Phosgene Safety Practices
for design, production and processing

Part 3
Related information and special safety practices

Section 3: Phosgene toxicity and toxicology

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III caveat

1. Introduction

1.1 Phosgene

Phosgene (carbonyl chloride) is a highly reactive, acylating colourless gas at ambient temperature and pressure. It is manufactured from the reaction of carbon monoxide and chlorine gas in the presence of activated charcoal as catalyst. These properties make phosgene amenable to an 'on-demand' design concept (see Part 1 section 2.2 Design Concepts).

Phosgene is an important and indispensable high production volume intermediate in the manufacture of building blocks of various types of plastics and numerous industrial materials, such as polyurethanes, polycarbonates and in the production of dyestuffs, pharmaceuticals and agrochemicals.

1.2 Toxicological attention for phosgene

In regard to its inhalation toxicity, phosgene has received extensive toxicological attention with focus on its mode of action to cause a non-cardiogenic acute pulmonary oedema upon short, high-level inhalation exposure in experimental animals (Borak & Diller, 2001; IPCS, 1998; Diller, 1985a, b; Duniho et al., 2002; Frosolono & Pawlowski, 1977; Frosolono, 1985; Sciuto, 1998; Pauluhn et al., 2007).

In biological reactions, phosgene is a wellknown acylating agent and is also capable of reacting with important cellular components of biomolecules, such as amino, hydroxyl and sulfhydryl groups (Duniho et al., 2002).

Typically, pulmonary toxicity as a result of acute lung oedema and lymph drainage overflow, reaches its climax 10-20 hours post exposure with full reconstitution. Systematic research on countermeasures to mitigate phosgene-induced acute lung injury has been published (Duniho et al., 2002; Sciuto et al., 1995; 1996; 2003; Sciuto and Hurt, 2004).

The low solubility and rate of hydrolysis of phosgene in aqueous media favours its penetration into the gas exchange region of the lung without the elicitation of subjective signs of irritation. Prevailing experimental evidence suggest that phosgene gas acts preferentially at the alveolar level in a concentration x exposure duration (C x t)-dependent manner. Its simple, direct mode of action is evidenced by a steep C x t-relationship (Pauluhn et al., 2007). Single and repeated sub-chronic exposures (Kodavanti et al., 1997) demonstrate that chronic effects appear to be contingent upon the 'acute-on-chronic' localised effects resulting in essentially identical no-observed-adverse-effect levels (NOAELs) independent of whether the duration of study is acute or subchronic (Pauluhn et al., 2007).

Opposite to more water soluble irritant gases, airway toxicity or a delayed-onset type of inhalation toxicity (e.g. obliterating bronchiolitis) has not been observed in experimental models of phosgene toxicity (Pauluhn, 2006a, b; Pauluhn et al., 2007).

1.3 Solid congeners of phosgene

More recent publications convey the message that using the solid congener of phosgene, triphosgene, may reduce the health risks potentially associated with phosgenation processes (Cotarca, 1999). This conclusion is based on the fact that triphosgene is asserted to be safer and more convenient to handle, transport and store because it is a solid with minimal vapour pressure at ambient temperature.

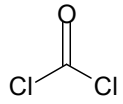
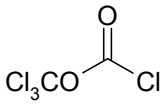
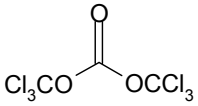
It has also been stated: "*The toxicity of both diphosgene and triphosgene is exactly the same as phosgene since both dissociate or depolymerise to phosgene on heating and upon reaction with any nucleophile*" (Cotarca and Eckert, 2004).

This may be a valid statement in context with chemical reactions; however, may not necessarily occur under physiological conditions in the lung.

The discoloration of phosgene indicator badges (dosimeters) has often been taken as indirect evidence that instant decomposition to phosgene occurs (Damle, 1993). However, based on the physical and toxicological characteristics relative to phosgene, these assumptions are subject to challenge because state-of-the-art published inhalation data of triphosgene are not available yet, nor is there any specific analytical method to quantify airborne triphosgene (Cotarca et al., 1996). Despite this the commercial scale of triphosgene has reached hundreds of tons per year a decade ago (Cotarca, 1999; Cotarca and Eckert, 2004).

The principal physicochemical differences between phosgene and the phosgene congeners are summarised in Table 1. The toxicological details of the congeners of phosgene are presented and discussed in section 5.

Table 1: *Physical characteristics*

substance	formula	appearance / phase	mol weight	melt/ boil point	saturated vapour		conversion factor
		[b]	[a] [g/mol]	[a] [°C]	concentration [mg/m³]	pressure	1 ppm= x mg/m³
phosgene		Gas	99	-128 / 7.4	$6.4x10^6$	0.16 MPa @ 20°C[a]	4.1
diphosgene		Liquid	198	-57 / 128	111000	1.3 kPa @ 20°C; decomposition @300°C[a]	8.1
triphosgene		Solid crystals, high sublimation potential,	297	80 / 206	4186[c]	0.263 mmHg @25°C[c], decomposition [d] >350°C	12.2

a) Physical data from wikipedia
b) Appearance/phase data are given as present at room temperature. All are insoluble in water.
c) http://lookchem.com/triphosgene/,
d) Triphosgene is thermally stable up to 131 °C. At higher temperatures this molecule can undergo a cyclic dissociation pathway to phosgene (Cotarca and Eckert, 2004).

1.4 Toxicity of inhaled phosgene

In laboratory animals, exposure to phosgene resulted in pathophysiological changes specifically in the bronchoalveolar region, which have been considered to be the cause of non-cardiogenic pulmonary oedema upon acute inhalation exposure (Diller 1985a,b; Diller et al., 1985; Duniho et al., 2002).

The solubility and rate of hydrolysis of phosgene favours its penetration into the gas exchange region of the lower respiratory tract (for anatomical details see Figure 1 and 2), including the layers constituting the alveolar blood barrier and reactions with nucleophilic molecules predominate hydrolysis. This may crosslink nucleophilic groups of peptides/proteins with resultant protein denaturation.

Similarly, modifications of structural matrix proteins of the lung, including collagen, may occur and then lead to long-term changes, suggestive of remodelling of the lung parenchyma. The more proximal airways are protected against phosgene by reactions in the mucus layer (Nash & Pattle, 1971).

After high-level acute exposure of experimental animals, the histopathological lesions were characterised by acute changes consisting of alveolar and interstitial oedema, fibrin and haemorrhage. This is followed by significant alveolar and interstitial flooding with inflammatory cell infiltrates and scattered bronchiolar and terminal airway degeneration and necrosis. Partial resolution of oedema and degenerative changes was followed by epithelial and fibroblastic regeneration (Gross et al., 1965; Duniho et al., 2002; Hatch et al., 2001).

Thus, the toxicity of inhaled phosgene is thought to be due to the direct effects of phosgene on the tissues of the gas exchange region. Due to its low water solubility phosgene is scrubbed to an insignificant extent in the upper respiratory tract or airways. So phosgene penetrates effectively the lower airways, including the alveolar region. This makes phosgene gas unusual (or similar to e.g. nitric oxides, ozone, perfluorated alkenes) compared to more soluble irritant gases, such as hydrochloric acid, which is preferentially be deposited in the less susceptible structures of the upper respiratory tract.

Prevailing experimental evidence suggest that acute lung injury occurs at moderate exposure doses as a results of the lungs response to injury, rather than an instant deterioration of cell membrane functionality. However, at exposure level high enough to titrate out all nucleophiles within the lining fluids of the lung, such direct damage may occur at excessively high dose levels. The potential release of HCl is unlikely to contribute to the acute inhalation toxicity of phosgene to any appreciable extent.

