

# Phosgene

[75-44-5]

## Supplement 2008

<b>MAK value (2007)</b>	<b>0.1 ml/m<sup>3</sup> (ppm) <math>\triangleq</math> 0.41 mg/m<sup>3</sup></b>
<b>Peak limitation (2000)</b>	<b>Peak limitation category I, excursion factor 2</b>
<b>Absorption through the skin</b>	–
<b>Sensitization</b>	–
<b>Carcinogenicity</b>	–
<b>Prenatal toxicity (1995)</b>	<b>Pregnancy Risk Group C</b>
<b>Germ cell mutagenicity</b>	–
<b>BAT value</b>	–
<b>1 ml/m<sup>3</sup> (ppm) <math>\triangleq</math> 4.111 mg/m<sup>3</sup></b>	<b>1 mg/m<sup>3</sup> <math>\triangleq</math> 0.243 ml/m<sup>3</sup> (ppm)</b>

A documentation from 1971 (see Documentation “Phosgene”, 1972) and supplements from 1984 (see Supplement “Phosgene” 1984), 1995 (see Supplement “Phosgene” 1995, only available in German) and 1996 (see Supplement “Phosgene” 1996) are available for phosgene. Based on the MAK value of 1996, the last peak limitation was established in 2000 (see Supplement “Phosgene” 2000).

More recent acute to subchronic inhalation studies in rats and acute studies in dogs with only head nose exposure make a reevaluation of the MAK value necessary. In this context, aspects making the scientific evaluation of earlier phosgene inhalation studies particularly difficult are given special attention. These aspects are:

1. Phosgene has a specific density about 3.5 times higher than air. Consequently, flowmeters used to dose phosgene streams can result in inaccurate calculations of nominal concentration. Phosgene-specific analytical methods were not used in earlier studies.

2. The higher density of phosgene can lead to inhomogeneous exposure atmospheres in whole-body inhalation chamber systems, i.e. nominally calculated concentrations need not necessarily correspond to the actual concentrations in the ani-

mals' breathing zone, particularly if the exposure time is relatively short. Numbers characterizing the time up to the steady-state concentration in the inhalation chamber ( $t_{95}$ ) were often not published. In addition, the stability of phosgene in whole-body inhalation chambers can be affected by air humidity and ammoniac released from faeces and urine. The nominal and analytically determined concentrations were very rarely compared, so that experimental implausibilities often escaped quantitative assessment.

3. Modern analytical processes to identify phosgene require sampling in the animals' breathing zone as well as phosgene-specific methods. The methods are based on the *in situ* derivatization of phosgene (chemical conversion of phosgene during sampling into a phosgene-specific, stable analytical indicator substance, which is then quantifiable using chromatographic methods). In studies with larger test animals (dogs or pigs, for example) sometimes whole-body, mask systems or endotracheal intubation systems were used. Apart from the disadvantages of whole-body exposure already mentioned above, exposure with masks or endotracheal devices often provide no possibilities for quantitative determination of the phosgene concentrations inhaled from the immediate breathing zone. In exposure systems without separation of inhaled and exhaled air or having a high dead volume relative to the tidal volume, one cannot exclude that phosgene is hydrolyzed or reinhaled from the atmosphere in the exposure system. In more recent inhalation studies (Pauluhn 2006 a, b), a "directed-flow nose-only" exposure principle was applied; this means that  $t_{95}$  is negligibly small versus the exposure duration, hydrolytic changes or inhomogeneities in the atmosphere in the exposure system cannot occur, and the respiratory minute volume of rats in exposure tubes is higher than in whole-body exposure systems.

4. The main retention localizations of the hydrophilic hydrolysis product HCl and of hydrophobic phosgene itself inside the respiratory tract are greatly different. The methodological variety of inhalation studies, especially studies with larger animals such as dogs, and involving the specific stress factors (hyperventilation), is considered to be an additional cause for the notably wide scatter band of results found. These methodological aspects have received special attention in more recent "directed-flow head-only" inhalation studies in dogs (Pauluhn 2006 c). In the re-evaluation of phosgene, only studies using validated methods were considered.

## 1 Toxic Effects and Mode of Action

The low water solubility of phosgene (Nash and Pattle aids penetration of the gas into the lower airways (bronchoalveolar region), without causing subjective irritation. The perception threshold is given as  $1.6 \text{ mg/m}^3$  (WHO 1998). High concentrations cause irritation to the eyes and the entire respiratory tract. Acute toxic concentrations of phosgene induce a non-cardiogenic pulmonary oedema in the distal airways (alveoli). The oedema-inducing effect on the distal airways occurs

with intermittent exposure patterns ( $\leq$  one working day) according to Haber's rule ( $C \cdot t = \text{constant}$ ), and the effect intensity behaves proportionally to the product from the inhaled concentration and the exposure duration ( $C \cdot t$ ). The clinical manifestation of phosgene-induced oedematous lung changes takes place with a latency period of several hours and normally reaches a maximum within 24 hours. The duration of the latency period behaves in a way inversely proportional to  $C \cdot t$  (Diller 1985). Acute pulmonary oedema with asphyxia, as well as severe subsequent bronchopneumonia, is considered to be the cause of lethality. For humans,  $C \cdot t$  products of about 2000 mg/m<sup>3</sup>·min (Diller and Zante 1982) or 3200 mg/m<sup>3</sup> · min (Cucinell 1974) are in the lethal range ( $LC_{t50}$ ). Phosgene reacts locally with the bronchoalveolar structures. This also applies to the liquid layers covering this part of the lungs (surfactant lining fluids). The high local reactivity of phosgene excludes a systemic availability, and no accumulation of the initial substance or its hydrolysis products can occur. Intermittent exposures, which temporarily decrease the detoxifying components in this area of the lungs, are tolerated without cumulative effects insofar as the buffer capacity available for this reaction is not exhausted and the reconstitution time between the intermittent exposures is sufficient.

