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RESEARCH ARTICLE

Measurement of various respiratory dynamics parameters following acute inhalational exposure to soman vapor in conscious rats

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Abstract

Respiratory dynamics were investigated in head-out plethysmography chambers following inhalational exposure to soman in untreated, non-anesthetized rats. A multipass saturator cell was used to generate 520, 560 and 600 mg × min/m^3 of soman vapor in a customized inhalational exposure system. Various respiratory dynamic parameters were collected from male Sprague-Dawley rats (300–350 g) during (20 min) and 24 h (10 min) after inhalational exposure. Signs of CWNA-induced cholinergic crisis were observed in all soman-exposed animals. Percentage body weight loss and lung edema were observed in all soman-exposed animals, with significant increases in both at 24 h following exposure to 600 mg × min/m^3. Exposure to soman resulted in increases in respiratory frequency (RF) in animals exposed to 560 and 600 mg × min/m^3 with significant increases following exposure to 560 mg × min/m^3 at 24 h. No significant alterations in inspiratory time (IT) or expiratory time (ET) were observed in soman-exposed animals 24 h post-exposure. Prominent increases in tidal volume (TV) and minute volume (MV) were observed at 24 h post-exposure in animals exposed to 600 mg × min/m^3. Peak inspiratory (PIF) and expiratory flow (PEF) followed similar patterns and increased 24 h post-exposure to 600 mg × min/m^3 of soman. Results demonstrate that inhalational exposure to 600 mg × min/m^3 soman produces notable alterations in various respiratory dynamic parameters at 24 h. The following multitude of physiological changes in respiratory dynamics highlights the need to develop countermeasures that protect against respiratory toxicity and lung injury.

Keywords

Head-out exposure, inhalation exposure, nerve agents, plethysmography, pulmonary function, respiratory toxicology, soman

History

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Introduction

Soman (pinacolyl methyl phosphonofluoridate, or GD) is considered to be one of the most toxic chemical warfare nerve agents (CWNAs) because it permanently deactivates acetylcholinesterase (AChE) complexes within minutes, resulting in accumulation of the neurotransmitter acetylcholine (ACh) in the synaptic cleft or neuromuscular junction. Overproduction of ACh at synapses results in prolonged stimulation of muscarinic and nicotinic receptors, leading to hypersecretions, convulsions, seizures, bronchoconstriction, respiratory depression and mortality. The rapid ''aging'' of AChE by soman results in the inability of available treatment regimens to reactivate the irreversibly inhibited AChE enzyme complexes that are formed (Shafferman et al., 1997; Worek et al., 2004). Immediate, aggressive therapeutic intervention following soman intoxication is critical (Talbot et al., 1988; Shafferman et al., 1997).

The various functions and structures of the respiratory system provide inhaled toxic materials with direct access to blood circulation and peripheral tissues. Inhalational exposures have been widely considered the primary exposure routes for the more volatile CWNAs (Greenfield et al., 2002; Niven & Roop, 2004; Sidell, 1996). The relatively high vapor pressure and ease of vaporization or aerosolization of soman make it a potential threat agent for inhalational exposure. It has been shown that variations in the rate of absorption and non-critical-site binding following intravenous, subcutaneous, intraperitoneal and intratracheal administration of 30 μg/kg of soman to anesthetized guinea pigs produce differences in times to ventilatory failure (Franz & Hilaski, 1990). Vaporized soman (13.14 mg/kg) and sarin (30 mg/kg) exposure in the upper airway of baboons resulted in persistent lung injury, increased lung resistance, decreased dynamic compliance and slow expirations (Anzueto et al., 1990). The nature of soman-induced toxicological effects on respiratory physiology and function following inhalational exposure needs to be investigated further and may be used as a potential biomarker for inhalational exposure to chemical threat agents.

