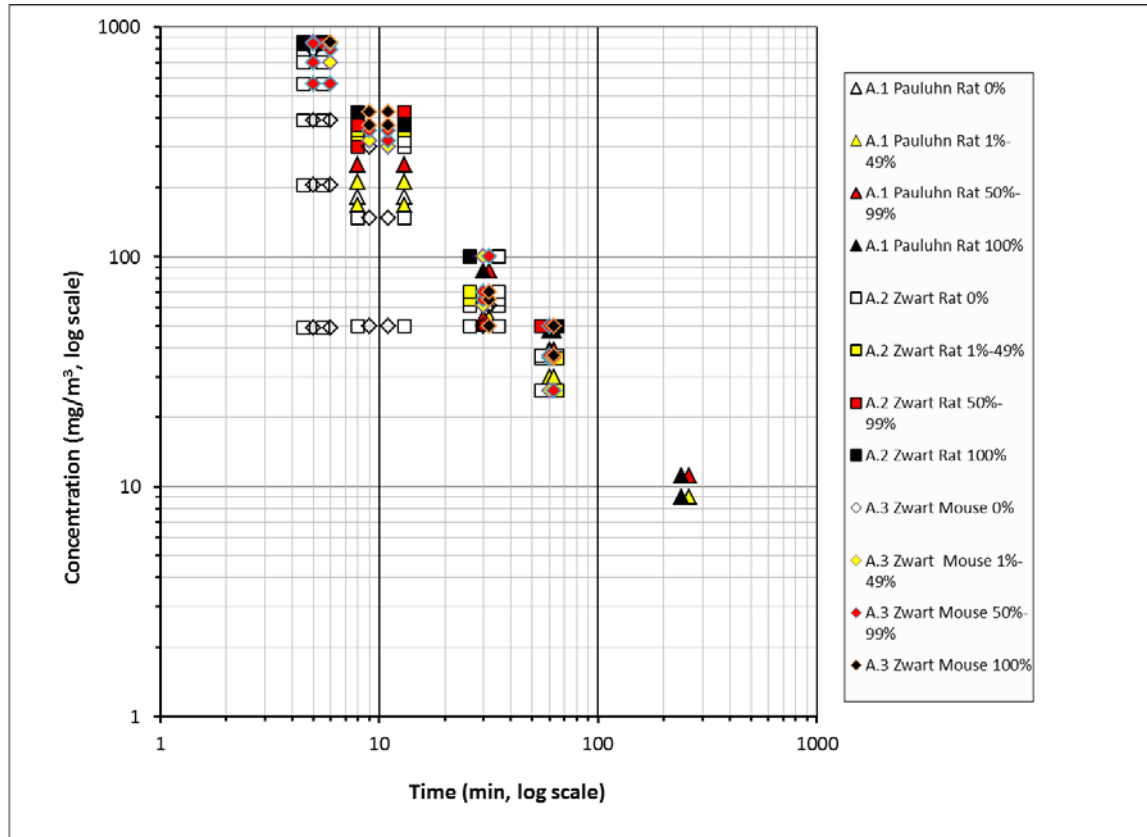
1  
2 **Figure 3** *LC<sub>50</sub> values of A.1, A.2, and A.3 datasets for phosgene, over time where*  
3 *available.*

4  
5  
6 **Table 2** *Data selected for the derivation of the animal probit function of phosgene*  
7 *(identical to table 2).*

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

8  
9 The data of the selected datasets are presented graphically below.

10  
11



1  
2 **Figure 4** Final data selected for derivation of the animal probit function of phosgene  
3 (identical to figure 2).  
4  
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6 **6. Derivation of the human probit function**

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

10 The species-specific n-value was 0.852 for the rat (arithmetic mean of A.1 and A.2)  
11 and 0.739 for the mouse. The mean n-value across species is the arithmetic mean of  
12 the species-specific mean n-values (without weight) and was calculated to be 0.796.  
13  
14

15 Second, the LC<sub>50</sub>-values of all A-studies were calculated for a common exposure  
16 duration of 30 minutes.  
17

18 The species-specific geometric mean LC<sub>50</sub>-values were calculated from the 30-min  
19 LC<sub>50</sub> values of studies A.1, A.2 for the rat and A.3 for the mouse. The species-specific  
20 LC<sub>50</sub>-values were 78.1 and 62.1 mg/m<sup>3</sup> for the rat and mouse, respectively. Finally,  
21 the geometric mean overall LC<sub>50</sub>-value was calculated as:  
22

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

23  
24 With  $\overline{LC_{50}}$  = geometric mean LC<sub>50</sub>-value across species  
25 LC<sub>50,i</sub> = LC<sub>50</sub>-value of study i.  
26 m = number of observations on LC<sub>50</sub>-values within a species (i=1...m).  
27 s = number of species for which LC<sub>50</sub>-values are pooled (j= 1...s).

1 Application of an overall assessment factor of 3 (determined by an interspecies factor  
2 of 3) would result in a calculated 30-min and 60-min human LC<sub>1</sub> of 7 and 3 mg/m<sup>3</sup>,  
3 respectively (probit function and resultant LC-values not shown). The 8h LC<sub>1</sub> would  
4 then be 0.22 mg/m<sup>3</sup>. This would be in conflict with human (occupational) data. Data  
5 of workers with a phosgene indicator badge revealed that an exposure below 50 ppm  
6 x min (corresponding to 0.43 mg/m<sup>3</sup> for an 8h exposure period) did not result in signs  
7 or symptoms of phosgene toxicity in 78 of 88 individuals; however, three cases of  
8 pulmonary edema were observed (AEGL, 2002).

9 Human information on lethal effects of phosgene poisoning is available. There are  
10 case reports and descriptions of accidents concerning phosgene and observations  
11 during the use of phosgene as a warfare agent during world war I. Diller and Zante  
12 (1982; as cited in AEGL 2002) derived a LC<sub>t1</sub> of approximately 300 ppm x min (equals  
13 to 1233 mg/m<sup>3</sup> x min), that would provide a human (unknown subtype population) 30  
14 min LC<sub>1</sub> of 41.1 mg/m<sup>3</sup>, assuming a linear C x t relationship. The authors Diller and  
15 Zante themselves reported that the reliability of the data in their literature review,  
16 especially on concentration levels, was insufficient and therefore the human  
17 information was considered as supportive information.