## 2 Mechanism of Action

### Local effects

Due to its low water solubility, phosgene gas is hazardous for the pulmonary structures of the respiratory tract. The extremely steep  $C \cdot t$  effect relationship is seen to be typical for a local, directly acting pulmonary irritant gas, which first reacts with surfactant components before producing a pulmonary oedema due to increased permeability (Pauluhn 2006 a). The effect of phosgene on the surfactant system has been investigated (Frosolono and Currie 1985; Jugg et al. 1999). Interventive measures based on surfactant reconstitution or oedema minimization by activating epithelial  $Na^+K^+$ -ATPases via the application of  $\beta$ -adrenergic agonists, have successfully been subjected to scientific investigation in animal models (Berthiaume et al. 2002; van Helden et al. 2004; Matthay et al. 2002; Mautone et al. 1985; Sciuto and Hurt 2004).

In biological systems, phosgene acylates amino, hydroxyl and sulfhydryl groups. In this context, HCl plays no significant role. Studies by Bauer et al. (1997) in guinea pigs confirm that protective sensory C fibres modulate the intensity of oedema formation as well as the latency period of oedema formation, for example through the release of tachykinin. There exist numerous acute inhalation studies with species comparisons (mouse, rat, hamster, guinea pig) in regard to early changes in bronchoalveolar lavage (BAL) fluid and, particularly, oedema formation. In this context, the determination of total protein in the BAL fluid was given a considerably higher pathognomonic value than lung weight determination. In rat studies, an

increase in protein concentration in the BAL fluid by a factor of 70 merely doubled the lung weights (Pauluhn 2006 b). In a comparative study in mice, rats and guinea pigs, species differences in the protein concentration of the BAL fluid were small (Sciuto 1998). The relative acute pulmonary irritation potential of phosgene and ozone was evaluated and compared after single 4-hour exposure on the basis of protein concentration in the BAL fluid in several animal species (mouse, hamster, rat, guinea pig and rabbit) (Hatch et al. 1986). Reanalysis of these data again revealed only minimal differences between hamster, rat and mouse in regard to their sensitivity to phosgene. Guinea pigs and rabbits were somewhat less sensitive. The same applies for dogs (Pauluhn 2006 c). Regarding lethality, however, there are no indications of any considerable species differences (NRC 2002).

Time-effect relationships were also investigated in the clearly irritating concentration range for a large number of lung parameters in mice and rats. Changes in regard to energy metabolism, glutathione synthesis and redox cell regulation, arachidonic acid metabolism as well as the release of proinflammatory cytokines were found partly in pulmonary irritating  $C \cdot t$  products (Currie et al. 1985, 1987 a, b; Deshpande et al. 1996; Duniho et al. 2002; Franch and Hatch 1986; Ghio et al. 2005; Madden et al. 1991; Sciuto et al. 2002, 2003 a, b, c, 2005). Reversible impairments in ATP concentration were found in the homogenized lungs of Sprague Dawley rats (Currie et al. 1985, 1987 a). The relationship between oedema formation and ATP concentrations was described, which is complex and difficult to interpret. These authors conclude that changes in  $Na^+K^+$ -ATPase activity cannot be causally related to a reduction in ATP concentration, and that the available data are not sufficient to describe the aetiopathological contexts of early changes in energy metabolism and oedema formation. A decrease in the ATP level occurred in rats immediately after single 4-hour exposure to  $0.05 \text{ ml/m}^3$ , without an increase in protein concentration in the BAL fluid (Currie et al. 1987 a). As the protein concentration in the BAL fluid was also not increased after repeated exposure to  $0.1 \text{ ml/m}^3$  (Hatch et al. 2001), the prognostic value of ATP reduction after acute exposure is not verified for the development of pulmonary oedema after repeated exposure, and can be considered as non-adverse.

After single exposure of male BALB/c mice ( $C \cdot t = 10780 \text{ mg/m}^3 \cdot \text{min}$ ;  $2156 \text{ mg/m}^3 \cdot 5 \text{ min}$ ) and processing of the lungs four hours after exposure, apoptotic changes in pneumocytes and inflammatory cells were found in the lungs (Li et al. 2006). Assessment of the relevance of these findings is made more difficult by the absence of a time-effect analysis; i.e. it is not known whether apoptotic cells as such are subject to phagocytosis or underwent secondary necrosis (Hussain et al. 1998). The latter could promote a reactive increase in the severity of inflammatory primary reactions.

Histological changes as sequels of acute (Diller et al. 1985; Duniho et al. 2002; Gross et al. 1965; Pauluhn 2006 b) and repeated (Kodavanti et al. 1997) phosgene exposure have been described in detail. Early histological changes such as alveolar fibrin deposits and epithelial damage as well as increased protein concentrations in

the BAL fluid showed the same behaviour in mice in regard to their temporal development up to 72 hours after single exposure for 20 minutes (Duniho et al. 2002).

### **Immunotoxicity/Infectiousness**

Inhaled substances that cause a decrease in phagocytosis activity or of bactericidal factors in the pulmonary environment, either directly by irritation and cytotoxicity or indirectly by transient loading of phagocytes with surfactant and lysed cells, potentially increase infectiousness. Due to considerable species differences and the influence of the temporal sequence of substance exposure and infection, the findings from bolus infection models in animal studies can ~~however~~ only be quantitatively applied to humans with great difficulty. The infectivity of inhaled bacteria is controlled by the joint action of different protective effects. These comprise mucociliary clearance, phagocytosis (neutrophilic granulocytes, alveolar macrophages), natural killer cells, specific immune components and intraluminal bacteriostatic factors (Gilmour and Selgrade 1993). A temporary influence on these factors can also occur due to a disturbance of surfactant homeostasis (Wang et al. 2006). The increased phagocytosis of apoptotic neutrophils by alveolar macrophages can produce temporary macrophage deactivation, thus increasing the survival time of bacteria/parasites in the lungs (Ribeiro-Gomes et al. 2004).