We have previously developed a head-out vapor-generating inhalational exposure system for conscious Sprague-Dawley rats (Wong et al., 2013). Advantages of head-only inhalational

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exposure models include the reduction of material usage, the minimization of skin and fur contamination, the ability to perform repeated brief exposures, and the ability to limit the pathways of agent or material entry into the subject (Phalen, 1976; Wong, 2007). This model involves the generation of nerve agent vapor by utilizing a saturator cell, wherein vapor is introduced into a custom-made glass exposure chamber to rats restrained in individual head-out plethysmography chambers. The exposure system is capable of acquiring respiratory parameters in real-time, before, during and after agent exposure. Studies by our lab have used this inhalation methodology for organophosphates and CWNA exposure to conscious rats for the assessment of respiratory toxicity, lung injury and respiratory dynamics and to evaluate therapeutic countermeasures (Perkins et al., 2013; Wong et al., 2013). Classical cholinergic and respiratory-induced toxicity following inhalational exposure to organophosphates and soman was observed in the following model using untreated and non-anesthetized animals. In this exposure model, the LCt_{50} of vaporized soman in rats was previously determined by probit analysis to be 593 mg × min/m^3 (Perkins et al., 2013). Studies using this model to evaluate the effects of CWNAs on respiratory dynamics may provide additional information on the respiratory toxicity following inhalational exposure to soman.

Historically, real-time collection of respiratory function parameters following inhalational exposure to CWNAs has been largely overlooked. The objective of this study is to assess respiratory dynamics in conscious, untreated rats following inhalational exposure to soman. Body weight loss, an indication of toxicity; pulmonary edema, determined by wet/dry ratio and real-time collection of various respiratory dynamic parameters within a head-out plethysmography chamber were used to demonstrate respiratory toxicity following inhalational exposure to soman. Additionally, no pre- or post-exposure treatments were provided in order to investigate a more realistic scenario of changes in respiratory dynamics following soman-induced respiratory toxicity. This study will provide valuable information on the toxicological effects of inhalational exposure to soman on respiratory dynamics as well as additional information that may be useful for the establishment of respiratory diagnostic measures and potential therapeutic countermeasures.

Materials and methods

Animals

Adult male Sprague-Dawley rats (300–350 g, Charles River Laboratories, Wilmington, MA) served as subjects. Animals were housed individually under standard conditions with a 12-h light/dark cycle, and food and water were available *ad libitum*. Rats were acclimated for 1 week prior to the exposure chamber. All rats were acclimated to the exposure chamber daily for a total of 7 days prior to exposure to optimize the habituation process and reduce novel environmental accommodation. Animals that failed to acclimate to the exposure chambers were removed from the study. The study protocol was approved by the Institutional Animal Care and Use Committee, USAMRICD, Aberdeen Proving Ground, Maryland. A total of 24 surviving rats [six vehicle control (perfluorohexane or PFH) rats, six rats for each 520, 560 and 600 mg × min/m^3 of soman] were used to evaluate respiratory parameters up to 24 h post-exposure for this study.

Chemicals

Soman, obtained as a stock solution (98.8% pure) from the US Army Edgewood Chemical Biological Center, Aberdeen Proving Ground, ML was diluted in PFH (to 1.45–1.57 mg/ml), and kept at −80 °C until use. PFH (99% pure) was obtained from Sigma-Aldrich (St. Louis, MO). Xylazine and ketamine were purchased from Webster Veterinary Supplies (Devens, MA). PFH, which permitted the use of less concentrated agent, was used in the inhalation studies to establish the soman LCt_{50} and resulted in no observed signs of toxicity (Perkins et al., 2013). Exposure to soman was performed in an approved fume hood with the appropriate personal protective equipment for the handling of CWNAs, as set forth by the US Army. At the end of all vapor exposures, any remaining soman and contaminated materials were decontaminated with 10% sodium hydroxide prior to disposal.