This has been further substantiated by the prophylactic parenteral or oral administration of nucleophiles. Especially hexamethylenetetraamine (HMT, see review Diller, 1980) and cysteine esters (Lailey et al. 1991) have been demonstrated to give measureable protection when given before exposure to phosgene. This, and related nucleophilic compounds, did not show the least protection if given after exposure to phosgene (Diller, 1980; Frosolono, 1985; Lailey et al. 1991; Potts et a., 1949). The protection provided when administered before exposure to phosgene, provides indirect evidence that the acylation and depletion of nucleophiles is more critical than any HCl release. This appears to be plausible as the LC50 of phosgene in acutely exposed rats (1741 mg/m³ x min; Pauluhn, 2006a) is orders of magnitude lower than that of HCl gas (211545 mg/m³ x min; Pauluhn et al., 2007).

1.5 Principal function of the respiratory system

To fully appreciate the toxicological mode of action of phosgene gas, the principal function of the respiratory system has to be recalled. The principle anatomical structures of the respiratory tract are illustrated in Figure 1 and 2.

To make the gas exchange in the lung efficient, thin membranes with a very large surface area are required. The surface area of this membrane is several times that of the outer protective surface of an individual. The barrier that separates the air surface of this membrane from the large vascular bed associated with it, is limited in thickness by the requirement of gas diffusion. The existence of such a large surface area creates a tremendous number of problems in terms of packaging and architectural organisation, to optimize such a large gas flow and volume change.

Another entire set of problems concerns the mechanisms needed to protect such a membrane from injury. The various, highly diverse structures making up the respiratory tract, provide the initial interface between the organism and the external air. This has led to a significant amount of interspecies variability in both architectural organisation and cellular composition.

Toxic agents may reach the lung both via the blood circulation and via the inhaled air. The latter is associated with a non-homogeneous deposition of the inhaled agent within the entire tract, depending on physical and physicochemical factors. The lung is designed to prevent dust from entering the alveoli. Inhaled biological substances, such as bacteria, are destroyed by highly destructive (bio) chemical processes which require tight control.

Any disturbance of these intricate control systems by inhaled toxicants, including the impairment of the clearance of the lung from inhaled substances, may initiate chronic disease. The uncontrolled rebound of these mechanisms may cause effects to the lung over and above the initial toxic event.

Figure 1: *Illustration of the lung showing details of the lobar structure, conducting airways and vessels. The acinus includes a terminal bronchiole and its respiratory bronchioles, alveolar ducts and alveolar sacs (alveoli).*

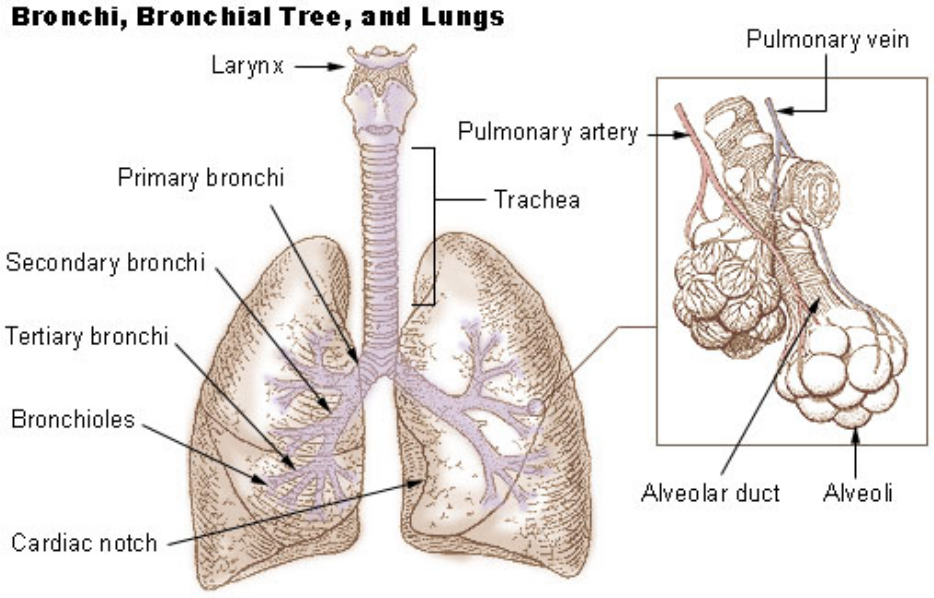
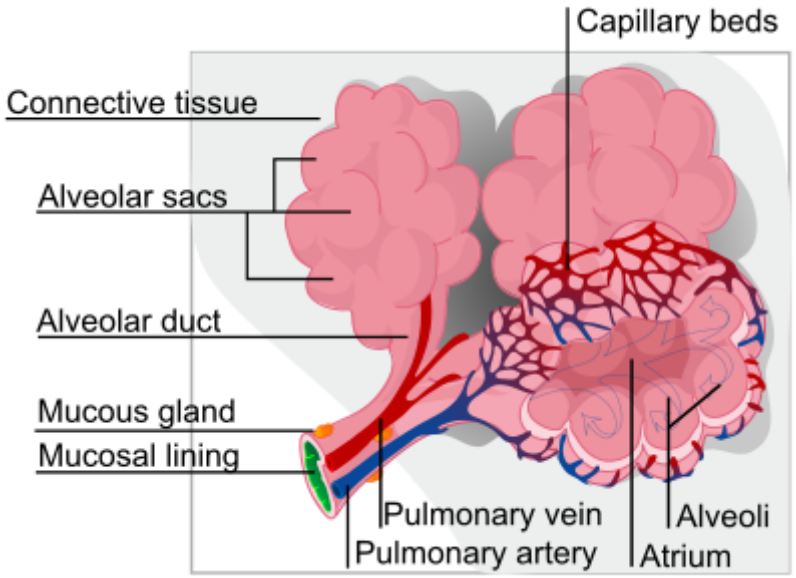


Illustration from: SEER Training Modules (NCI, 2011), in public domain, free of known copyright restrictions.

The high susceptibility of the alveolar walls is related to their extreme surface area to cellular mass ratio. Alveolar walls which separate two adjacent alveoli consist of two

layers of alveolar epithelium on separate basement membranes, enclosing the interstitial space. This space contains the pulmonary capillaries, elastin and collagen fibres and nerve endings. The interstitial space is asymmetrically disposed with relation to the capillaries. On one side of the capillary the space tends to be thicker and to contain abundant collagen fibres. This may be considered as the 'service side' of the capillary. This forms the connective tissue framework which maintains the geometry of the alveolus. On the other side of the capillary, endothelium and epithelium are closely apposed. The total thickness of the alveolar capillary membrane is usually less than 0.4 μm. This may be considered as the 'active side' of the capillary and the gas exchange function is presumably more efficient on this side.

Figure 2: *Illustration of the pulmonary alveoli showing details.*



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1.6 Toxicological mode of action of phosgene

The alveolar surface of the lungs is lined with a complex and highly surface-active material: the pulmonary surfactant. The surface film that lines the alveoli prevents alveolar collapse. It also provides some 'buffering capacity' for the chemical scavenging and inactivation of phosgene in this region of the lung. The major functions of surfactant in the alveolar lining layer are diverse. By stabilisation of the fluid balance and reducing the contractile forces in the curved air-liquid interface, it prevents fluid passing from the blood capillaries into the alveoli.

Disturbance of the surfactant system by noxious agents, such as phosgene, can take place at different stages.

A compromised surfactant layer may lead to an increased permeability of the air-blood barrier and subsequent intrusion of plasma proteins into the alveoli. This can cause 'alveolar flooding' when excessive enough. Plasma proteins per se may further decrease the function of this layer, causing extra-vascular fluid accumulation with increasing post exposure time. Thus, the early acute injury of the air-blood barrier leads to a pulmonary oedema, cell destruction and inflammation which may block the oxygenation of blood with subsequent death.

The length of this 'clinical latency period' is inversely related to the inhaled dose of phosgene. Thus, depending on the exposure intensity and duration, the pulmonary oedema reaches its maximum about one day after exposure, or shorter. It may last for several days. Pulmonary inflammation may persist for a longer period. Lung tissue remodelling leading to fibrosis (replacement of functionally active tissue by matrix tissue) may be a likely long-term sequel.