18  
19 Pauluhn (2006b) discussed whether the dog is a better model for human phosgene  
20 inhalation toxicity than rodent species. He concluded that "dogs are considered more  
21 human-like and a better model for humans", whereas "Small rodents are associated  
22 with the higher ventilation rate and with rodent-specific sensory bronchopulmonary  
23 defense reflexes (i.e. reflex bradypnoea)." Pauluhn (2006b) compared the  
24 bronchoalveolar lavage (BAL) fluid proteins that are indicative for pulmonary injury  
25 after phosgene intoxication both in rats (Pauluhn, 2006c) and dogs (Pauluhn, 2006b),  
26 in addition to other parameters including lung weights, arterial blood gases and lung  
27 histopathology. Focusing on the BAL liquid proteins -Pauluhn considers this parameter  
28 the most sensitive predictor for pulmonary injury- Pauluhn showed that rats had 10-  
29 fold higher levels at similar C x t products (appr. 1050 mg/m<sup>3</sup> x min, which is the  
30 LC<sub>t01</sub> in rats in Pauluhn, 2006a). It should be noted, however, that Pauluhn  
31 hypothesized that the 10-fold higher protein exudation into the BAL at this high dose  
32 could be a reflection of the rat (or rodent) specific defence mechanism, as the major  
33 part of the lungs is "lavaged and proteinaceous secretions from airways may  
34 contribute markedly to the total protein and inflammatory cells detected in BAL."  
35 Hence, absolute differences between BAL fluid proteins may not provide information  
36 on susceptibility differences between species. The comparison between rats and dogs  
37 as to what dosage produces 150% BAL proteins compared to background levels  
38 showed a 3-fold lower C x t product in rats (117 mg/m<sup>3</sup> x min) compared to dogs  
39 (375 mg/m<sup>3</sup> x min) (Pauluhn, 2006b, see Figure 8 therein (see also appendix II),  
40 which the author considered to be a better indication for differences in susceptibility.  
41 "The occurrence of pulmonary responses suggestive of mild oedema and inflammation  
42 at 495 mg/m<sup>3</sup> x min in dogs compares favourably with similar observations in humans  
43 at >600 mg/m<sup>3</sup> x min by the National Research Council, 2002, and Diller & Zante,  
44 1982" (as cited by Pauluhn, 2006b). However, as Diller and Zante stated themselves,  
45 the concentration levels reported in their literature review are unreliable (cf. above).  
46 Pauluhn's studies provide no information that allows a direct comparison of lethality  
47 rates at different concentration-time combinations between rodents and dogs.

48  
49 There is no reliable lethality study in dogs. In Table 4, ranges of 30-min LC<sub>50</sub> values  
50 are presented for several species including rats and dogs, mostly derived from C-  
51 studies unless indicated otherwise. LC<sub>50</sub> values suggest that LC<sub>50</sub> values for dogs are a  
52 factor of 3 to 4 higher than those of rats.



1 **Table 4** Ranges of 30-min LC<sub>50</sub> values for several species.

Species	LC <sub>50</sub> , 30 minutes (mg/m <sup>3</sup> )	
Mouse	21 – 62*	4 5
Rat	64* – 95*	6
Guinea pig	74 – 580	7 8
Rabbit	82 – 452 (20-min LC <sub>50</sub> ); 411 – 555 (30-min LC <sub>70</sub> )	9
Dog	247 – 288	10 11

12 \* Values derived from A studies.

13  
14 Based on the information above, it appears that dogs are less susceptible than rats  
15 and mice to adverse respiratory system effects of phosgene inhalation exposure. The  
16 anatomy of human (and dog) lungs is fundamentally different from rodent lungs, with  
17 humans having a 'leaf tree' structure and rodents having a 'pine-tree' structure. Since  
18 no reliable dog inhalation lethality study is available, the rat and mouse data have  
19 been used as point of departure for probit function derivation as calculated above. It  
20 should be noted that the most susceptible species have been used to derive a PoD,  
21 while dogs, having the same anatomy as humans, are thought to be a better (lung)  
22 model for humans and are considerably less susceptible than rats.

23  
24 In addition, the literature review by Diller and Zante suggests that the human  
25 response (pulmonary oedema and lethality effect levels) following phosgene  
26 inhalation exposure seems to occur at higher exposures than in the rodent studies,  
27 even though the exact concentrations on human lethal response are considered to be  
28 unreliable by Diller and Zante as they rely on case reports.

29  
30 The Point of Departure for the human probit function is a 30-minute geometric mean  
31 animal LC<sub>50</sub> value of 69.6 mg/m<sup>3</sup> and an arithmetic mean n-value of 0.796. Starting  
32 from these rodent data and applying an interspecies factor of 1 would lead to a 30-  
33 min LC<sub>01</sub> of 22 mg/m<sup>3</sup>. However, the lowest reported human lethal concentration-time  
34 of 100 ppm×min corresponds to 14 mg/m<sup>3</sup> for 30 minutes. Therefore, an interspecies  
35 factor of 2 is applied for the derivation of the probit function despite the fact that the  
36 derived probit function would still be in conflict with the 8-hour occupational data.

37  
38 The human equivalent LC<sub>50</sub> was calculated by applying the following assessment  
39 factors:

41 **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.

42  
43 The estimated human equivalent 30-minute LC<sub>50</sub> value is  $69.6 / 2 = 34.8 \text{ mg/m}^3$ .

44  
45 The experimentally determined n-value was **0.796** (see above). Assuming a  
46 regression coefficient (b×n) of 2 for the slope of the curve, the b-value can be  
47 calculated as  $2 / n = 2.514$ .

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

4  
 5  **$Pr = -10.7 + 2.51 \times \ln (C^{0.80} \times t)$  with C in mg/m<sup>3</sup> and t in min.**

6  
 7 The derived human probit function has a scientifically sound basis. The probit function  
 8 is based on two studies and three datasets in the rat and mouse with A quality,  
 9 containing in total 60 C x t combinations with 10 animals (5 per sex) per C x t  
 10 combination.

