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Acute inhalation toxicity of ammonia: Revisiting the importance of RD_{50} and $LCT_{01/50}$ relationships for setting emergency response guideline values

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ABSTRACT

This study examined the acute median lethal concentration (LC_{50}) and the non-lethal threshold concentration (LC_{01}) of ammonia in male and female Wistar rats nose-only exposed at exposure durations of either 1 or 4 h. Additional attributes characterizing the acute toxicity of inhaled ammonia were determined during a post-exposure period of 2 weeks. The objective of this study is to further refine the methodology applied to derive Emergency Response Planning Guideline (ERPG) values on potent sensory irritants in a controlled rat bioassay. In the more susceptible male rats the 1- and 4-h LC_{50} (LC_{01}) were 12,303 (10,067) and 4923 (4028) mg/m^3, respectively. At sublethal exposure levels the ventilation of rats was about one third of normal breathing. This change in ventilation and inhalation dosimetry was adjusted for Cxt-dependent lethal endpoints whereas sensory irritation-related phenomena were *C*-dependently adjusted. In summary, the outcome of this study shows that *C*- and $C \times t$-dependent causes of toxicity need to be appreciated when extrapolating across species with species-specific inhalation dosimetry. It also appears to be indispensable that each exposure metric must be disentangled when translating $C \times t$-dependent lethality and reflexively-induced, sensation-based *C*-dependent point of departures. For one hour exposure periods, these PODs were derived to be 1500 and 500 ppm, respectively.

1. Introduction

Ammonia is a colorless gas at ambient temperatures and pressures with a characteristic sharp, irritating, pungent odor that increases awareness acting as a warning signal of potentially dangerous exposure. Such phenotypes of 'sensory irritation' occur when inhaled chemicals interact with free endings of somatosensory nerves located in the eyes and nose. The afferents are mediated by the trigeminal nerve (Bryant and Silver, 2000; Doty and Cometto-Muñiz, 2003). Ammonia is a naturally occurring substance that plays a vital role in protein metabolism. The endogenous production of ammonia in the intestine has been reported to be 4.2 g/man/day. This endogenous production might cause background levels up to 2 mg ammonia/m^3 in the exhaled air of humans (DFG, 1986). In man it also is an important component of the balanced acid–base, electrolyte system. The toxicity of ammonia has been reviewed in great detail elsewhere (AEGL, 2007; DFG, 1986; IPCS, 1986, 1990). Ammonia is a high production volume chemical with many industrial applications. It is also used for the remediation of gaseous emissions of highly toxic reactive chemical intermediates and may deliberately be expelled under controlled conditions as a component of the 'ammonia steam curtain' to chemically neutralize accidental spills of phosgene gas (IPCS, 1998).

Previously published acute inhalation toxicity studies on 45-min directed-flow nose-only exposed mice and rats in concentrations up to 1243 mg ammonia/m^3 (1740 ppm) in either dry air, steam-humidified air (approx. 95% rel. humidity) or as aqueous aerosol tolerated the exposure without evidence of macroscopic evidence of respiratory tract irritation, changes in body and lung weights (Li and Pauluhn, 2010). The evoked changes on breathing patterns resembled those known to occur following exposure to '*upper respiratory tract sensory irritants*', rapid both in onset and reversibility. Time-course changes were characterized by diminution (adaptation) rather than progression. Reflex stimulation from the lower airways (i.e., frank apnea) was not observed up to the maximum concentration examined. In rats, the respiratory decrease 50%, RD_{50}, was 1361 and 1267 ppm in dry and wet air, respectively (Li and Pauluhn, 2010). Thus, the sensory irritation patterns of inhaled ammonia do not change to any appreciable extent when inhaling ammonia as gas or dissolved in an aqueous aerosol. These data were extended further by acute directed-flow nose-only rat inhalation studies of 1- and 4-h duration (this paper) to evaluate at which

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exposure concentration × time (C × t) relationship any breakthrough of ammonia gas into the lower airways would occur. Typical findings of such a pattern of airway injury is expected to be associated with laryngitis, tracheobronchitis with airflow obstruction, bronchiectasis, and mortality more delayed in onset. Functionally, typical apnea periods as a result of alveolar nociceptor stimulation are also expected to occur at lethal exposure levels.

The available information on concentration-dependent effects (sensory irritation and RD_{50}) and $C \times t$-dependent lethality (time-adjusted median lethal concentration, LCt_{50}, and non-lethal threshold concentration, LCt_{01}) are believed to be of paramount importance for the translation of findings from rats to humans. In this context it is important to recall that mask (mouth/nose)-exposed humans exposed for 30-min to 500 ppm ammonia, a concentration reported to be permissible for more than half to one hour, elaborated an increased respiratory response (up to 2.5-fold increased hyperventilation) to inhaled ammonia with the time required to attain steady-state from 10 to 27 min (Silverman et al., 1949). At this exposure condition some subjects showed a reflexively-induced lacrimation (eyes not directly exposed) in the absence of coughing (no apparent stimulation of laryngeal sensory nerves). The latter was reported to occur at 1000 ppm (Silverman et al., 1949) with more severe effects (laryngospasms and edema of glottis) at 1700 ppm (DFG, 1986; Silverman et al. (1949) conclude that when the rise in concentration of ammonia in the absorbing lining fluids/surfaces of the upper respiratory tract were becoming more saturated with this gas, the retained percentage decreased to 20%. Consequently, the deduction that inspired ammonia would go progressively deeper into the respiratory tract was not borne out by the symptoms observed. Presumably the inspired ammonia continued to be removed by the upper passages, which then returned progressively larger amounts to expired air as it passed over these surfaces, consistent with a wash-in wash-out phenomenon. Collectively, the most significant effects were on respiration and subjective reactions and 500 ppm was considered to be physiologically undesirable but rapidly reversible.