Current workplace and emergency response planning guideline levels of phosgene are based on controlled animal inhalation toxicity studies. They take either into account the increased protein extravasation into the alveoli (early oedema evidenced by protein in bronchoalveolar lavage (BAL)) or its chronic sequel. This is chronic pneumonitis and evidence of increased collagen deposition, including focal fibrosis. Elevated levels of BAL protein appear to arise from increased permeability of the lung to plasma proteins caused by damage to the alveolar capillary barrier.

While this interpretation is undoubtedly valid at high exposure-concentrations, there continues to be a lack of understanding of the possible contribution of alterations in the normal fluxes of proteins at lower exposure concentrations. Collectively, in most of the acute experimental scenarios, i.e., high-level exposures of short duration, concentration alone appears to drive the toxic response more significantly rather than cumulative dose over extended exposure periods (e.g., days).

2. Toxicity data base

No reliable, quantitative human data exist. Human data are limited to descriptive effects from accidental exposures. Therefore, the current human data base is considered to be not appropriate for the derivation of workplace limits or acute emergency exposure guideline levels. Despite the availability of human data, all derivations of exposure limits so far are based on controlled animal exposure studies.

There has been increased numbers of publications addressing the specific aspects of phosgene-induced pulmonary toxicity. Accordingly, current exposure limits were adjusted (see Part 3 – "Regulatory levels and limits for phosgene", Table 3). Most animal models address effects associated with acute inhalation exposure, whilst in only one single study the subchronic inhalation toxicity over 3 months of exposure was investigated (Kodavanti et al., 1997).

State-of-the-art studies addressing the systematic analysis of concentration x time (C x t) relationships of interest for Emergency Response Planning are scarce. Most focus on short exposure durations using animal species not commonly used in guideline inhalation toxicity studies.

There is a large amount of acute inhalation data in many experimental species. Despite the wealth of acute inhalation exposure data available, only few of them utilised standardised, well validated GLP-like procedures in terms of the controlled exposure of experimental animals, the analytical characterisation of exposure atmospheres and the endpoints measured.

The higher density of phosgene relative to air (3.4 times), makes concentration gradients within improperly designed/ventilated whole-body chambers more likely to occur than in highly dynamic nose-only chambers. Hence, the specific density of this gas makes it potentially prone to inaccurate measurements of nominal airflows and in-homogeneities of exposure atmospheres in larger inhalation chambers.

Most the inhalation studies performed in the past utilised whole-body exposure systems and only few of them observed these aspects by determining phosgene close to the breathing zone area of experimental animals by state-of-the-art analytical procedures. Very short durations of exposure may not necessarily provide uniform exposure atmospheres in larger inhalation chambers.

In concert, almost all endpoints of interest have already been addressed in research-type toxicity tests with phosgene. Only few addressed all endpoints of interest in one single study, including analysis of dose/concentration-response, their time-course and full reversibility. The last point appears to be particularly important. Dose-response and evidence of reversibility needs to be demonstrated in order to comply with the contemporary paradigms applied for the derivation of Emergency Response Levels (vide infra). Moreover, the animal species often selected in research environments (mostly mice due to the small size and cost) are not commonly used for guideline inhalation toxicity studies.

In terms of relevance to humans, the strengths and weaknesses of animal models have to be critically assessed. One essential factor appears to be related to dosimetry (inhaled dose over time). In this context, mice are often markedly more susceptible when compared to humans, because their respiratory minute ventilation per unit body weight significantly exceeds that of humans. Especially for emergency type of short-term exposures, slow and more human-like breathing animals (15-25 breaths/minute) with larger dead-space volumes in their respiratory tract may respond differently when compared to mice (250-300 breaths/minute). This means,

the critical rate of the delivery of phosgene to the pulmonary region may be appreciated more appropriately in larger species, such as dogs, compared to mice.

What is known about the structure of the mouse lung probably has important bearing on its function. For example, the total lung capacity (TLC) of the mouse is about 1 ml compared to 6000 ml of a human. The alveoli and the thickness of the blood-air barrier of the mouse are smaller than those of humans. This might have important implications for both gas exchange and parenchymal lung mechanics. An important additional difference between mice and humans is the paucity, or even complete absence, of mucosal glands relative to their large-airway size (Irvin and Bates, 2003). However, due to the reasons mentioned above, mice studies are abundant in the published scientific domain.

Moreover, analysis of the validity of Haber's rule – which means that different C x t relationships produce the same outcome – in small laboratory rodents is complicated. This is caused by the fact that high concentrations of phosgene stimulate protective reflexes in rodents and especially mice ('breath-hold' and decrease of respiratory ventilation). Similar changes do not occur in humans or more human-like animal species, such as dogs (cf. figure 4 of Pauluhn, 2006a). This again may suggest that the response to high exposure concentrations of short duration is difficult to model in (very) small laboratory animals. Changes in the dosimetric uptake of inhaled phosgene make extrapolations from short-term to longer-term exposure, and vice versa, error prone and therefore call for experimental data that validate or refute the assumptions made.

3. Acute toxicity data (LC50 and LC01)

Current paradigms used in risk characterisation utilize the non-lethal threshold concentration (LC01) as starting point. This value is the highest (statistically derived) exposure level that does not cause lethality. Commonly, this value is obtained through dividing the LC50 (statistically derived median lethal concentration) by 3, as default (NRC, 2001).

3.1 Acute inhalation study

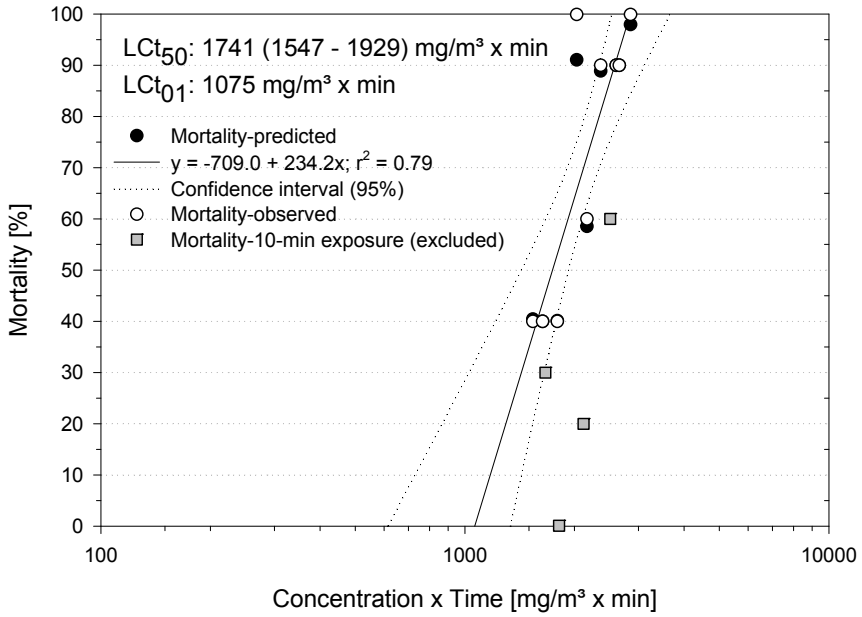
In an acute inhalation study, groups of young adult Wistar rats were acutely exposed to phosgene gas using a directed-flow nose-only mode of exposure (Pauluhn, 2006a). The exposure durations used were 10, 30, 60 and 240 minutes and the corresponding C x t products bracketed a range from 1538 – 2854 mg/m³ x min.

For details see figure 4 of this study (Pauluhn, 2006a). That figure shows means of respiratory parameters of 8 exposed for 30-min or 10-min (210 mg/m³) to phosgene. Each exposure was preceded and followed by a 15-min and 30-min exposure period, respectively.

The post exposure period was 2 weeks. Subgroups of rats were subjected to respiratory function measurements. With few exceptions, mortality occurred within 24 hours after exposure.