11  
 12 The calculated human 60 min LC<sub>0.1</sub> (Pr = 1.91) calculated with this probit equation is  
 13 3.2 mg/m<sup>3</sup> and the calculated human 60 min LC<sub>1</sub> (Pr = 2.67) is 4.7 mg/m<sup>3</sup>.

14  
 15 **Table 6** LC-values calculated with the derived probit function compared with existing  
 16 acute inhalation exposure guidelines.

Estimated level	30 min (mg/m <sup>3</sup> )	60 min (mg/m <sup>3</sup> )
0.1% lethality, this probit	7.7	3.2
1% lethality, this probit	11.2	4.7
AEGL-3 <sup>2</sup> (2002, final)	6.2	3.1
ERPG-3 <sup>2</sup> (2008)		6.2
LBW (2016)	9.4	4.2

17  
 18 Compared with equivalent (inter)national guideline levels as presented in the table  
 19 above, the lethal levels derived with this probit function are similar.

20  
 21  
<sup>2</sup> AEGL and ERPG values were converted from ppm to mg/m<sup>3</sup> with the conversion factor calculated in section 1. Therefore, the AEGL and ERPG values in mg/m<sup>3</sup> 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 <sub>2</sub> O. 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 (t <sub>95</sub> )	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	<i>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<sup>3</sup>).</i>
Particle size distribution measurement in breathing zone of the animals in case of aerosol exposure	N/A
Assessment of Reliability	<b>A</b> <i>Studies is well performed and considered several C x t combinations.</i>

1  
2**Results**

Species	Concentration (mg/m <sup>3</sup> )		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

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11**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<sup>3</sup>, 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)

12

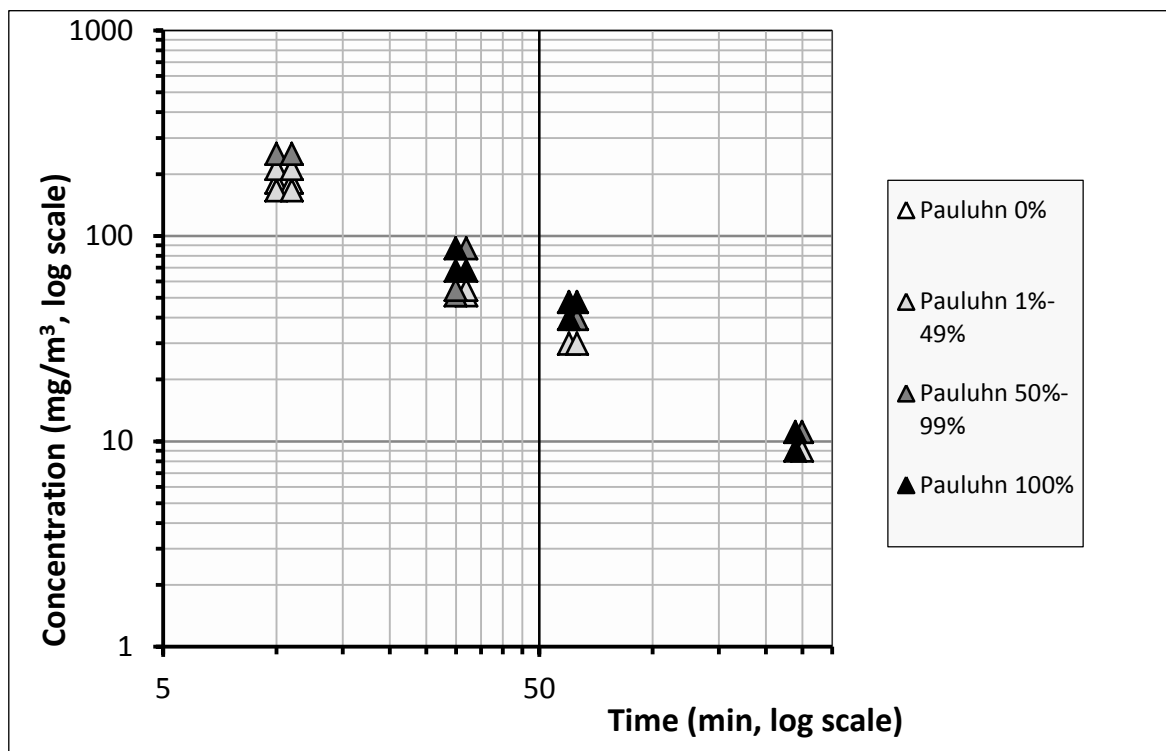
1 The LC<sub>50</sub> values for both sexes did not differ by more than a factor 2. This does not  
 2 support that sex differences exist in the lethal response. For this reason the data from  
 3 both sexes were pooled and analyzed to derive the animal probit function.

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<i>Duration (minutes)</i>	<i>LC<sub>50</sub> (mg/m<sup>3</sup>) 95%-C.I. Male</i>	<i>LC<sub>50</sub> (mg/m<sup>3</sup>) 95%-C.I. Female</i>	<i>LC<sub>50</sub> (mg/m<sup>3</sup>) 95%-C.I. Sexes combined</i>
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)

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



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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.

1 **Study ID: A.2**2 **Author, year: Zwart, 1990**

3 Substance: phosgene

4 Species, strain, sex: SPF-bred Wistar-derived rats, males and females

5 Number/sex/conc.: 5

6 Age and weight: Age unknown, males weighed between 119-181 g and females

7 103-137 g.

8 Observation period: 14 days

9 **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	<b>A</b> Well performed study. Multiple concentration levels and durations were tested.

1 **Results**

Species	Concentration (mg/m <sup>3</sup> )		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

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**Probit function**

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The probit function and associated LC-values have been calculated using the

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DoseResp program (Wil ten Berge, December 2016) as

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

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with C for concentration in mg/m<sup>3</sup>, t for time in minutes and S for sex (0 = female, 1

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= male).

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

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The LC<sub>50</sub> 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.