The lowest threshold concentration for effects of phosgene gas has been determined in animal models with immediate infection after the end of exposure. Following acute 6-hour exposure of F344 rats to 0.4 or 0.8 mg/m<sup>3</sup> ( $C \cdot t$  product: 144 and 288 mg/m<sup>3</sup> · min), the survival of *Streptococcus* in the lung was increased. The phagocytosis index of latex particles was reduced at 0.8 mg/m<sup>3</sup>. Higher phosgene concentrations (4 mg/m<sup>3</sup>) were accompanied by an increase in the infectivity. In contrast, 18 hours after exposure no differences could be found between rats exposed to phosgene and those exposed to air in regard to infections in the animals (Yang et al. 1995).

F344 rats were whole-body exposed to phosgene at concentrations of 0.4 or 0.8 mg/m<sup>3</sup> for 6 hours/day on 5 days/week for 4 or 12 weeks, or to 2 mg/m<sup>3</sup> on 2 days/week, and then investigated in the *Streptococcus* infection model. In addition, animals were infected 4 weeks after the end of the 12-week exposure (recovery group) (Selgrade et al. 1995). The experimental details of this subchronic inhalation study have been described separately (Kodavanti et al. 1997). Minimal effects were observed at 0.4 mg/m<sup>3</sup> and above or a  $C \cdot t$  product of 144 mg/m<sup>3</sup> · min. Compared with the acutely exposed animals (Selgrade et al. 1989), the bacterial clearance showed neither adaptive nor additive effects. In the recovery group, all changes were found to be reversible (Selgrade et al. 1995).

### 3 Toxicokinetics and Metabolism

Phosgene gas is partly hydrolyzed in the respiratory tract (half-life 0.026 seconds at 35°C; see Supplement “Phosgen” 1995, only available in German), and direct local damage occurs at the main site of deposit, though no damage to systemic organs or accumulation occurs (Documentation “Phosgene”, 1972).

### 4 Effects in Humans

No new data have become known since the last documentation.

## 5 Animal Experiments and in vitro Studies

### 5.1 Acute toxicity

#### Lethality

Table 1 shows the more recent studies on the acute inhalation toxicity of phosgene. The studies by Pauluhn (2006 a) aimed at determining the median lethal concentration ( $LC_{50}$ ) for different exposure times (10–240 minutes). These studies meet the requirements of OECD test guideline 403, and were carried out according to Good Laboratory Practice (GLP).

Young adult Wistar rats were head nose-only exposed for 10, 30, 60 or 240 minutes. The exposure atmospheres were characterized using phosgene-specific analytical methods. The nominal concentrations were analytically confirmed. The post-exposure period was 2 weeks. The corresponding  $C \cdot t$  products comprised a range of 1538 to 2854  $mg/m^3 \cdot min$ . A  $C \cdot t$  product-dependent mortality occurred in the majority of cases within 24 hours after exposure, and was causally attributed to an acute pulmonary oedema. The median  $LC_{50}$  values are given in Table 1. The calculated  $LC_{01}$  values were 105.3, 29.2, 21.1 and 5.0  $mg/m^3$  after 10, 30, 60 and 240 minutes exposure time, respectively. In the first 10 minutes of the exposure, a reflex-evoked respiratory depression in ventilation occurred, which normalized as exposure duration increased. Respiratory function measurements revealed an increased apnoea time at 1.2  $mg/m^3$  and above. For calculation of the  $LCt_{50}$  values averaged through all exposure times, the results from 10-minute exposures were excluded, as these values reflect no higher tolerance, but merely the sequel of a rodent-specific hypoventilation due to reflex-mediated stimulation. The average  $LCt_{50}$  (95% confidence interval) and  $LCt_{01}$  values were 1741 (1547–1929)  $mg/m^3 \cdot min$  and 1075  $mg/m^3 \cdot min$ , respectively, with a mean  $LCt_{50}/LCt_{01}$  ratio of 1.6 (Pauluhn 2006 a), from which a very steep concentration-effect relationship can be concluded.

**Table 1** Acute inhalation toxicity of phosgene

Species, strain, (number ♂/♀)	Exposure type	Exposure duration [min]	LC <sub>50</sub> (95% confidence interval) [mg/m <sup>3</sup> ]	LCt <sub>50</sub> [mg/m <sup>3</sup> · min]	References
Rat, WIS, (5/5)	NO	10	253 (194–331)	2533	Pauluhn 2006 a
Rat, WIS, (5/5)	WB	10	346 (327–366) <sup>1</sup>	3460	Zwart et al. 1990
Mouse, SWI, (5/5)	WB	10	327 (315–341) <sup>1</sup>	3270	Zwart et al. 1990
Rat, WIS, (5/5)	NO	30	54.5 (48–62)	1635	Pauluhn 2006 a
Rat, WIS, (5/5)	WB	30	76.9 (70.4–84.0) <sup>1)</sup>	2307	Zwart et al. 1990
Mouse, SWI, (5/5)	WB	30	40 (concentration – effect relationship unclear) <sup>1</sup>	1200	Zwart et al. 1990
Rat, WIS, (5/5)	NO	60	31.3 (28–35)	1878	Pauluhn 2006 a
Rat, WIS, (5/5)	WB	60	43 <sup>1)</sup>	2580	Zwart et al. 1990
Mouse, SWI, (5/5)	WB	60	22 (12–41) <sup>1</sup>	1320	Zwart et al. 1990
Rat, WIS, (5/5)	NO	240	8.6	2064	Pauluhn 2006 a

<sup>1</sup> Recalculation of data using Probit analysis (sexes combined);

WIS: Wistar rat; SWI: Swiss mouse; WB: whole-body exposure;

NO: head nose-only exposure;

LCt<sub>50</sub>: LC<sub>50</sub> · exposure time

In the study by Zwart et al. (1990) 5–6-week-old Wistar rats or about 2-month-old Swiss mice were whole-body exposed in a small tubular chamber. There are no detailed descriptions in regard to analytical quantification method and nominal concentrations.