The LC50 and the estimated LC01 for 10, 30, 60 and 240 min were 253.3 (105.3), 54.5 (29.2), 31.3 (21.1) and 8.6 (5.3) mg/m³, respectively. With regard to the fixed outcome Cn x t product, the exponent n was found to be ≈0.9 for both the LC50 and the LC01. Due to an apparent rodent-specific transient depression in ventilation (figure 4 of Pauluhn, 2006a), results from 10 min exposures were excluded for the calculation of average C x t products. The average LCt50 (and confidence interval 95%) and LCt01 were 1741 (1547-1929) mg/m³ x min and 1075 mg/m³ x min, respectively (Figure 3), with an LCt50/LCt01 ratio of 1.6.

Figure 3: *LCt50 and LCt01 values from single-exposure rat nose-only inhalation studies using C x t products from 30, 60 or 240 minute exposures. Mortality data from 10-min exposures were excluded from the regression analysis due to an instant, although transient, decrease in the respiratory minute volume (as depicted in figure 4 Pauluhn, 2006a)*



In an ancillary study one group of rats was exposed to 1008 mg/m³ x min (at 4.2 mg/m³ for 240 min; post-exposure period 4 weeks). Emphasis was on the time-course of non-lethal endpoints (BAL) and histopathology of the lungs of rats sacrificed at the end of the four-week post exposure period. The climax of BAL-protein was on the first post exposure day and exceeded approximately 70-times the control without causing mortality. The changes in BAL-protein resolved within 2 weeks. Histopathology did not show evidence of lung remodeling or progressive, potentially irreversible changes 4 weeks post exposure.

In summary, the analysis of the C x t dependent mortality revealed a steep C x t mortality relationship. The C x t product in the range of the non-lethal threshold of 1008 mg/m³ x min, indicators of pulmonary injury in BAL were markedly increased, however, resolved almost entirely within the 4 week post exposure period.

In this study, test atmospheres were monitored in the breathing zone area of animals using two independent analytical methods:

1) continuous real-time monitoring used a calibrated non-dispersive infrared spectroscopy (Binos)

2) a phosgene-specific 2-HMP-GC method (impinger containing a toluene solution of 2-hydroxymethyl-piperidine (2-HMP) as reagent for derivatisation).

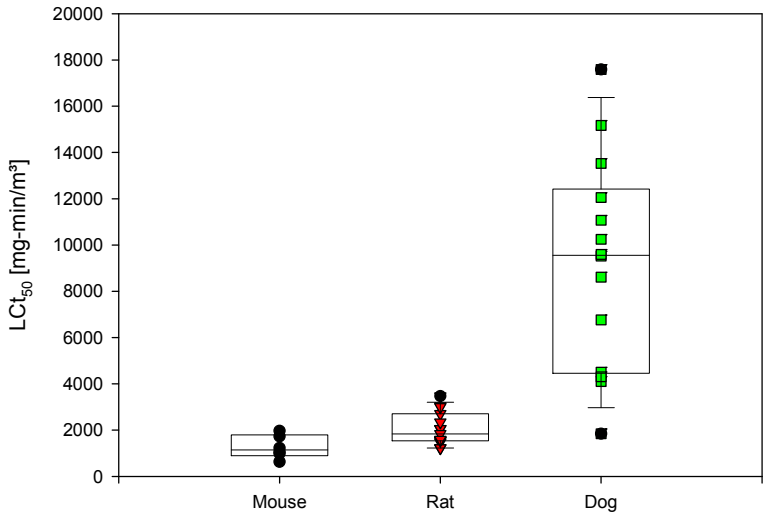
The comparison of nominal and actual concentrations determined by two different analytical methodologies gave essentially identical results (Pauluhn, 2006a).

The results of this study and that reported by Zwart et al. (1990) demonstrated a very steep dose-mortality curve which almost approached a step function. For quantal data, the variability determines the slope of the concentration/(dose)-response and is considered to be typical for an acute, directly acting pulmonary irritant acting through a simple chain of events.

3.2 Animal lethality studies

Animal lethality studies are abundant, but are of varying quality. Many are incompletely reported, therefore, the focus here is solely on the key studies. Even though there are limitations concerning these studies, they appear to suggest that species with high respiratory minute volume per body weight are more susceptible than larger ones. Thus, in terms of risk characterisation, due to the similarity of breathing patterns, LCt50 data obtained in dogs may outweigh those obtained in smaller rodents and especially mice (Figure 4).

Figure 4: *LCt50 – Species Comparison (LCt50 from individual studies, boundaries reflect Tukey-Box plots and medians).*



Thus, the C x t = k relationship appears to be generally valid, although at very high concentrations a transiently increased breath holding (decrease in ventilation) has been observed. This leads to higher than predicted LC50 values for very short exposure durations. Overall, the uncertainty associated with exposure durations shorter than 30 minutes, calls for the use of animal species with more human-like breathing (e.g., dogs), rather than species experiencing reflexively-induced changes in breathing patterns.

Zwart et al. (1990) exposed (whole body) groups of five male and five female Swiss mice and rats to varying concentrations of phosgene for 5, 10, 30, or 60 minutes. The test atmosphere was monitored at the inlet and outlet of a glass exposure chambers but not within the chamber. Details regarding the test substance, the calibration and specificity of analytical detection system, including verification of the uniformity of exposure atmospheres, are lacking entirely. The relationship of nominal and actual concentrations and the humidity in the inhalation chamber have not been reported.

For mice, ten minute LC50 values were 77 and 61 ppm for males and females, respectively. Thirty minute LC50 values were 18 and 11 ppm for males and females, respectively and 60 minute LC50 values were determined to be 9 and 5 ppm for males and females, respectively. For rats (males and females combined), the ten minute LC50 value was 64 ppm, the thirty minute LC50 value was 13 ppm and the 60 minute LC50 value was 6.7 ppm using a probit analysis. Based on the alternative calculation method used by the authors, the ten minute LC50 value was 80 ppm, while the thirty and 60 minute LC50 values were 20 and 12 ppm, respectively.

4. Non-lethal pulmonary toxicity

Gross et al. (1965) exposed 117 male Wistar rats to a range of phosgene concentrations from 0.5 to 4 ppm, for various time periods between 5 minutes and 8 hours. With regard to irreversible effects occurring after single exposure the Gross et al. (1965) study is considered superior to all other currently existing studies.

Rats were placed in individual cages and were introduced into the lacquered wooden exposure chamber when the desired concentration had been achieved. Phosgene atmospheres were generated by passing nitrogen through a reservoir of paraffin oil which had been saturated with phosgene. Constant monitoring of the chamber atmosphere was accomplished by using bubblers containing nitro-benzylpyridine with subsequent spectrophotometric detection. The poor methodological description does not allow any judgment whether at the onset of exposure, high levels of phosgene were purged from the paraffin reservoir, where air samples were taken relative to the location of animals and whether the bubbler sampling periods were adequately spaced to detect possible short-term high-level excursions.

This study did not expose control rats to paraffin alone purged with nitrogen under identical conditions. The majority of rats were killed four days post exposure. The lungs were subjected to histopathological examination. Any observed inflammatory changes (termed chronic pneumonitis) were graded as slight, moderate or severe depending on the amount of alveolar tissue involved.

Slight changes in alveolar epithelium were identified following exposure to 0.5 ppm of phosgene for 120 minutes (60 ppm x min). The lowest phosgene CT product to produce signs of slight or moderate inflammation was 3 ppm for 5 minutes (15 ppm x min). Moderate or severe inflammatory change was observed following exposure to 3 ppm of phosgene for 20 minutes (60 ppm x min). Evidence of acute pneumonia was observed following exposure to 0.8 ppm of phosgene for 60 minutes (48 ppm x min) although these pathological changes were more commonly seen at C x t products of 108 ppm x min and above.