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<i>Duration (minutes)</i>	<i>LC<sub>50</sub> (mg/m<sup>3</sup>) 95%-C.I. Male</i>	<i>LC<sub>50</sub> (mg/m<sup>3</sup>) 95%-C.I. Female</i>	<i>LC<sub>50</sub> (mg/m<sup>3</sup>) 95%-C.I. Combined</i>
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)

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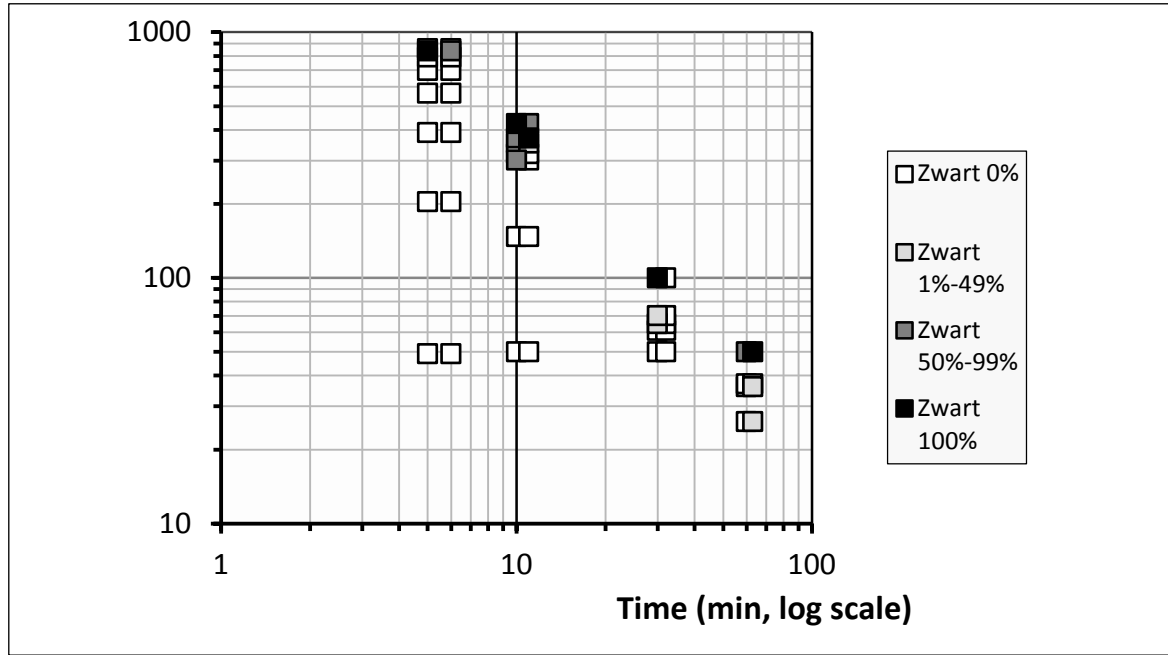
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A graphical overview of the data is presented below. Each concentration-time combination represents one point in the plot.

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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.

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1 **Study ID: A.3**

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3 **Author, year: Zwart, 1990**

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

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

17  
18 **Evaluation of study quality**

Criteria	Comment
Study carried out according to GLP	Yes.
Study carried out according to OECD 403 guideline(s)	<i>No statement of compliance with OECD guideline 403 provided.</i>
Stability of test compound in test atmosphere	<i>Not specified</i>
Use of vehicle (other than air)	<i>No</i>
Whole body / nose-only (incl. head/nose-only) exposure	<i>Whole body</i>
Type of restrainer	<i>N/A</i>
Pressure distribution	<i>Not specified</i>
Homogeneity of test atmosphere in breathing zone of animals	<i>An adjustable flow of test substance was mixed with airflow.</i>
Number of air changes per hour	<i>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)</i>
Equilibration time (t95)	<i>1.2-1.9 min</i>
Start of exposure relative to equilibration	<i>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).</i>
Actual concentration measurement	<i>Concentrations were measured continuously, by IR analysis and gas chromatography.</i>
Particle size distribution measurement in breathing zone of the animals in case of aerosol exposure	<i>N/A</i>
Assessment of Reliability	<b>A</b> <i>Well-performed study. Multiple concentration levels and durations were tested.</i>

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1 **Results**

Species	Concentration (mg/m <sup>3</sup> )		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

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**Probit function**

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

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

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

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<i>Probit function</i>	<i>Species</i>	<i>A</i>	<i>b</i>	<i>C</i>	<i>d</i>	<i>n-value</i>
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)

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The LC<sub>50</sub> 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.

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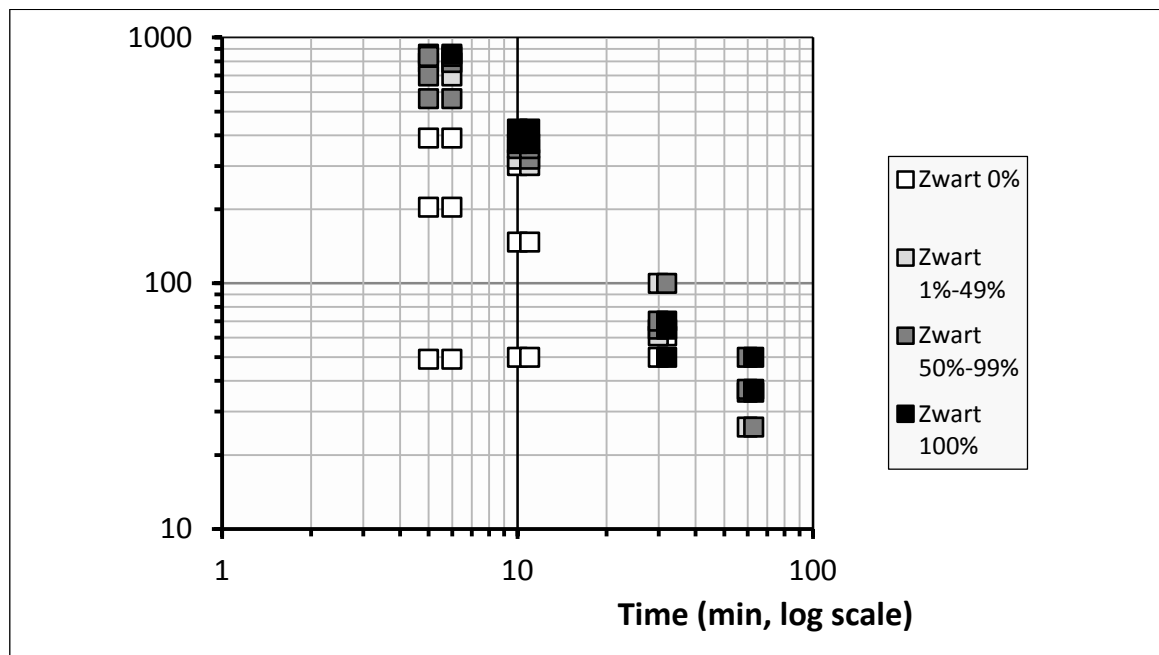
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Duration (minutes)	$LC_{50}$ ( $mg/m^3$ ) 95%-C.I.	$LC_{50}$ ( $mg/m^3$ ) 95%-C.I.	$LC_{50}$ ( $mg/m^3$ ) 95%-C.I.
	<b>Male</b>	<b>Female</b>	<b>Combined</b>
10	326 (273 – 401)	231 (178 – 276)	275 (229 – 317)
30	73.6 (59.3 – 89.5)	52.2 (36.9 – 64.0)	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)

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



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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.