Vapor inhalational model configuration and vapor generation

This study used a recently developed head-out vapor-generated model for control vehicle and soman inhalational exposure of unanesthetized, untreated rats (Wong et al., 2013). Soman vapor was generated in a multipass glass saturator cell as described previously (Muse et al., 2006). The saturator cell was placed in-line with a nitrogen gas flow-over source to dilute and carry the control vehicle or dilute soman. The saturator cell was filled (5–6 mL) with soman (1.45–1.5 mg/mL) with or without PFH, and filtered air was used to push the vapor mixture into the customized 11 L glass exposure chamber. The exposure chamber consisted of an inlet port connected to a mixing tube, an outlet port and two slots for placement of the head-out plethysmography chambers (Figure 1). A 0–15 standard liters per minute (SLPM) flow controller (Brooks, Hatfield, PA) was used to regulate the carrier gas nitrogen (0–5 SLPM) entering into the saturator cell and a second 0–30 SLPM flow controller (Brooks, Hatfield, PA) was used to regulate exhaust and pull air (7.5 SLPM) through the mixing tube. The duration of exposure time and the inlet carrier gas flow were regulated to achieve soman exposure doses within this exposure system (Perkins et al., 2013). The air exiting the exposure chamber was decontaminated in a 10% sodium hydroxide solution and filtered through a charcoal filter connected directly to a vacuum pump.

Head-out inhalation exposure procedure

Age-matched, conscious, restrained Sprague-Dawley rats were placed into a single plethysmography chamber (600-2100-001, Data Sciences International, St. Paul, MN) and exposed to control or soman vapor (Perkins et al., 2013). The exposure groups consisted of animals that survived to 24 h after exposure to one of three doses of soman (520, 560 and 600 mg × min/m^3). Changes in chamber pressure, caused by

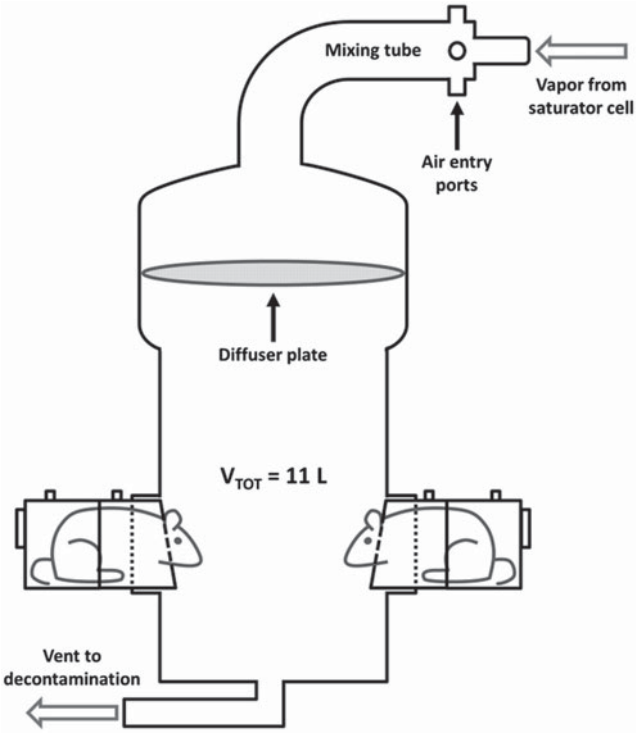

Figure 1. Illustration of the head-out inhalation exposure chamber. The custom glass chamber (V = 11 L) consists of two slots for placement of conscious rats restrained within head-out plethysmography chambers. Ambient air mixes with vapor generated from a saturator cell to dilute the soman or vehicle control vapor, which exits at the bottom of the chamber.