Pauluhn (2006b) described a study where young adult male Wistar rats were acutely exposed to phosgene gas for either 30 or 240 minutes using a directed flow nose-only mode of exposure. In 30 minute exposed rats the concentrations were 0.94, 2.02, 3.89, 7.35 and 15.36 mg/m³ which relate to C x t products of 28.2, 60.6, 116.7, 220.5 and 460.8 mg/m³ x min. In 240 minutes exposed rats the concentrations were 0.96, 0.387, 0.786, 1.567 and 4.2 mg/m³ which relate C x t products of 47.0, 92.9, 188.6, 376 and 1008 mg/m³ x min. Six rats/group were sacrificed on post-exposure days 1, 3, 7, 14 and 84, whilst the rats of the 1008 mg/m3 x min group where sacrificed on post-exposure days 1, 7, 14 and 28.

The focus of measurements was directed toward indicators of inflammatory response and increased transmucosal permeability in bronchoalveolar lavage (BAL), including lung weights. Lungs from rats sacrificed at the end of the post-exposure period were additionally examined by histopathology. Mortality did not occur at any C x t product. The most pronounced changes were related to C x t – dependent increases in the following markers in BAL: protein, soluble collagen, poly-morpho-nuclear leukocytes counts (PMN) and alveolar macrophages with foamy appearance. These indicators were maximal on the first post-exposure day. Total cell counts and alveolar macrophages containing increased phospholipids reached their climax around post-exposure day 3. At 1008 mg/m³ x min the most sensitive indicators in BAL, i.e. protein, PMN and collagen, resolved within 2 weeks, whereas at lower C x t products they reached the level of the control by post-exposure day 7. At 1008 mg/m³ x min (day 28) histopathology revealed a minimal to slight hyper-cellularity in terminal bronchioles with focal peribronchiolar inflammatory infiltrates and focal septal

thickening. At lower C x t products (day 84), the rats from all groups were indistinguishable. Sirius red stained lungs did not provide evidence of late-onset sequelae, such as fibrotic changes or collagen deposition. At similar C x t products the changes in BAL were slightly less pronounced using 30 minute exposure periods when compared to 240 minute exposure periods.

In summary, the phosgene-induced transmucosal permeability caused a C x t dependent increase of several BAL-indicators of which those of protein, PMN and soluble collagen were most pronounced. Exposure intensities up to 116.7 mg/m³ x min did not cause changes different from those observed in controls, whilst at 188.6 mg/m³ x min distinct differences to the control existed. Despite the extensively increased airway permeability, histopathology did not provide evidence of lung tissue remodeling or irreversible sequelae.

BAL protein has been used extensively as an indicator of acute non-lethal lung toxicity. The German Maximum Workplace Limit Value (DFG-MAK) had precautionary been lowered in 1996 from 0.1 ppm to 0.02 ppm, based on a single 4-hour inhalation study in rats and endpoints suggestive of lower respiratory tract irritation. The EU-SCOEL occupational exposure level (IOELV, see Part 3 – “Regulatory levels and limits for phosgene”, Table 3) and the German MAC limit values are identical.

Other key studies were made in rats with somewhat variable results. Hatch et al. (2001) shows that when rats were whole-body exposed BAL-protein peaked 24-hours after exposure. Rate exposed 1x4-hours to 0.25 ppm (60 ppm x min) elaborated an increase four-times the control. Those exposed 1x6-hours to 0.1 and 0.2 ppm (36 and 72 ppm x min) displayed an increase twice and 12-times the control group. BAL fluid protein elevation following exposure to phosgene, appears to return to normal soon after exposure to the low levels of phosgene, but is slower to return after higher phosgene exposures. Pauluhn (2006b) exposed (nose-only) rats for 30 and 240 min to 0.5 (15 ppm x min) and 0.1 ppm (24 ppm x min), respectively and did not report any remarkable changes of the same endpoint.

5. Mechanistic studies: phosgene and phosgene congeners

The objective of study was to compare the key attributes of acute inhalation toxicity of diphosgene and triphosgene (Pauluhn, 2011) with that of previously investigated phosgene (Pauluhn, 2006a,b) utilizing an internationally harmonised testing protocol (OECD, 2009).

In this comparison the exposure was always to the gas or vapour phase irrespective of the physical property of the substance. Due to the marked differences of the starting material (pressurised phosgene gas), highly volatile liquid diphosgene and solid crystalline triphosgene, attempts were made to expose rats to the vapour phase without using any solvent or vehicle.

Robust, validated analytical methods are yet not available for diphosgene and triphosgene. The established phosgene-based methodology (OSHA method no. 61) can not differentiate these congeners of phosgene from phosgene per se. Therefore, at the atmosphere generation state, these compounds were handled solely in pure nitrogen (dry) under temperature conditions where decomposition does not occur.

This has been empirically verified by FT-IR. Phosgene badges may also be suitable to show the presence/absence of congeners of phosgene in a dichotomous, non-quantitative manner. However, more quantitative approaches as published for phosgene (Niessner, 2010) are absent. The lack of any specific analytical method in an environment where triphosgene and diphosgene may coexist with phosgene is considered to be a distinctive disadvantage of these phosgene precursors.

Similar to phosgene, the solid triphosgene has very low water solubility. When immersed in water, there was no significant change in pH (HCl release) and hence decomposition into phosgene occurred (Cotarca and Eckert, 2004). During a pre-test run desiccated hexane was used as vehicle for triphosgene (data not shown). The aerosolisation of 1% triphosgene in hexane (15 ml hexane/m³) resulted in a concentration of 76-95 mg triphosgene/m³ as vapour (no aerosol detected in the inhalation chamber).The exposed rats showed the same mortality pattern as those exposed at 65 mg/m³ vapour (animals were found dead shortly after the exposure day 0 up to the first post-exposure day). Key findings are summarised in Table 2.

Table 2: *Indices of acute inhalation toxicity of phosgene, di- and tri-phosgene gas/vapour phase of rats nose-only exposed for 240 minutes*

Substance	Sex	LC_{50} [mg/m³]	[ppm]	LC_{50} [mmol]	LC_{01} [mg/m³]	[ppm]	LC_{50}/LC_{01} Ratio
Phosgene[a]	combined	7.2	1.8	0.07	4.5	1.1	1.6
Diphosgene	males	13.9	1.7	0.07	8.4	1.0	1.7
Triphosgene	combined	41.5	3.4	0.14	24.1	2.0	1.7

a) LC_{50} recalculated from studies combining different C x t products (for details see Pauluhn, 2006a). The recalculated LC_{50} was adjusted to 240 min.

The findings presented in Table 2 support a conclusion that triphosgene reacts within the lung instantly as intact molecule and not via its putative degradation products which would have increased rather than decreased its molar toxic potency. The mode of action of triphosgene appears to be different from that of diphosgene and phosgene, due to its property to elicit more persistence signs of respiratory distress, increased necropsy findings suggestive of prolonged/persistent lung injury and a unique additional delayed mortality pattern typical of central airway injury (bronchiolitis obliterans). Under the conditions of tests, triphosgene vapour

atmospheres generated at approximately 40°C followed by subsequent dilution with dry air of at least 1:50, were still in the lethal range. From that perspective, triphosgene appears to be the most insidious substance as its solid physical state conveys the erroneous perception of insignificant exposure. Moreover, triphosgene sublimates at any elevated temperature, making unexpected exposure more likely to occur. Based on the molar LC50s', the survival patterns and occurrence of toxic signs phosgene and diphosgene exhibited essentially identical toxic potencies.

Despite the analytical challenges to characterize the parent molecule, the data presented in Table 2 demonstrate unequivocally that diphosgene and triphosgene are both stable in air and do not decompose to phosgene in the lung.

Consistent with the range of nucleophilic substitution reactions shown by Cotarca et al. (1996), this reactivity appears cause instant acylation of nucleophiles by the intact molecule. Injury patterns appear to be contingent on the site(s) of initial retention and chemical reaction. The cause of the apparent lower molar toxicity of triphosgene appears to be consistent with its biphasic mortality patterns. This is suggesting that the retention of vapour is not restricted to the pulmonary region alone. In other words, some triphosgene may also react within the bronchial airways causing different injury patterns.