## 1 Study ID: C studies

### 3 Acute lung injury models

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

### 14 Non-human primates

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

### 23 Sheep

24 Two Dorset crossbred wethers/group were exposed to 5620, 10,000, 17,800, or  
25 31,600 mg x min/m<sup>3</sup> phosgene gas for 10 minutes and observed for 24 hours (Keeler  
26 et al., 1990a). Sheep were exposed through a cone sealed over the nose and mouth  
27 after the gas passed through a 5-L anesthesia bag and one-way valve. The 10-minute  
28 LC<sub>50</sub> was estimated to be 13,300 mg x min/m<sup>3</sup> (1330 mg/m<sup>3</sup>) using Thompson and  
29 Weil analysis. All sheep had shallow breathing with some breath holding, as well as  
30 periods of dyspnea during and after exposure. All concentrations produced pulmonary  
31 oedema in the sheep grossly and microscopically. Gross findings in the decedents  
32 included the trachea filled with a mixture of stringy mucous and frothy material and  
33 moist, heavy lungs. Histopathology findings included oedema filled alveoli,  
34 perivascular spaces, and interlobular septa. The sheep that survived 24 hours had  
35 less prominent gross lesions with mild to moderate alveolar oedema.

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

### 50 Rats

51 A total of 118 male Wistar rats were exposed to phosgene at 0.5 to 4.0 ppm (2.1 to  
52 16.4 mg/m<sup>3</sup>) for 5 min to 8 h (Rinehart 1962; Rinehart and Hatch 1964). The  
53 exposures were varied to give CT products between 12 and 360 ppm x min (49.3 and  
54 1480 mg/m<sup>3</sup> x min) and were carried out in 1,700-L wooden exposure chambers  
55 operating at a constant ventilation rate of 1,000 L/min. The chamber surfaces were  
56 lacquered, and thus, potential loss of phosgene by reaction with the wooden surface  
57 was minimized. Details on the exact concentrations and exposure durations were not

1 provided. This system provided for air "turnover" every 2 min and a 99% equilibrium  
2 time of 8 min. Air samples were taken frequently during exposures, and adjustments  
3 were made when necessary to maintain constant phosgene concentrations. An L(CT)<sub>0</sub>  
4 of 180 ppm x min (740 mg/m<sup>3</sup> x min), a 75-min LC<sub>50</sub> of 4 ppm, and a 125-min LC<sub>100</sub>  
5 of 4 ppm were determined. The authors concluded that different combinations of  
6 concentration and time exposure giving equal products of C × T constitute equally  
7 effective doses.

8  
9 Gross et al. (1965; as cited in Glass et al. 2009) extended the study of Rinehart and  
10 Hatch (1964) and exposed an additional 117 Wistar rats to concentrations of  
11 phosgene ranging from 0.5 to 4.0 ppm (2.1 to 16.4 mg/m<sup>3</sup>) for 5 minutes to 8 hours,  
12 with 18 unexposed control rats. Most of the animals were sacrificed 4 days post  
13 exposure; however, there was a group of 15 rats exposed to 1.7 ppm (7.0 mg/m<sup>3</sup>)  
14 phosgene for 120 minutes and sacrificed in groups of three at 4, 8, 24, and 48 hours  
15 or 1 week post exposure. Another group was exposed to 2.2 ppm (9.0 mg/m<sup>3</sup>) for 80  
16 minutes and sacrificed 3 months post exposure; these rats developed chronic  
17 pneumonitis. Pneumonitis was defined as slight mural thickening of respiratory  
18 bronchioles with involvement of adjacent alveoli; moderate alveolar involvement in a  
19 peribronchiolar zone; and severe mural thickening of the respiratory bronchiole  
20 accompanied by obliteration of adjoining alveoli. The lowest concentration producing  
21 moderate pneumonitis was 0.8 ppm (3.3 mg/m<sup>3</sup>) for 1 hour (48 ppm x min; 197  
22 mg/m<sup>3</sup> x min) and severe chronic pneumonitis occurred at 1.5 ppm (6.2 mg/m<sup>3</sup>) for  
23 40 minutes (60 ppm x min; 247 mg/m<sup>3</sup> x min). In the group exposed to 1.7 ppm for  
24 120 minutes, severe pneumonitis was observed in 2/3 rats 48 hours post exposure,  
25 with most other time points indicating slight or moderate changes; in rats sacrificed 1  
26 week after exposure, 2/3 rats had slight and 1/3 had moderate pneumonitis.

27  
28 Box and Cullumbine (1947; as cited in AEGL, 2002) investigated phosgene-induced  
29 lethality in rats after the rats had experienced an exposure to phosgene at a nonlethal  
30 concentration. Rats were divided into two groups (12 per group). Half of each group  
31 was exposed to 19.2 ppm (78.9 mg/m<sup>3</sup>) phosgene for 10 min and the other half  
32 served as a control group. Five days later, the pre-exposed and control rats were  
33 exposed to phosgene at 55.2, 60, 75.6, and 105.6 ppm (227, 247, 311 and 434  
34 mg/m<sup>3</sup>) for 10 min. The rats were then observed for the next 48 h for deaths. The  
35 pretreated rats had a reduced percentage of mortality (33%) compared with the  
36 control animals (74%). Thus, partial protection from phosgene-induced lethality was  
37 obtained by the phosgene pretreatment.