chest movement experienced during breathing while inside the plethysmography chamber, were detected by the pressure transducer and used to monitor various parameters of respiratory function. All respiratory data were collected by the 7700 Platform and Ponemah Performance Analysis software (data acquisition hardware: ACQ7700XE, software: Ponemah v.4.9, Data Sciences International, Valley View, OH). Immediately before exposure, all rats were allowed to acclimate to the exposure chamber for 10 min. Respiratory parameter readings were then recorded for each animal before exposure (10 min), during exposure (20 min) and during off-gassing (10 min). Data collection occurred at 15-s intervals. The Ponemah Performance Analysis software utilized a small rejection index to exclude external noise. The respiratory dynamics parameters that were measured were respiratory frequency (RF); peak inspiratory flow (PIF) and peak expiratory flow (PEF), the maximum inspiratory and maximum expiratory flow rates occurring in one breath; inspiratory (IT) and expiratory (ET) time, the times spent inhaling and exhaling during each breath, respectively; tidal volume (TV), the volume of air inspired in a single breath; and minute volume (MV), the product of tidal volume and respiratory rate calculated on a breath-by-breath basis.

At the conclusion of the off-gassing period, surviving animals were returned to their cages and observed for 24 h. Animals were weighed prior to agent exposure and again at 24 h post-exposure to calculate the percentage of body weight loss. All animals were then returned to the head-out plethysmography chambers for 10 min to record 24-h post-exposure respiratory parameters. Prior to euthanasia, animals were deeply anesthetized using an intramuscular (i.m.) injection of ketamine (90 mg/kg) in combination with xylazine (10 mg/kg). The thoracic cavity was opened and exsanguination performed via cardiac puncture. During tissue harvesting, the left lung lobe was tied off using surgical string, then harvested, weighed and dried at 100 °C for 7 days to determine the wet/dry weight ratio.

Data analysis

Statistical analyses of the differences between the vehicle control and soman-exposed animals at 24 h post-exposure were performed using the GraphPad Prism V5.04 software (Graph Pad Software Inc., San Diego, CA). Volume-based respiratory parameters (TV, MV, PIF and PEF) for each exposure dose were first normalized to the time-matched body weight for each individual animal. All respiratory parameters were then normalized to the average of their pre-exposure values. For each of the respiratory parameters at each of the three time points (pre-exposure, exposure and 24 h post-exposure), one factor analysis of variance followed by Turkey's multiple comparison test was used to determine the differences between groups. Probability (p) and p values ≤ 0.05 were considered statistically significant.

Results

Effects of vapor inhalational exposure to soman

In exposed animals, all three doses (520, 560 and 600 mg × min/m^3) of soman showed signs of CWNA-induced cholinergic crisis characterized by licking, chewing, salivation, lacrimation and increased urination. Mild-to-severe tremors and convulsion were observed in animals exposed to 560 and 600 mg × min/m^3. No signs of toxicity were observed in vehicle-exposed animals. Exposure of animals to 520, 560 and 600 mg × min/m^3 resulted in 100, 75 and 50% survival at 24 h post-exposure. The respiratory parameters of a total of six animals that survived 24 h post-exposure were evaluated for each of the soman doses and the control-exposed animals. Mortality following inhalational exposure to 560 and 600 mg × min/m^3 of soman was observed 2–3 min post-exposure; therefore, the following study only evaluated the respiratory parameters of animals that survived the 24-h post-exposure period. No mortality was observed in animals exposed to 520 mg × min/m^3 of soman. Severe convulsions and copious oronasal secretions were observed in all of the animals that did not survive soman exposure.

Body weight loss and lung edema

Average percentage of body weight loss was 1.5, 5.1 and 9.5% in animals exposed to 520, 560 and 600 mg × min/m^3 of soman, respectively, as compared to 0.6% in control animals (Figure 2A). Animals exposed to 520 and 560 mg × min/m^3 had a lower body weight at 24 h post-exposure, but the loss was not significant. Significant losses in body weight were observed in animals exposed to 600 mg × min/m^3 of soman in comparison to the control group. Alterations in lung edema,

measured as wet/dry weight ratio of the left lung lobe, were not drastic in animals exposed to 520 mg × min/m^3 of soman in comparison to controls (Figure 2B). However, animals exposed to 560 and 600 mg × min/m^3 of soman experienced a significant increase in lung edema.