Minute differences in the physicochemical properties of these congeners of phosgene may change their primary site of initial deposition within the lung. Due to the inhomogeneous distribution and abundance of nucleophilic scavengers in the lining fluids or cell surfaces, the manifestations of toxicity may differ from one congener to the other. Similar bi-phasic mortality patterns have been observed following single exposures to highly reactive and somewhat less lipophilic aliphatic and aromatic mono-isocyanate vapours (Bucher et al., 1987; Pauluhn, 1989; Pauluhn and Eben, 1992; Pauluhn et al., 1995).

So far it cannot be judged unequivocally whether triphosgene contains yet incompletely chlorinated by-products of higher volatility and reactivity, as it would be suggested by the mortality patterns observed. The armamentarium of currently available analytical techniques needs to be improved to better characterize both triphosgene per se as well its putative, yet ill-defined more volatile by-products. Notably, the triphosgene used in this study contained off-gassing component(s) reactive with the 2-HMP scavenger which caused a high initial concentration at the commencing of study. However, when using preconditioned triphosgene (purging of test material with nitrogen during one dummy exposure, without animals when used for the first time) more stable airborne concentrations could be attained. It cannot be excluded that these off-gas by-products would have increased the acute lethal toxic potency of triphosgene.

Suffice it to say, the attainment of the thermodynamic equilibrium may not readily attained from a solid substance used under laboratory-scale conditions. However, in larger containers transported and shipped, headspace concentrations in the lethal range are likely to occur. Compared to phosgene, triphosgene appears to be more difficult to handle due to its tendency to sublimate and off-gassing by-products which appears to be particularly critical in the absence of any specific analytical method. Moreover, the scrubbing methods to detoxify/decompose phosgene in these studies appeared to be least efficacious for triphosgene (vapour break through even when using three gas scrubbers containing aqueous solutions of sodium hydroxide in line).

It seems reasonable to conclude that each phosgene congener appears to act as an independent chemical entity and not necessarily phosgene-like. Although difficult to trace back in detail, it appears as if the readily available triphosgene has often been

used as a substitute of phosgene in inhalation toxicity and intervention studies. This new evidence calls for a redirection of this approach.

In summary, the toxic mode of action of triphosgene is distinctly different to that of phosgene. Repeated inhalation exposure studies are needed to judge the contribution of airway irritation relative to pulmonary irritation by using better methodologies characterizing the different injury patterns within the respiratory tract. It seems timely to consider closing the substantial data gaps of triphosgene and not to 'bridge' or 'read across' its putative toxicity to phosgene.

6. Mechanistic studies: research on intervention strategies

The activity of nucleophiles to alleviate effects of phosgene exposure was investigated in animals by Pauluhn and Hai (2011). This study utilised the inhalation route for administration of three different nucleophilic drugs after an exact 90 sec phosgene exposure.

The results show that strong nucleophiles both with and without antioxidant properties cannot readily neutralise effects of phosgene or reconstitute acylated proteins or peptides, even when the phosgene exposures were very short and treatment with the nucleophiles commenced instantly after the cessation of exposure to phosgene.

This supports the conclusion that phosgene acts via a direct and instant pathway of acylation of nucleophilic components. It also underscores the protective nature of constitutive nucleophiles in the lining fluids of the lung. In this context, the rat appears to be a conservative animal model as humans have higher levels of potentially scavenging proteins/peptides in their lung lining fluids than rats (Slade et al., 1993). However, as known from previous single, high-level inhalation studies, the ensuing acute lung injury is rapidly repaired and long-term sequelae to high-level exposures do not occur (Pauluhn, 2006b).

The nucleophilic aerosolised compounds were “dried” in the inhalation chamber following dispersion due to the use of dry air. Therefore, exposure was to a dry powder aerosol and not necessarily an aqueous aerosol. This study demonstrates that the susceptibility of the pulmonary region of the lung to the drug aerosols was already elevated shortly after exposure as evidenced by elevated BAL-protein in drug-treated animals. Rats not pre-exposed with phosgene tolerated a similar exposure without any evidence of adversity. This corroborates the notion that injury appears to be fixed concomitant to the exposure to phosgene. Treatment regimens commencing as short as possible after the exposure to phosgene, despite the absence of any clinical evidence of lung damage, are likely to be successful against the secondary responses to the original injury, which are not fully evolved yet.

As antioxidants have been shown to be effective in mitigating phosgene-induced lung injury (Ji et al., 2010, Wang et al., 2010), these countermeasures appear to operate on the secondary response to injury rather than primary pathomechanisms.

In summary, the pathology of oedema formation appears to be dependent on multiple factors secondary to initial injury. Therefore, it appears unlikely that in severe lung injury the use of single therapy strategies will be sufficient to address all facets involved in the resolution of injury (Berthiaume, 1998; Berthiaume et al., 1999; 2002; Matthay et al., 2002a, b).

Other mitigating factors, such as the reduction of oxidative stress or anti-inflammatory drugs, might provide additional protection to maintain and restore the functional capacity of the alveolar epithelium to remove excessive fluid from the alveolar space. These issues have been thoroughly investigated in multiple animal models (Berthiaume et al., 1999; van Helden, 2004; Lailey et al., 1991; Sciuto und Hurt, 2004).

More research is needed to understand the complex cycle caused by the secondary responses to injury in regard to

- the pulmonary inflammatory response and oedema,
- the associated neurogenic changes in microvascular control leading to lung oedema (Ivanhoe & Meyers, 1964; Chen, 1995),

- overflow of lymphatic drainage (Parker et al., 1981)
- cardiovascular-neural dysregulation associated with a translocation of blood from the systemic to the pulmonary circulation (Allison, 1991; Benoit et al., 1985; Sarnoff et al., 1953).

In regard to pulmonary inflammation, redox-active nucleophilic pro-dugs of GSH (N-acetylcysteine) have been shown to be efficient to attenuate phosgene-induced acute lung injury through the down-regulation of oxidative stress (Ji et al., 2010, Wang et al., 2010; Chen et al., 2009).

Further details of specialised rationales for treatment are included in section 5.3.2 ‘Treatment Strategies’.

7. Research on exposure mitigation strategies 'the ammonia curtain'

Young adult male Wistar rats, a species commonly used in inhalation toxicity studies and OF1 mice, a species often used in sensory irritation studies, were simultaneously nose-only exposed for 45 min to ammonia in concentrations from 92 to 1243 mg/m³.

This study examined airway reflexes by the changes in respiratory patterns elicited by ammonia in either dry, steam humidified (approx. 95% rel. humidity) or aqueous aerosol containing atmospheres. This served the objective to explore whether high concentrations of anhydrous ammonia and/or high humidity and aqueous aerosol change the predominant nasal deposition site to more distal locations in the lung.

Animals from all groups tolerated the exposure without evidence of respiratory tract irritation, changes in body and lung weights. The evoked changes on breathing patterns resembled those known to occur following exposure to 'upper respiratory tract sensory irritants', rapid in onset and reversibility. Reflex stimulation from the lower airways was not observed in any group. While mice showed some adaption during the 45 min exposure period, rats displayed more stable changes in respiratory patterns. In this species humidity- or aqueous aerosol-related changes in sensory irritation potency did not occur to any appreciable extent. The respiratory decrease 50%, RD50, was 972 and 905 mg/m³ in dry and wet air, respectively. In contrast, mice appeared to more susceptible to ammonia in presence of dry air (the RD50, was 582 and 732 mg/m³ in dry and wet air, respectively). Further details have been published elsewhere (Li and Pauluhn, 2010).

For risk assessment this and other studies in larger species (Coon et al., 1970) have been used, because smaller rodent species are less tolerant to ammonia than the more human-like oronasally breathing larger animal species. Ammonia is a direct acting irritant gas acting at the point of initial contact. Such a deposition pattern does not call for any species-specific adjustments for differences in dosimetry nor does local or systemic metabolism play any role.