In summary, the more recent studies reveal the typical picture of a gas with direct pulmonary action and a very steep concentration-mortality relationship and death by oedema formation with hypoxia/asphyxia. There were no indications of delayed findings, such as obliterant bronchiolitis. Dyspnoea in the surviving rats had subsided by the end of the 2-week post-exposure period (Pauluhn 2006 a).

In an acute inhalation study in anaesthetized pigs with endotracheal exposure ( $C \cdot t = 2443 \text{ mg/m}^3 \cdot \text{min}$ ;  $244.3 \text{ mg/m}^3$ , 10 minutes), four of five animals died of

pulmonary haemorrhagic oedema within the observation period of 24 hours (Brown et al. 2002).

### **Sublethal endpoints**

Rats exposed to phosgene in an acute study (4 hours,  $2 \text{ mg/m}^3$ ,  $C \cdot t = 480 \text{ mg/m}^3 \cdot \text{min}$ ) were re-exposed after an exposure-free period of one to four weeks. In re-exposed rats, the protein concentrations in the BAL fluid were markedly lower than in non-pre-exposed rats, indicating an increased tolerance (adaptation) (Hatch et al. 2001). The protein concentration in the BAL fluid after 6-hour whole-body exposure of male F344 rats to  $1440 \text{ mg/m}^3 \cdot \text{min}$  was about 100 times the normal values. This exposure level was tolerated without lethality (Hatch et al. 2001). Further concentration-effect relationships were determined after 4-hour exposure in Sprague Dawley rats, however with lower  $C \cdot t$  products (Currie et al. 1987 b). As more recent and comprehensive GLP studies in rats with “directed-flow nose-only” exposure techniques are available, the results of these earlier studies are not evaluated in more depth.

In an earlier study in rats (Gross et al. 1965; Rinehart and Hatch 1964), histopathological changes after different  $C \cdot t$  products were investigated for a recovery period of up to 3 months. According to the descriptions of Rinehart and Hatch (1964), a reservoir with 500 ml paraffin oil was charged with phosgene and then, by bubbling nitrogen through the reservoir, conducted into a wooden whole-body exposure chamber. According to the authors’ description, it was found that the analytical characterization of exposure atmospheres using spectrophotometry presented methodological problems. Furthermore, the authors were not sure whether the observed chronic pneumonitis is to be considered as clearly phosgene-induced or whether the changes were confounded by bacterial or viral pneumonia. On account of these uncertainties, a higher experimental value is ascribed to a more recent GLP study in rats (Pauluhn 2006 b), also having a recovery period of up to 3 months.

In this inhalation study, male Wistar rats were exposed for either 30 or 240 minutes using a directed-flow nose-only mode of exposure. Changes in effect markers over time in the BAL fluid and in histopathology were recorded over recovery periods of about 4 or 12 weeks. In 30-minute-exposed rats the exposure concentrations were 0.94, 2.02, 3.89, 7.35 and  $15.36 \text{ mg/m}^3$ , the corresponding  $C \cdot t$  products 28.2, 60.6, 116.7, 220.5 and  $460.8 \text{ mg/m}^3 \cdot \text{min}$ , respectively. In rats exposed for 240 minutes, they were 0.196, 0.387, 0.786, 1.567 and  $4.2 \text{ mg/m}^3$  with corresponding  $C \cdot t$  products of 47.0, 92.9, 188.6, 376 and  $1008 \text{ mg/m}^3 \cdot \text{min}$ , respectively. Six rats per group were used to investigate the BAL fluid on days 1, 3, 7, 14 and 84 of the recovery period. Rats exposed to  $1008 \text{ mg/m}^3 \cdot \text{min}$  were examined on days 1, 7, 14 and 28 of the recovery period. All  $C \cdot t$  products were tolerated without mortality. The most pronounced changes were found in the markers protein, soluble collagen and polymorphonuclear leukocyte count in the BAL fluid. Maximum changes occurred on the first day of the recovery period, whereas the total cell counts in the



BAL fluid and the number of alveolar macrophages containing phospholipids reached their climax on day 3 after exposure. At  $1008 \text{ mg/m}^3 \cdot \text{min}$ , histopathology of the lungs revealed a minimal to slight hypercellularity in the terminal bronchioles with focal peribronchiolar inflammatory infiltrates and focal septa thickening at the end of the 4-week recovery period. In the case of low  $C \cdot t$  products, the rats were normal after a recovery period of about 12 weeks. There were no indications of fibrotic changes or increased collagen deposition. At similar  $C \cdot t$  products the changes in the BAL fluid were slightly less pronounced after 30-minute exposure compared to 240-minute exposure. The cause of these findings was seen to be related to an increased apnoea time (hypoventilation) during the initial phase of exposure. On the basis of the most sensitive indicators in the BAL fluid (protein, polymorphonuclear leukocyte count, soluble collagen),  $C \cdot t$  products up to  $116.7 \text{ mg/m}^3 \cdot \text{min}$  did not cause changes different from those in controls, whereas a significant increase in protein concentration in the BAL fluid was found at  $188.6 \text{ mg/m}^3 \cdot \text{min}$ . There were no indications of potentially irreversible effects up to an exposure level of  $1008 \text{ mg/m}^3 \cdot \text{min}$  (Pauluhn 2006 b).

To obtain a better assessment of the pathodiagnostic importance in humans of low-level protein changes in the BAL fluid after acute exposure of rats, Beagle dogs were similarly exposed by inhalation using a head-only mode of exposure (Pauluhn 2006 c). The exposure concentrations were 9, 16.5 and  $35 \text{ mg/m}^3$  with resultant  $C \cdot t$  products of 270, 495 and  $1050 \text{ mg/m}^3 \cdot \text{min}$  (exposure time: 30 minutes, 4 dogs per group). At the time of maximum oedema occurrence, i.e. about 24 hours after the end of exposure, the lung weights and the arterial blood gases were determined, the smallest lung lobe completely lavaged, and all lung lobules, including bronchi and trachea, histologically examined. Mortality did not occur at any  $C \cdot t$  product. Increased lung weights and elevations in protein, collagen in the BAL fluid as well as a significant increase in the polymorphonuclear leukocyte count were found at  $1050 \text{ mg/m}^3 \cdot \text{min}$ . Marginal changes occurred at  $495 \text{ mg/m}^3 \cdot \text{min}$  and above. Arterial blood gas changes (decreased arterial  $\text{pO}_2$ ) were found only at  $1050 \text{ mg/m}^3 \cdot \text{min}$ . Histopathological investigation of the lungs revealed distinctive, though only mild, inflammatory reactions at the bronchoalveolar level at  $495 \text{ mg/m}^3 \cdot \text{min}$  and above. Pulmonary serofibrinous exudates and oedema were only found in dogs exposed to  $1050 \text{ mg/m}^3 \cdot \text{min}$  (Pauluhn 2006 c).