### 38 39 **Mouse**

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

### 45 46 **Pigs**

47 Five young adult female white pigs/group were anesthetized and exposed to either air  
48 or 244 mg/m<sup>3</sup> (60 ppm) phosgene for 10 minutes (Brown et al., 2002; as cited in  
49 Glass et al. 2009). Prior to exposure, pigs were anesthetized, and arterial and venous  
50 catheters were placed. After the surgery, the pigs were allowed to equilibrate for 1  
51 hour, then phosgene was administered by the endotracheal tube and the  
52 concentration of phosgene was monitored continuously using an infrared gas  
53 analyzer. At 30 minutes post exposure, anesthesia was deepened to allow  
54 intermittent positive pressure ventilation to occur for up to 24 hours. Cardiovascular  
55 and respiratory measurements were taken every 30 minutes and blood was obtained  
56 for arterial and mixed venous blood gas analysis at 0, 10, 30 minutes and then every  
57 hour. All control animals survived the full 24 hours; only one treated pig survived the

1 entire 24 hours, the rest died 16–23 hours post exposure. Histopathology was  
 2 performed on all pigs. Control animals had minimal passive congestion of the lungs  
 3 and the treated animals had widespread pulmonary edema with bronchial epithelial  
 4 necrosis. Lung wet weight/body weight was significantly ( $p < 0.001$ ) increased in the  
 5 treated pigs. From 6 hours post exposure on, arterial pH, PaO<sub>2</sub> and lung compliance  
 6 were all significantly ( $p < 0.01$ ) decreased with treatment, with oxygen delivery and  
 7 consumption decreased from hour 15 on.

## 8 9 **Dogs**

10 Underhill (1920; as cited in Glass et al. 2009) exposed a total of 327 dogs  
 11 (breed/age/sex not provided) to phosgene at concentrations ranging from 44 to 120  
 12 ppm (181 to 493 mg/m<sup>3</sup>) for 30 minutes. Limited details were given as to the type of  
 13 exposure chamber besides stating that it was an airtight chamber through which a  
 14 mixture of gas and air flowed with frequent analysis of samples taken to check  
 15 concentration. Unlike their behavior during exposure to other gasses, the dogs  
 16 remained lying quietly in the chamber during the phosgene exposures. The LC<sub>50</sub>  
 17 (estimated concentration lethal to 50% of animals) for 30 minutes was approximately  
 18 61–70 ppm (247 – 288 mg/m<sup>3</sup>) based on the number of dogs that died in the first 3  
 19 days. Winternitz et al. (1920; as cited in AEGL 2002) reported on the histopathology  
 20 of these dogs. Of those exposed, 68% of the dogs died within the first 48 hours with  
 21 most deaths occurring between 12 and 24 hours. These dogs showed little variation in  
 22 histopathology, along with voluminous, heavy lungs, a frothy exudate found in the  
 23 lower trachea, and right heart dilation. Systemic effects other than those in the  
 24 cardiorespiratory system were not identified. Dogs that died 3–10 days after dosing  
 25 were found to have more respiratory infections in the deep lung.  
 26 Pauluhn (2006c) cites a reference of Cucinell et al. 1974, where dogs were exposed  
 27 for 20 minutes and a LC<sub>t50</sub> of 4200 mg/m<sup>3</sup>×min was reported.

## 28 29 **Several species**

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

38  
 39 LC<sub>50</sub> values (ppm (mg/m<sup>3</sup>)) for several species (Boyland et al., 1946; as cited in Glass  
 40 et al. 2009)

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

41 \* Dogs were exposed for 30 and 60 minutes.

## 42 43 **Overview of acute lethality studies in several species.**

44 The tables have been copied from AEGL; not all mentioned studies were included in  
 45 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 <sub>20</sub>	Shils 1943
NR	NR	10	60	LC <sub>40</sub>	Shils 1943
NR	NR	1	1,625	LC <sub>50</sub>	Chasis 1944
NR	44/NR	10	38-75	LC <sub>50</sub>	Box and Cullumbine 1947a
NR	NR	12	30	LC <sub>50</sub>	Chasis 1944
NR	NR	15	35	LC <sub>50</sub>	Cameron and Foss 1941
NR	NR	20	15	LC <sub>50</sub>	Kimmerle and Diller 1977
Wistar	40/NR	30	10-15	LC <sub>75</sub>	Henschler and Laux 1960
NR	NR	20	25	LC <sub>50</sub>	Rothlin 1941
NR	NR	12	85	LC <sub>60</sub>	Shils 1943
NR	NR	10	40	LC <sub>70</sub>	Kimmerle and Diller 1977
NR	32/NR	10	39-103	LC <sub>75</sub>	Box and Cullumbine 1947a

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**TABLE 1-7** *Continued*

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

3

NR, not reported.

4

5

6

**TABLE 1-6** Acute Lethality of Phosgene in Mice

Time (min)	LC <sub>50</sub> (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

7



**TABLE 1-8** Acute Lethality of Phosgene in Guinea Pigs

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

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2**TABLE 1-9** Acute Lethality of Phosgene in Rabbits

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

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

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**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 <sub>25</sub>	Cameron and Courtice 1946
NR	NR	1	2,100	LC <sub>50</sub>	Chasis 1944
NR	NR	10	45	LC <sub>50</sub>	Kimmerle and Diller 1977
NR	24/NR	15	60-70	LC <sub>50</sub>	Underhill 1920
NR	NR	20	502	LC <sub>50</sub>	Chasis 1944
NR	6/NR	30	100-175	LC <sub>50</sub>	Patt et al. 1946
NR	NR	30	78	LC <sub>55</sub>	Postel and Swift 1945
Mongrel	18/NR	3	745-880	LC <sub>70</sub>	Coman et al. 1947
NR	94/NR	20	135	LC <sub>70</sub>	Freeman et al. 1945
NR	42/NR	30	98	LC <sub>70</sub>	Postel and Swift 1945
Mongrel	15/M,F	30	124	LC <sub>90</sub>	Schultz 1945
Mongrel	32/NR	10	39-103	LC <sub>75</sub>	Box and Collumbine 1947
Mongrel	NR	3	734	LC <sub>99</sub>	Coman et al. 1947
Mongrel	NR	30	90	LC <sub>99</sub>	Coman et al. 1947

NR, not reported.