For this highly water soluble gas, it has been demonstrated that the primary site of deposition is the upper respiratory tract (nasal passages), including eyes, for non-lethal endpoints. A break though of ammonia into the lower airways did not occur in the 45-min rodent sensory irritation study up to 1243 mg/m³ x 45 min or 8-hour/day repeated inhalation study up to 770 mg/m³ from Coon et al. (1970). Due to a lack of experimental data at higher exposure levels, as a precautionary principle, this concentration is considered to be the dose-rate related ceiling for any exposure duration. An interspecies assessment factor of 1 is also supported by the REACH R.8 for direct local effects. At non-lethal concentrations, injury at the deposition site of ammonia is restricted to the proximal, directly exposed nasal airways, which also supports an interspecies assessment factor of 1.

In summary, it was shown that the sensory irritation potency of ammonia does not increase when inhaling wet atmospheres, nor does this gas penetrate into the lower airways up to 1243 mg/m³ x 45-min. Hence, at those concentrations of ammonia present under controlled phosgene mitigation measures, no toxicological interaction of phosgene (lower respiratory tract toxicant) and ammonia (upper respiratory tract toxicant) is likely to occur.

Accordingly, the following maximum concentrations are considered to be implicitly conservative 770, 507 and 359 mg/m³, respectively. These concentrations are equivalent to approximately 1100 ppm (10 min), 724 ppm (30 min) and 513 ppm (60 min). This derivation is supported by the experienced-based RAM TRAC corporation

recommendation of 696 and 492 ppm for 30 and 60 min, respectively (Michaels, 1998).

Collectively, for the specific and equally exceptional case ‘mitigation measures by controlled discharge of ammonia (ammonia curtain)’ the following (rounded) approximate ceiling levels are deemed adequate to prevent irreversible effects to occur in a general, informed subpopulation of the general public in the circumference of a chemical plant: 1000 ppm (10 min), 700 ppm (30 min) and 500 ppm (60 min).

Suffice it to say, all concentrations exceed the LDSA. Hence, the subpopulation involved need to be informed of these readily perceptible events causing potentially mild, but distinct ocular/nasal irritation at yet innocuous exposure levels of ammonia. For exposures exceeding 1 hour the AEGL (2007) levels should be observed.

8. Discussion and interpretation of toxicological evidence

The results from recent acute inhalation toxicity studies in rats with phosgene have been detailed elsewhere (NRC, 2002; Pauluhn, 2006a, b, c). In acute studies it has been shown that rats experience an instant change in respiratory patterns when exposed to phosgene. The observed effects were considered typical for the stimulatory effects on pulmonary receptors (Pauluhn, 2006a).

The transient decrease in ventilation was thought to be the cause of the departure from Haber's rule at short, high-concentration exposures. Moreover, the stimulation of sensory bronchopulmonary defence reflexes is known to modulate airway mucosal inflammation (Lee & Widdicombe, 2001) and may be an important aspect to interpret changes in the mucosal permeability observed in rats following exposure to irritant substances (Persson et al., 1996).

In this context, dogs are considered to be more human-like and have extensively been used in context of inhalation toxicity studies with phosgene (NRC, 2002; Cucinell, 1974; Okonek et al., 1987). Also the lavage procedure per se appears to be somewhat closer to that used in humans. The lobar lavage applied in dogs minimises the confounding contribution of secretions from the airways as compared with total lung lavage technique applied in rats.

Beagle dogs are often the species of choice as an experimental model the study of pulmonary responses and play a key role in the toxicological characterisation of inhalation pharmaceuticals (DeGeorge et al., 1997). Their lungs bear a reasonable resemblance to human lungs (Heyder & Takenaka, 1996; Kreyling et al., 1999). The structural and functional characteristic of their respiratory system is well documented (Heyder & Takenaka, 1996; Takenaka et al., 1996; 1998). Briefly, submucosal glands are observed throughout the bronchial tree of beagles.

In contrast to human and dog lungs, bronchial glands are not found in small rodents. At the level of the terminal bronchioles, the lining cells of beagle dogs are mainly nonciliated (Clara cells) whereas ciliated cells are rare. In the acinus (composed of airways distal to the terminal bronchiole) of beagle lungs, several generations of alveolated bronchioles (i.e. respiratory bronchioles) exist; human acini have similar morphology.

However, the lungs of small rodents have either no respiratory bronchioles or, at most, one generation (Heyder & Takenaka, 1996). Also the number of alveolar pores, important for collateral ventilation, is similar in a canine and a human alveolus, but smaller in a rat alveolus. The acinar region is the main target site for irritant agents causing inflammation of the lower airways, such as phosgene (Kodavanti et al., 1997). Hence, these interspecies differences in acinar morphology are important and must be considered when comparing pulmonary responses to inhaled phosgene across species. In contrast to most of the published dog inhalation studies with phosgene (for overview see NRC, 2002), the study from Pauluhn (2006c) utilised the same head-only mode of exposure and experimental methodologies as used in previous rat inhalation studies (Pauluhn, 2006a,b).

This dog study compared concentration x exposure time (C x t) relationships of inhaled phosgene in dogs over a single exposure period of 30 minutes. The exposure regimen used in this dog inhalation study matched that used in previous acute inhalation studies with phosgene gas in rats (Pauluhn, 2006a,b).

Emphasis was directed toward the assessment whether the wide range of extravasated plasma protein concentrations observed in the bronchoalveolar lavage fluid of rats following exposure to phosgene, can also be duplicated in dogs, a

species with breathing patterns, airway reflexes and an acinary morphology more similar to humans. In his context it is important to recall, that in rats the extravasated protein in BAL fluid has been shown to increase up to ~100-times the control without causing mortality (Hatch et al., 2001), whilst in morbid patients with acute respiratory distress syndrome (ARDS) a ~30-fold increase was lethal (Pittet et al., 1997).

Therefore, it is subject to speculation whether the effect-magnitude observed in rats is solely related to the irritant-related disturbance/damage of the blood-air barrier system, or whether it is superimposed by rodent-specific neurogenic, reflexively-induced factors that contribute additionally to the magnitude of changes. Opposite to humans, the rodent-specific mucosal defence system might favour a reflexively-induced secretion of proteins into the lumen of airways (Persson et al., 1996).

Alternatively, also differences the lavage procedures might be causative for the marked differences observed (Pauluhn, 2006c; Pauluhn and Hai, 2001). In rodents the entire lung up the carina is lavaged and proteinaceous secretions from airways may contribute markedly to the total protein and inflammatory cells detected in BAL. In contrast, in this dog study only one isolated lobe was lavaged. Although difficult to identify unequivocally the relative contribution of adaptively increased airway secretions from an adverse disturbance of the alveolar blood-air barrier, it is conceived that some of these uncertainties can be analysed in dogs in a more predictive manner for humans than can be done using rats.

The mean respiratory minute volume of dogs was approximately 0.4 L/(min x kg bw) was not at variance from measurements reported by other authors (Bide et al., 2000). Thus, the average ventilation of dogs is 2-3 times less than that of rats. The average ventilation of dogs was about twice as high as reported for man (0.22 L/(min x kg bw); Bide et al., 2000). From these measurements it is also surmised that beagle dogs appear to have different neural reflex mechanisms as compared to rats. Collectively, the comparison of indicators of acute lung injury in BAL total protein support a respiratory minute volume-dependent degree of pulmonary damage which recapitulates the conclusions drawn by other authors (Mautone et al., 1985). These authors showed that mongrel dogs exposed for 20 min to 94 ppm (7708 mg/m³ x min) experienced a marked shallow breathing pattern and apO2 values decreased from 79 (pre-exposure) to 60.5 mmHg (post exposure), however, marked differences in exposure technology makes more quantitative comparisons difficult.

The preponderance of studies with phosgene using specific indicators in bronchoalveolar lavage to define the extent of acute pulmonary injury, demonstrated that the concentration of protein in BAL fluid was amongst the most sensitive endpoints to probe for alterations of the blood-air barrier (Pauluhn, 2006b). Other authors have also suggested that the measurement of protein in BAL fluid is a better indicator of the presence of increased epithelial permeability in the lung than the counts of polymorphonuclear leukocytes (Pittet et al., 1997). This study suggests further that the quantification of phosgene-induced acute pulmonary injury is more aptly addressed by measurements of changes of BAL protein, an indicator integrating the increased exudation of proteins into alveoli and airways, than histopathology or arterial blood gases.