Comparative analysis of the most sensitive endpoint, i.e. protein concentration in the BAL fluid from acute inhalation studies in rats with exposure durations of 0.5 to 6 hours with double logarithmic transformation showed a linear relationship of  $C \cdot t$  (Pauluhn 2006 c). This summarizing analysis confirmed that phosgene-induced early pulmonary changes after single exposure are determined by the intensity of exposure and thus the inhaled phosgene dose, and not by the concentration alone. A nearly identical functional relation was also present in the dog study, although identical  $C \cdot t$  products produced, in the dog, an increase in protein concentration in the BAL fluid about 5 times lower than in the rat. Protein concentrations in the BAL fluid of phosgene-exposed rats were about 70 to 100 times higher than in the controls, and were tolerated without mortality (Hatch et al. 2001;

Pauluhn 2006 b, c). In contrast, a protein increase by a factor of about 30 in the BAL fluid in morbid patients with acute respiratory distress syndrome (ARDS) was evaluated as being in the lethal range (Pittet et al. 1997). This apparent discrepancy could be attributed in rats to rodent-specific, bronchopulmonary protective reflexes (Persson et al. 1996), which increase the protein concentration in the BAL fluid in addition to irritant damage of the air blood barrier as well as the specific lavage technique (lavage of the entire lung including the airways) (Pauluhn 2006 c). More recent studies in anaesthetized adult pigs (Brown et al. 2002) also support this conclusion, as the time to death and the level of lung damage in pigs is similar to that found in dogs. In contrast, in regard to subclinical biochemical endpoints, species differences are obvious (Sciuto 1998). Reflex-mediated, neurogenic vasodilatation or the concentrations of antioxidants (e.g. glutathione) in the surfactant fluid films of the airways are in the foreground here. The rat has a glutathione concentration in the BAL fluid about 20 times lower than in humans (Hatch 1992).

For these reasons, in principle a greater weight is given to the derivation of workplace-relevant threshold values from inhalation studies in dogs, since the anatomy and physiology of the lung, as well as the breathing pattern, in dogs are more similar to those of humans than is that of the rat (see also Section 5.2, subheading “Evaluation of Inhalation Toxicity”).

## **5.2 Subacute, subchronic and chronic toxicity**

Groups of 8–12 male F344 rats, 2 months old, were exposed for 6 hours/day. The air control animals and the 0.4 and 0.8 mg/m<sup>3</sup> groups (144 and 288 mg/m<sup>3</sup> · min) were exposed 5 times/week, the 2 mg/m<sup>3</sup> group twice per week and the 4 mg/m<sup>3</sup> group once per week for 4 or 12 weeks. Additionally, another 5–9 rats per group were exposed for 12 weeks and subsequently observed for 4 weeks. The exposure to 2 mg/m<sup>3</sup> took place in 2-m<sup>3</sup> whole-body Hazelton inhalation chambers with an air supply of 1 m<sup>3</sup>/min. The phosgene concentration in the exposure chambers was determined using gas chromatography, and samples were taken at various locations within the chamber. All groups were dissected. The right lung was weighed and histologically examined (2 mg/m<sup>3</sup> group: no histological study took place due to an inadvertent loss of study material). The volume of the excised, fixed lung was determined by measuring the displacement volume of the left lung lobe after 30 minutes instillation with glutaraldehyde/cacodylate buffer inflated to 25 cm H<sub>2</sub>O transpulmonary pressure. No mortality occurred in any group. The body weights were significantly decreased at 2 mg/m<sup>3</sup> and above and the lung weights concentration-dependently increased at 0.8 mg/m<sup>3</sup> and above. The lung volume was increased in all exposure groups. The histological changes in the rats exposed for 4 weeks consisted of mild terminal bronchiolar thickening and an increase in the number of intraluminal inflammation cells in the 0.4 mg/m<sup>3</sup> group. These borderline changes in individual animals were often more pronounced after an exposure lasting 4 weeks than after one lasting 12 weeks (see Table 2 and Table 3). Qualitatively, the findings

**Table 2** Effects of phosgene after repeated inhalation by male F344 rats (Kodavanti et al. 1997)

No. of animals	Exposure	Findings
8–12/ group	<b>6 hours/day, 4 or 12 weeks</b> 0.4 mg/m <sup>3</sup> , 5 days/week; 0.8 mg/m <sup>3</sup> , 5 days/week; 2 mg/m <sup>3</sup> , 2 days/week; 4 mg/m <sup>3</sup> , 1 day/week	<b>0.4 mg/m<sup>3</sup>:</b> minimal histological effects with low incidence in the bronchoalveolar region; no difference between 4- and 12-week exposure; all changes reversible <b>at 0.8 mg/m<sup>3</sup> and above:</b> relative lung weight increased, clear changes in the region of the terminal bronchioles/alveoli, collagen deposition <b>at 2 mg/m<sup>3</sup> and above:</b> body weight gain reduced <b>4 mg/m<sup>3</sup>:</b> more pronounced histological changes than after 0.8 mg/m <sup>3</sup> , collagen and elastin in the lung homogenate significantly increased