This evidence in man demonstrates that rodent bioassays need to be designed and interpreted with caution. The concentration-dependent efferent sensory irritation-related "subjective" endpoints can be modeled best by the analysis of respiratory patterns concomitant to exposure (Li and Pauluhn, 2010). These common trigeminal afferents trigger rodent-specific efferents with substantial hypoventilation, changes in acid–base status, and hypothermia. Due to their small thermal inertia, unlike humans, small laboratory rodents undergo a typical hypothermic response when exposed to this type of respiratory tract irritants. Consequently, the ten-fold lower body weights makes mice even more thermolabile than rats (Gordon et al., 1988; Gordon, 1993; Pauluhn and Mohr, 2000). Hence, mice appear to be the least suitable species to extrapolate from to man because of their extreme hypothermic response and associated physiological response when exposed to sensory irritants (Gordon et al., 2008) superimposed by the alkalinity of ammonia. With regard to the integrated injury, the hypoventilation in rats and the opposite hyperventilation in man, require a thoughtful and likewise complex derivation of assessment factors to extrapolate empirical data from rats to humans keeping in mind that some of the rodent-specific findings have no direct counterpart in humans. The purpose of this paper is to devise a strategy to achieve this objective.

2. Methods

2.1. Test material

Anhydrous ammonia (NH_3, ammonia 3.8; contained in a 2 L cylinder) was from Linde, Düsseldorf, Germany. The purity was specified to be ⩾99.98%. The following default factors were used for mass to volumetric conversions: 1 mg/m^3 = 1.4 ppmV; 1 ppmV = 0.7 mg/m^3.

2.2. Animals, diet, and housing conditions

Healthy male and female SPF-bred Wistar rats of the strain Hsd Cpb:WU from the experimental animal breeder Harlan-Nederland (NL), AD Horst, were used. Animals were placed in polycarbonate cages containing bedding material. Both feed and water were given *ad libitum* except during inhalation exposures. At the commencement of study, the average body weights were 180 (±15) g. Animal rooms were maintained at approximately 22 °C with relative humidity of 40%–60% and a 12-h light cycle beginning at 0600 h. The studies described were in accordance with contemporary, internationally harmonized testing standards/guidelines (OECD, 2009a,b, Available at: http://oberon.sourceoecd.org) and EG Guideline 92/69/EWG which also detail the animal welfare aspects to be applied to this type of study. The experiments were performed in an animal care-approved laboratory in accordance with the German Animal Welfare Act and European Council Directive 86/609/EEC (1986) as well the updated Directive 2010/63/EU as of 22 September 2010. All procedures applied were GLP (Good Laboratory Practice) compliant (OECD, 1997).

2.3. Experimental design

Previous acute inhalation studies on rats (small whole body chamber, exposure durations from 10 to 60 min, analytical characterization of test atmospheres by acidimetric titration) demonstrated that male rats were markedly more susceptible to ammonia gas than female rats (Appelman et al., 1982). These authors reported a 60-min LC_{50} of 16,600 ppm which differed appreciably from the 60-min LC_{50} of 7338 ppm published by MacEwen and Vernot (1972). In many of the past studies only one sex was used. The difference of study outcomes was discussed controversially in expert panels due to the absence of any recently published inhalation study complying with more recent testing guidelines OECD (2009a,b). This study attempts to reconcile this controversy in using a rigorously validated directed-flow nose-only exposure system (Pauluhn, 1994; Pauluhn and Thiel, 2007) equipped with real-time analytical procedures (FTIR) and physical atmosphere characterization (humidity, temperature) to overcome the shortcomings of past studies. The higher susceptibility of male rats to this irritant gas is not uncommon in this bioassay (Pauluhn, 1993). This study utilized both sexes to verify/refute the findings from Appelman et al. (1982). The mathematical procedures applied where adopted from these authors (Appelman et al., 1982; ten-Berge, 1986; Zwart and Woutersen, 1988; Zwart et al., 1990). Exposure concentrations were related to those reported by Appelman et al. (1982) with the objective to minimize the number of experimental animals.

This study examined the concentration × exposure time ($C \times t$) relationships of anhydrous ammonia gas in rats for the acute median lethal concentration (LC_{50}) and non-lethal threshold concentrations (LC_{01}), i.e., the highest calculated level that does not cause lethality. An ancillary group of rats was exposed to examine the maximum depression in ventilation occurring at subthreshold lethal exposure levels. At the end of the acclimatization period of at least 5 days, which included also acclimatization to the exposure restrainers, rats were randomly assigned to the respective exposure groups, each consisting of 5 rats/sex/group. Exposure duration were 60 and 240 min on day 0, followed by a post-exposure period of 2 weeks. The male rats of the ancillary lung function group were sacrificed on the first post-exposure day. Body weights were recorded before exposure, on days 3, 7, and 14. Rectal temperatures