The relationship of the C x t dependent increases of protein in BAL fluid relative to the concurrent control over a wide range of concentrations and exposure durations from rat inhalation studies is given in Pauluhn (2006c) and Pauluhn, Hai (2011). C x t products were from 30 to 360 min exposures. Shorter exposure durations were omitted due to reflexively-induced changes in ventilation observed at higher concentrations in rats (Pauluhn, 2006a). The magnitude of the phosgene-induced increase of protein in BAL fluid was approximately 10-times less pronounced in dogs when compared to rats receiving identical C x t products.

For detailed information see figure 8 in Pauluhn (2006c). That figure shows concentration x time dependence of total protein in bronchoalveolar lavage fluid protein (obtained from the lobus accessorius) in head-only exposed dogs to phosgene, whole-body exposed Fischer 344 rats (Hatch et al., 2001) or nose-only exposed Wistar rats (Pauluhn, 2006b) to various concentrations of phosgene bracketing exposure durations from 30 to 360 minutes. Rats and dogs were sacrificed approximately 24 hours following exposure.

In Pauluhn and Hai (2011) figure 1 gives similar BAL-protein relationships as presented in figure 8 of Pauluhn (2006c). Mortality data were from rat inhalation studies utilising exposure durations from 30 to 360 minutes (Pauluhn, 2006a). The C x t-relationship of this intervention study was close to the non-lethal threshold C x t (900 mg/m³ x min).

As hypothesized above, it may be that the constituents in the airway mucous might contribute fairly significantly to the extracellular material washed out by BAL in rats and that rats may be different from and dogs (or humans) in the percentage contributed by airway fluid vs. alveolar fluid (Hatch, 1992).

The slopes of the C x t – effect curves from rats and dogs were essentially identical. The interpolated points of departures for protein in BAL, based on changes exceeding the background of control rats by 50% (95th percentile) were 117 and 375 mg/m³ x min for rats and dogs, respectively (background variability not calculated for dogs because of the small number of samples).

Based on this comparative analysis, rats displayed an approximately three times higher susceptibility to elaborate phosgene-induced increases in BAL protein than dogs.

Also the occurrence of pulmonary responses, suggestive of mild oedema and inflammation at 495 mg/m³ x min in dogs (Table 3), compares favourably with similar observations in humans, at >600 mg/m³ x min (NRC, 2002; Diller and Zante, 1982). Moreover, the compilation of toxicological indices of phosgene in Table 1 supports the conclusion that also the chronic outcome of repeated exposures to phosgene is apparently contingent upon acute irritation-related rather than cumulative effects.

Table 3: *Summary of effect levels in rats and dogs exposed to phosgene gas*

C x t [mg/m³xmin]	Regimen & Effect	Reference
4200	LCt_{50} in dogs; 1 x 20-min exposure, no experimental details	Cucinell et al. (1974)
1741	LCt_{50};1 x 10, 30, 60 and 240 min nose-only exposure of rats	Pauluhn (2006a)
1476	1 x 6-hour whole-body exposure of rats to 4.1 mg/m³; BAL-protein ≈100-times control; no mortality	Hatch et al. (2001)
1476	1 x 6-hour/week whole-body exposure of rats to 4.1 mg/m³ for 4 and 12 weeks; no mortality	Kodavanti et al. (1997)
1075	LCt_{01};1 x 10, 30, 60 and 240 min nose-only exposure of rats	Pauluhn (2006a)
1050	1 x 0.5-hour head-only exposure of dogs; BAL-protein 10 to 15-times control; fibrinous inflammation, oedema, no mortality	Pauluhn (2006c)
1008	1 x 4-hour nose-only exposure of rats to 4.1 mg/m³; BAL-protein ≈70-times control; no mortality, no evidence of irreversible effects during a follow-up period of 4 weeks	Pauluhn (2006a)
495	1 x 0.5-hour head-only exposure of dogs; BAL-protein ≈2-times control; minimal inflammation at the level of the terminal bronchioles	Pauluhn (2006c)
375	1 x 0.5-hour head-only exposure of dogs; estimated point of departure of BAL protein	Pauluhn (2006c)
270	1 x 0.5-hour head-only exposure of dogs; NOAEC	Pauluhn (2006c)
164	Compilation of 1 x 0.5 to 6-hour nose-only/whole-body exposures of rats ; BAL-protein 3-times the levels of controls	Pauluhn (2006b), Hatch et al. (2001)
148	1 x 6-hour/day; 5 times/week whole-body exposure of rats for 4 and 12 weeks to 0.41 mg/m³; minimal histopathological changes in terminal bronchioles	Kodavanti et al. 1997)
117	Compilation of 1 x 0.5 to 6-hour nose-only/whole-body exposures of rats; estimated point of departure of BAL protein	Pauluhn (2006b), Hatch et al. (2001)

Based on the comparison of the phosgene-induced changes of protein in BAL fluid from dogs one may conclude that similar changes in rats must be at least three times higher to be of pathophysiological significance.

Furthermore, the comparison of BAL-protein with mortality data (Pauluhn, Hai 2011) shows that 'point of departure' estimations based on minimal increases in BAL-protein are implicitly conservative in regard to mortality (implicit safety factor based on the more susceptible rat approximately 3x10).

9. References

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10. Abbreviations and acronyms

AEGL	Acute Exposure Guideline Level (US)
ALI	acute lung injury
ARDS	Acute Respiratory Distress Syndrome
BAL	Bronchoalveolar Lavage
BAR	A unit of pressure which is about the same as atmospheric pressure (1 bar = 14.054 psi)
°C	Degree Celsius
C x t	Dose = Concentration x time (mg/m3 x min)
DFG	German Research Foundation (**D**eutsche **F**orschungs**g**emeinschaft)
EU	European
FT-IR	Fourier Transform Infrared Spectroscopy
GC	Gas chromatograph
GLP	Good Laboratory Procedures
g/mol	molecular weight in gram per mol
GSH	Glutathione
HCl	Hydrogen chloride
HMP	Hydroxymethylpiperidine
HTM	hexamethylenetetraamine
IOELV	Indicative Occupational Exposure Limit Values
kPa	kilopascal = 10^3 Pa = 10^{-2} bar
LC	Lethal Concentration
LC01	Non-lethal Threshold Limit; Lethal Concentration, 1% Lethality
LC50	Medium Threshold Limit; Lethal Concentration, 50% Lethality
LCt01	Dose at Non-lethal Threshold Limit; Lethal Concentration, 1% Lethality
LCt50	Dose at Medium Threshold Limit; Lethal Concentration, 50% Lethality
LDSA	Level of Distinct Sensory Awareness
MAC	Maximum Allowable Concentrations
MAK	German MAC (**M**aximale **A**rbeitsplatz**k**onzentrationen
mg/m3	concentration in milligram per cubic meter
ml	millilitre
mmHg	pressure in millimeters of mercury
mmol	mili-mole
Mol	mole, the SI unit for an amount of substance
MPa	mega-pascal = 10^6 Pa = 10 bar
N2	Nitrogen
NAC	N - acetylcysteine
NOAEC	No Observed Adverse Effect Concentration
O2	Oxygen
OECD	Organisation for Economic Co-operation and Development
OSHA	Occupational Safety and Health Administration
Pa	the SI derived unit of pressure. 1 Pa = 10^{-5} bar
pH	Measure of the acidity of a solution (potential of Hydrogen)
PPM	Parts per Million
PPM-MIN	dose in PPM times Minutes
PSI	Pounds per Square Inch
RD50	50% response dose
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals a European Union Regulation addressing the production and use of chemical substances,

SaO2	Oxygen saturation
SCOEL	Scientific Committee on Occupational Exposure Limits
TLC	Total Lung Capacity
μm	micrometre = one-millionth of a metre