**Table 3** Histopathological results in the lungs (incidence and intensity; Kodavanti et al. 1997)

Phosgene concentration [mg/m <sup>3</sup> ]	4 weeks				12 weeks			
	0	0.4	0.8	4	0	0.4	0.8	4
n	12	8	8	6	12	8	8	8
Alveolar effusion	0	0	0	2 (0.33)	0	0	0	1 (0.13)
Alveolus, interstitial thickening	0	2 (0.25)	5 (0.63)	6 (1.83)	0	2 (0.25)	4 (0.5)	8 (2.13)
Bronchus, epithelial alteration	0	1 (0.13)	2 (0.5)	2 (0.33)	0	0	1 (0.13)	1 (0.25)
Bronchus, inflammation	1 (0.08)	2 (0.25)	2 (0.4)	3 (0.83)	0	0	0	1 (0.13)
Terminal bronchioles/alveoli: inflammatory cell influx	2 (0.17)	3 (0.38)	8 (1.0)	6 (3.0)	1 (0.08)	3 (0.38)	8 (1.13)	8 (2.13)
Terminal bronchioles/peribronchiolar alveoli, epithelial alterations	2 (0.17)	4 (0.5)	5 (0.63)	6 (2.50)	0	1 (0.13)	7 (0.88)	8 (2.38)
Terminal bronchioles/peribronchiolar: increased collagen staining	1 (0.08)	1 (0.13)	8 (1.0)	6 (1.0)	2 (0.17)	2 (0.25)	8 (1.0)	8 (2.0)

n: number of animals examined; assessment key for the severity score of lesions: 0: not remarkable, 1: minimal, 2: slight/mild, 3: moderate, 4: moderately severe; 5: severe; [based upon relative evaluation of lesions whose maximum score from phosgene exposure was 3]; values in brackets gives average values/group

obtained in the 0.4 mg/m<sup>3</sup> group could also be found in the control group. Clearer and more frequent changes were present at 0.8 mg/m<sup>3</sup> and above, and particularly at 4 mg/m<sup>3</sup>. Concentration-dependently increased collagen (Masson's trichrome stain) was described at 0.8 mg/m<sup>3</sup> and above. A time-dependent exacerbation was only observed in the 4 mg/m<sup>3</sup> group. All histological changes in the 0.4 mg/m<sup>3</sup> group were found to be reversible after the 4-week recovery period. Biochemical indices for increased collagen (hydroxyproline, prolyl hydroxylase) and elastin (desmosine) in the lung homogenate could only be found in the 4 mg/m<sup>3</sup> group (Kodavanti et al. 1997). The concentration-dependent increased "fixed" lung volume (left lobe) is possibly attributable to an insufficient fixation duration (measurement immediately after the 30 minutes instillation fixation). As a result of inflammatory reactions, exudates etc., not all lung areas are necessarily identically fixed within 30 minutes. As it is difficult to distinguish artificial and phosgene-induced changes clearly from each other, this finding is not plausible and as not interpreted as a lung change of the more restrictive type (collagen deposition).

In a further publication, the protein concentrations in the BAL fluid in rats from the study of Kodavanti et al. (1997) were compared with those obtained after acute exposure to the same concentrations. While at a single 6-hour exposure to 0.8 mg/m<sup>3</sup> ( $C \cdot t$ : 288 mg/m<sup>3</sup>·min) the protein concentration was 10 times higher than in the controls, no increase could be established compared with controls after repeated exposure (6 hours/day, 5 days/week, 4, 8 or 12 weeks) (Hatch et al. 2001). This indicates adaptation to repeated exposure.

### **Evaluation of inhalation toxicity**

The animal inhalation studies, on which this evaluation is based, are compared with empirical data from humans in Table 4.

From this data it can be seen that the borderline findings obtained in rats in the bronchoalveolar region after 12-week exposure (6 hours/day, 5 days/ week) with a daily  $C \cdot t$  product of 144 mg/m<sup>3</sup>·min correspond causally to the changes found (total protein concentration in the BAL fluid) after acute exposure with a  $C \cdot t$  product of 164 mg/m<sup>3</sup>·min; i.e., the pulmonary changes induced by phosgene gas depend on the acute intensity of exposure on the exposure day or working day, and are not cumulatively toxic. Marginal increases in protein concentrations in the bronchoalveolar lavage fluid of rats can also develop as the sequel of reflexory, protective reactions in the airways. An increased intraluminal plasma extravasation into the alveoli as a result of reflexory neurogenic stimulation could be accompanied by dysfunction of the surfactant and subsequent disturbances of the coagulatory and fibrinolytic cascades (Ruppert et al. 2003) and secondary morphological reactions (adaptive increased surfactant synthesis with morphological changes in the bronchoalveolar region). In the latter case, compensatory reactions in the bronchoalveolar region would then originate from a species-specific, protective, reflex-mediated and not necessarily irritation-induced aetiopathology. The