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## 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<sup>3</sup> 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 <sup>3</sup> )	Actual concentration <sup>a</sup> (mg/m <sup>3</sup> )	Concentration × time (mg/m <sup>3</sup> × 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 <sup>b</sup>	4.2	4.20	1008	28

<sup>a</sup> Actual breathing zone concentration.

<sup>b</sup> Data duplicated from Pauluhn (2006).

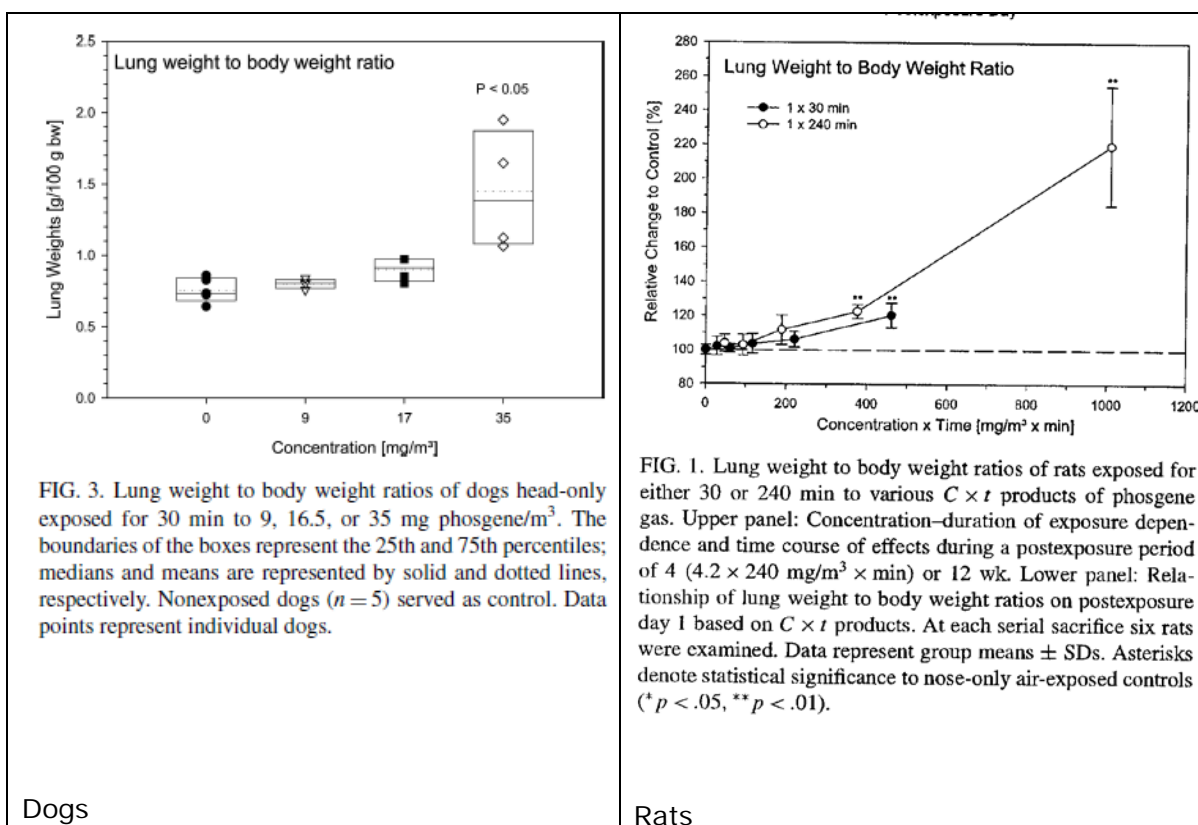
Atmospheres were generated by diluting phosgene in synthetic air with filtered ambient air and consequently passing the gas mixture into the nose-only chamber. Sampling took place at various exposure ports and continuously measured by infrared analyser or CM4 toxic gas paper tape monitor. Characterisation 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<sup>3</sup> and Hsd Jan: DOBE for 16.5 mg/m<sup>3</sup> and 35 mg/m<sup>3</sup>) were exposure to phosgene head-only for 30 minutes to 9 mg/m<sup>3</sup>, 16.5 mg/m<sup>3</sup> and 35 mg/m<sup>3</sup>. The respective C × t products were 270, 495, and 1050 mg/m<sup>3</sup> × min. The objective of this "proof of principle" study was to compare the magnitude of changes of selected nonlethal endpoints (lung weights, bronchoalveolar lavage

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

21 Test atmosphere analyses were the same as reported in the rat study (Pauluhn,  
 22 2006c, and as described above).

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



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

11  
12 Focusing on the BAL liquid proteins as -since this parameter is considered the most  
13 sensitive predictor for pulmonary injury, Pauluhn showed that rats had 10-fold higher  
14 levels at similar C x t products (approx. 1050 mg/m<sup>3</sup> x min, which is the LCt<sub>01</sub> in rats  
15 in Pauluhn, 2006a). It should be noted, however, that Pauluhn hypothesized that the  
16 10-fold higher protein exudation into the BAL at this high dose could be a reflection of  
17 the rat (or rodent) specific defence mechanism, as major part of the lungs is "lavaged  
18 and proteinaceous secretions from airways may contribute markedly to the total  
19 protein and inflammatory cells detected in BAL." Hence, absolute differences between  
20 BAL fluid proteins may not provide information on susceptibility differences between  
21 species. The comparison between rats and dogs as to what dose produces 150% BAL  
22 proteins compared to background levels showed a 3-fold lower C x t product in rats  
23 (117 mg/m<sup>3</sup> x min) compared to dogs (375 mg/m<sup>3</sup> x min) (Pauluhn, 2006b, see  
24 Figure 8 therein and copied in below), which according to the author is considered to  
25 be a better indication for differences in susceptibility.