**Table 4** Summary of toxicological endpoints from different C · t products in rats, dogs and humans

C · t product/day [mg/m <sup>3</sup> · min]	Species, exposure, effects	References
4200	dog, exposure: 1 · 20 min, no experimental details, LC <sub>T50</sub>	Cucinell 1974
about 2000	humans, estimated LC <sub>T50</sub>	Borak and Diller 2000
1845	dog, whole-body exposure: 1 · 10 min, analytical characterization of exposure atmosphere, LC <sub>T50</sub>	Diller and Zante 1982
1741	rat, nose-only exposure: 1 · 10, 30, 60 or 240 min, analytical characterization of exposure atmosphere, LC <sub>T50</sub>	Pauluhn 2006 a
1440	rat, whole-body exposure: 1 · 6 hours, 4.1 mg/m <sup>3</sup> , analytical characterization of exposure atmosphere, protein concentration in BAL fluid about 100 times higher than in controls, no mortality	Hatch et al. 2001
1440	rat, whole-body exposure: 1 · 6 h/week, 4.1 mg/m <sup>3</sup> for 4 and 12 weeks, analytical characterization of exposure atmosphere, no mortality	Kodavanti et al. 1997
about 1200	humans, estimated LC <sub>T01</sub>	Borak and Diller 2000
1075	rat, nose-only exposure: 1 · 30, 60 or 240 min, analytical characterization of exposure atmosphere, LC <sub>T01</sub>	Pauluhn 2006 a
1050	dog, head-only exposure: 1 · 0.5 h: analytical characterization of exposure atmosphere, protein concentration in BAL fluid 10 to 15 times higher than in controls, no mortality, fibrinous inflammation, oedema	Pauluhn 2006 c
1008	rat, nose-only-exposure: 1 · 4 hours, 4.1 mg/m <sup>3</sup> , analytical characterization of exposure atmosphere, protein concentration in BAL fluid about 70 times higher than in controls, no mortality, no indications of irreversible effects after 4-week recovery period	Pauluhn 2006 b
about 600	humans, estimated exposure intensity inducing a clinically manifest oedema	Borak and Diller 2000
495	dog, head-only exposure: 1 · 0.5 hours, analytical characterization of exposure atmosphere, protein concentration in BAL fluid 2 times higher than in controls, minimal inflammatory reactions in the terminal bronchiolar region	Pauluhn 2006 c
375	dog, head-only exposure: 1 · 0.5 hours, analytical characterization of exposure atmosphere, calculated threshold concentration for increased protein concentrations in the BAL	Pauluhn 2006 c

**Table 4** (Continued)

C · t product/day [mg/m <sup>3</sup> · min]	Species, exposure, effects	References
270	dog, head-only exposure: 1 · 0.5 hours, analytical characterization of exposure atmosphere, exposure tolerated without specific effects	Pauluhn 2006 c
164	rat, summarizing assessment of acute inhalation studies, nose-only or whole-body exposure: 1 · 0.5 to 6 hours (Hatch et al. 2001; Pauluhn 2006 b), protein concentration in BAL fluid 3 times higher than in controls	Pauluhn 2006 c
144	rat, whole-body exposure: 1 · 6 hours/day, 5 times/week, 4 and 12 weeks, 0.41 mg/m <sup>3</sup> , minimal histological changes in the terminal bronchioles, protein concentration in BAL fluid not increased	Hatch et al. 2001; Kodavanti et al. 1997
117	rat, summarizing assessment of acute inhalation studies in rats, nose-only or whole-body exposure: 1 · 0.5 to 6 hours (Hatch et al. 2001; Pauluhn 2006 b), protein concentration in BAL fluid not significantly increased	Pauluhn 2006 c

stimulation of sensory (protective) bronchopulmonary reflexes manifests itself among other factors in the form of increased plasma exudation and mucine secretion of the respiratory mucosa (*lamina propria*) and it accounts for the elementary protective function of the airways, particularly in smaller rodent species, which show no marked glandular structures in the lower airways due to their narrow respiratory lumina (Lee and Widdicombe 2001; Persson et al. 1996). In humans, similar changes generally do not occur on a reflex-mediated basis, but only in connection with inflammatory reactions (Folkesson et al. 1996; Persson et al. 1996).

In acute inhalation studies in Wistar rats, a reflexory, transient change in the breathing pattern lasting 10–15 minutes during exposure to phosgene was found. The observed effects were typical for a stimulation of pulmonary capillary J-receptors (Pauluhn 2006 a), whose stimulation results in alveolar oedemas and apnoea. The transient depression of ventilation is assessed as being the cause for a dosimetrically produced deviation from Haber's rule after high short-term exposures in this species.

When, in addition, the fact is duly accounted for that, in the rat, the relative proportion of airways to alveolar surface is about 4% (in humans this proportion is only 0.5%; Hatch 1992), this means that low-level protein increases in the BAL fluid in the rat are not necessarily prognostic for irritating toxic, alveolar pulmonary oedema in humans. In other words, an animal species such as the dog, in which no reflexory increased plasma extravasation into the airways takes place, allows more

relevant statements for humans in regard to the "protein concentration in the BAL fluid" endpoint than the rat model would make possible. In this context, it is noteworthy that, independent of the aetiopathology, every excessive, chronic plasma exudation can be accompanied by secondary effects such as an intra-alveolar fibrin and collagen deposition with its typical sequel reactions.

These considerations make it clear that the rat, as an established model in inhalation toxicology, is suitable for determining concentration-effect relationships in a model-specific way. However, one disadvantage of the rat model is that protein contents measured in the BAL fluid allow no clear differentiation of respiratory and alveolar shares particularly in the NOAEC/LOAEC range. The fluid portion originating from the surfactant fluids of the alveoli is about 1/3 of the total fluid volume of airways and alveoli in the rat and about 2/3 in humans (Hatch 1992). Studies using BAL in the dog are to be considered more predictive in assessing the increased intraalveolar plasma exudation in humans than the corresponding data for rats as, in the dog, only an isolated lobe is lavaged and not the total lung with all respiratory areas. Hence, in regard to the extrapolation of phosgene-specific pulmonary effects for humans, the dog represents the most suitable species.

In this context, it is important that the dog also represents the non-rodent species of choice in the toxicological characterization of inhalation pharmaceuticals (DeGeorge et al. 1997). The lungs of Beagle dogs show anatomical structures similar to those of humans (Heyder and Takenaka 1996; Kreyling et al. 1999; Takenaka et al. 1996, 1998). Their structural as well as functional properties have been researched and documented. In the same way as humans, Beagles have mucus-forming cells in their bronchial tract. The centriacinar region (i.e. the area between terminal bronchioles with alveoli and alveolar ducts) is the most affected target structure in the lower airways for phosgene. In the dog, the acinar morphology corresponds in principle to that of humans. The rat has no alveolar bronchioles. The number of alveolar pores in the dog, important for collateral ventilation, is also closest to that in humans. In addition, the breathing pattern in dogs corresponds to that of humans, as dogs also breathe via the nose in the state of rest and oro-nasally in the state of excitation (Amis et al. 1996). On the basis of both the rodent-specific and the phosgene-specific components, the results of the dog studies are given a higher weighting than the rat studies.