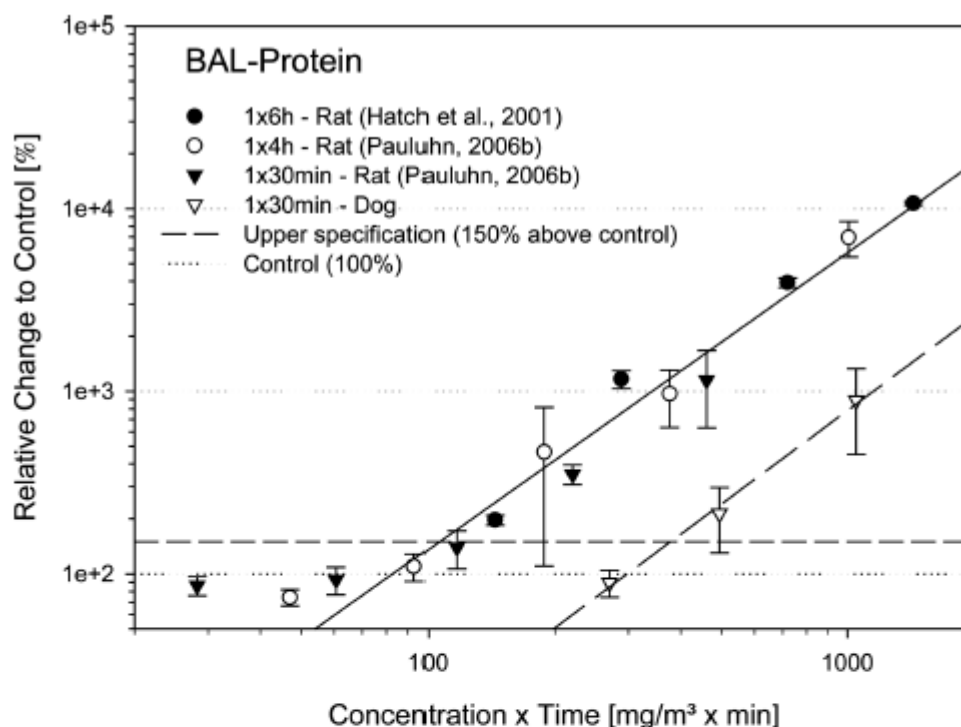


FIG. 8. Concentration  $\times$  time dependence of total protein in bronchoalveolar lavage fluid protein (obtained from the lobus accessorius) in head-only exposed dogs to phosgene (dashed line:  $y_{\text{dog}} = -2.2 + 1.7x$ ;  $r^2 = .99$ ), whole-body exposed Fischer 344 rats (Hatch et al., 2001),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

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### 1 Appendix 3 Reference list

- 2  
3 Bradley, B.L. and Unger, K.M. 1982. Phosgene inhalation: A case report. *Texas Med.*  
4 1982;78:51-53.  
5  
6 Chemiekaarten. Ed 32. Den Haag. TNO/SDU uitgevers, 2017.  
7  
8 Diller, W.F. and Zante, R. 1982. Dosis-wirkungs-beziehungen bei Phosgeneinwirkung  
9 auf Mensch und Tier. *Zbl. Arbeitsmed.* 1982;32: 360-368.  
10  
11 ERPG (2008). Emergency response planning guideline for phosgene (2008 update).  
12 American Industrial Hygiene Association.  
13  
14 Glass D, McClanahan M, Koller L, and F Adeshina. (2009) Provisional Advisory Levels  
15 (PALs) for phosgene (CG). *Inhalation Toxicology* 2009;21(S3): 73–94.  
16  
17 Keeler JR, Hurt HH, Nold JB, and Lennox WJ. (1990a). Estimation of the LC<sub>50</sub> of  
18 phosgene in sheep. *Drug and Chemical Toxicology*, 1190;13:229-239.  
19  
20 National Research Council. Acute Exposure Guideline Levels for Selected Airborne  
21 Chemicals. Volume 2. Washington, DC. The National Academies Press, 2002.  
22  
23 Pauluhn J. (2006a). Acute Nose-Only Exposure of Rats to Phosgene. Part I:  
24 Concentration x Time Dependence of LC<sub>50S</sub>, Nonlethal-Threshold Concentrations, and  
25 Analysis of Breathing Patterns. *Inhalation Toxicology*. 2006; 18:423-435.  
26  
27 Pauluhn J. (2006b). Acute Head-Only Exposure of Dogs to Phosgene. Part III.  
28 Comparison of Indicators of Lung Injury in Dogs and Rats. *Inhalation Toxicology*.  
29 2006; 18:609-621  
30  
31 Pauluhn J. (2006c). Acute nose-only exposure of rats to phosgene. Part II.  
32 Concentration × time dependence of changes in bronchoalveolar lavage during a  
33 follow-up period of 3 months. *Inhal. Toxicol.* 2006;18:595–607.  
34  
35 Pauluhn J., A. Carson, D. L. Costa, T. Gordon, U. Kodavanti, J. A. Last, M. A. Matthay,  
36 K. E. Pinkerton, A. M. Sciuto. (2007) Workshop Summary: Phosgene-Induced  
37 Pulmonary Toxicity Revisited: Appraisal of Early and Late Markers of Pulmonary Injury  
38 From Animal Models With Emphasis on Human Significance. *Inhalation Toxicology*  
39 2007;19:789-810.  
40  
41 Plahovinsak JL, Perry MR, Knostman KA, Segal R, Babin MC. (2015) Characterization  
42 of a nose-only inhaled phosgene acute lung injury mouse model. *Inhal Toxicol.*  
43 2015;27(14):832-40. doi: 10.3109/08958378.2015.1117549  
44  
45 Rinehart WE and Hatch T. (1964). Concentration-Time product (CT) as an expression  
46 of dose in sublethal exposures to phosgene. *Industrial Hygiene Journal* 1964:545-553  
47  
48 RIVM 2017. Interventiewaarden gevaarlijke stoffen.  
49 [http://www.rivm.nl/rvs/Normen/Rampen\\_en\\_incidenten/Interventiewaarden](http://www.rivm.nl/rvs/Normen/Rampen_en_incidenten/Interventiewaarden).  
50  
51 Ruijten M.W.M.M., J.H.E. Arts, P.J. Boogaard *et al.* Methods for the derivation of  
52 probit functions to predict acute lethality following inhalation of toxic substances.  
53 RIVM report 2015-0102. Bilthoven, RIVM, 2015.  
54  
55 Zwart A, Arts JHE, Klokman-Houweling JM. and Schoen ED. (1990). Determination of  
56 concentration-time-mortality relationships to replace LC<sub>50</sub> values. *Inhalation*  
57 *toxicology*, 1990;2:105-117.