In the light of the profile of phosgene effects, the detection of total protein in the BAL fluid is superior to morphological methods, because changes on the level of surfactant fluids in the lung are difficult to establish. Due to these considerations, species- and method-specific dependencies are to be taken into account in order to clearly differentiate the pathodiagnostic important effects of phosgene from homeostatic effects.

### 5.3 Local effects on skin and mucous membranes

Phosgene is an irritant to the eyes and upper respiratory tract (see Supplement “Phosgene” 1984). No recent data are available on this subject.

### 5.4 Allergenic effects

No data are available on allergenic effects.

### 5.5 Reproductive toxicity

#### 5.5.1 Fertility

No data are available on fertility.

#### 5.5.2 Developmental toxicity

No data are available. In the 1995 Supplement (see Supplement “Phosgen” 1995, only available in German), the developmental toxicity of phosgene was assessed as follows:

“For the hydrolysis of carbonyl chloride (phosgene) in aqueous solution at 35°C, a rate constant of the first pseudo order of  $26.7 \text{ sec}^{-1}$  was measured (Manogue and Pigford 1960). From this, a half-life of 0.026 seconds can be calculated for hydrolysis. Reaction with amino, hydroxy and thiol groups takes place even more rapidly *in vitro* (US EPA 1986). It is therefore improbable that carbonyl chloride, after exposure at the level of the MAK value, can find its way into the systemic circulation. The observed damage to the brain at very high exposure concentrations are caused by secondary effects from the anoxia resulting from pulmonary oedema (Diller 1985). Classification of carbonyl chloride in Pregnancy Risk Group C is therefore justified, in spite of the fact that no studies on reproduction toxicity have yet become available.”

### 5.6 Genotoxicity

In a modified mutagenicity test with gases in *Salmonella typhimurium* TA98 and TA100, phosgene was negative in the presence and absence of metabolic activation (see Supplement “Phosgene” 1984).

No more recent data are available. On account of the rapid hydrolysis and as no alkylation of DNA can be expected due to its structure, no genotoxic potential is assumed for phosgene.



## 5.7 Carcinogenicity

No data are available on carcinogenicity.

## 6 Manifesto (MAK Value, Classification)

The few occupational case reports available, offer no quantitative conclusions about retrospective exposures and consequently cannot be used to establish the MAK value for phosgene. For this reason, a threshold level for occupational exposure can only be established on the basis of available animal studies. In the Supplement of 1996 (see Supplement "Phosgene" 1996), the decreased ATP content of the lungs of rats after single exposure to  $0.05 \text{ ml/m}^3$  was used as the most sensitive parameter. At this concentration, the protein concentration in the BAL fluid was not increased; this only occurred at  $0.1 \text{ ml/m}^3$  (Currie et al. 1987 a). Since later studies with repeated exposure showed no increased protein concentration in the BAL fluid even at  $0.1 \text{ ml/m}^3$  (Hatch et al. 2001), the relevance of an ATP decrease after acute exposure is not proof for the development of pulmonary oedemas following repeated exposure and can therefore be considered as non-adverse.

The minimal histological effects in the bronchoalveolar region of rats at  $0.1 \text{ ml/m}^3$  showed no time-dependent increase between 4 and 12 weeks exposure (Kodavanti et al. 1997). Therefore, acute studies can also be used for the derivation of a threshold value for phosgene. As discussed in Section 5.2, the results of studies with dogs are more meaningful for humans than those with rats.

Single exposure of dogs for 30 minutes showed a  $C \cdot t$  product of  $270 \text{ mg/m}^3 \cdot \text{min}$  with no significant changes in the BAL fluid or adverse histological findings (Pauluhn 2006 c). Based on the validity of Haber's rule across a wide range of concentrations and exposure times during single exposure, this would correspond to an 8-hour exposure concentration of  $0.56 \text{ mg/m}^3$  ( $0.14 \text{ ml/m}^3$ ). As demonstrated above, extrapolation to chronic exposure is not considered necessary. As the MAK value is derived on the basis of the dog as being the more relevant species for extrapolation to the human situation, and as the respiratory minute volume per kg body weight in dogs at  $0.36 \text{ l/min}$  and kg (Bide et al. 2000) is about as high as that in humans ( $0.30 \text{ l/min}$  and kg) under usual workplace conditions ( $10 \text{ m}^3$ , 8 hours, 70 kg), the data from the dog study are transferable to the workplace situation. Phosgene reacts, unmetabolized, locally at the site of retention and without marked inter-individual differences. This has been confirmed by the values for protein concentration in the BAL fluid, located very near to each other, in the four examined dogs, at  $270 \text{ mg/m}^3 \cdot \text{min}$  (Pauluhn 2006 c). On the basis of the 8-hour concentration without adverse effects of  $0.14 \text{ ml/m}^3$  calculated for the dog, the MAK value is thus established at  $0.1 \text{ ml/m}^3$  according to the "preferred value approach".

The local effects in the lower lung region are the critical effect; phosgene is therefore classified in Peak Limitation Category I. Due to the  $C \cdot t$  dependence of the

effects, the previous excursion factor 2 has been retained. The resulting concentration is below the odour perception threshold of  $0.4 \text{ ml/m}^3$  and the concentration which still induces borderline, transient, species-specific respiratory reflexes in the rat after short-term exposure ( $0.3 \text{ ml/m}^3$ ).

Due to its rapid hydrolysis and the absence of systemic availability (see Supplement "Phosgen" 1995, only available in German phosgene remains in Pregnancy Risk Group C, ) even though there are no data on the corresponding endpoints. It is still not designated with "Sh", "Sa" or "H", and its classification in one of the categories for Germ Cell Mutagens is not necessary. There are no data on carcinogenicity. It is not possible to estimate whether carcinogenic effects can occur due to the local effects of phosgene in the respiratory tract. As explained in Section 5.6, no genotoxic potential is assumed. Phosgene is therefore not classified in one of the categories for carcinogens.

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completed 12.10.2006