Head-out plethysmography chamber signal following exposure to soman

A representative portion of recordings of several breaths before, during, and 24 h after exposure using a head-out plethysmography chamber in control and 600 mg × min/m^3 soman-exposed animals is shown in Figure 3. Breathing patterns in control animals remained comparable during all collection time points. Irregular breathing patterns of animals exposed to 560 and 600 mg × min/m^3 of soman were observed during and 24 h after exposure in comparison to control.

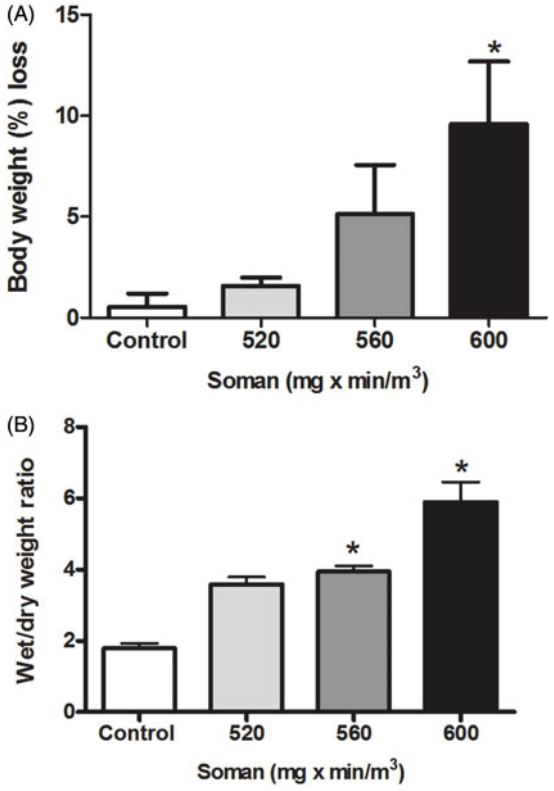

Figure 2. Body weight loss and lung edema. (A) Percentage body weight loss. There was a significant increase in animals exposed to 600 mg × min/m^3 of soman in comparison to the control group. (B) Lung lobe edema. Lung edema increased in all soman-exposed animals, with a significant increase in animals exposed to 560 and 600 mg × min/m^3 of soman in comparison to the control group. Control ($n = 6$), 520 mg × min/m^3 ($n = 6$), 560 mg × min/m^3 ($n = 6$) and 600 mg × min/m^3 soman ($n = 6$). Asterisk indicates statistical significance ($p < 0.05$ when compared to control) between control and exposure groups.

Effects of soman inhalation on RF, TV and MV

The RF, TV and MV before, during and 24 h after exposure to soman in surviving animals and in vehicle controls are shown in Figure 4(A–C). There was an increase in RF for all soman-exposed animals and a significant increase ($p < 0.05$ when compared to control) at 24 h in animals exposed to 560 mg × min/m^3 of soman. During exposure, TV increased in all soman-exposed animals in comparison to control-exposed animals. At 24 h, TV significantly increased in animals exposed to 600 mg × min/m^3 of soman. Exposure to 560 and 600 mg × min/m^3 of soman resulted in significant increases in MV at 24 h post-exposure in comparison to controls. No significant increases in RF, TV and MV were observed in soman-exposed animals during the exposure period as compared to control-exposed animals.

IT and ET following inhalational exposure to soman

The effects of soman inhalation on IT and ET in surviving animals before, during and 24 h after exposure are shown in Figure 5(A and B). There were no significant changes in IT or ET during and 24 h after exposure to soman in comparison to the control group. However, comparison of animals exposed to 520, 560 or 600 mg × min/m^3 of soman during the exposure period resulted in decreases (49, 41 and 30%) at the 24-h post-exposure period in IT in comparison to the control groups. The ET in animals exposed to 600 mg × min/m^3 of soman decreased at 24 h in comparison to controls, but the decrease was not significant.

Effects of soman inhalation on PIF and PEF

The PIF and PEF before, during and 24 h after exposure to soman and vehicle controls in surviving animals are shown in Figure 6(A and B). There was a significant increase in PIF at

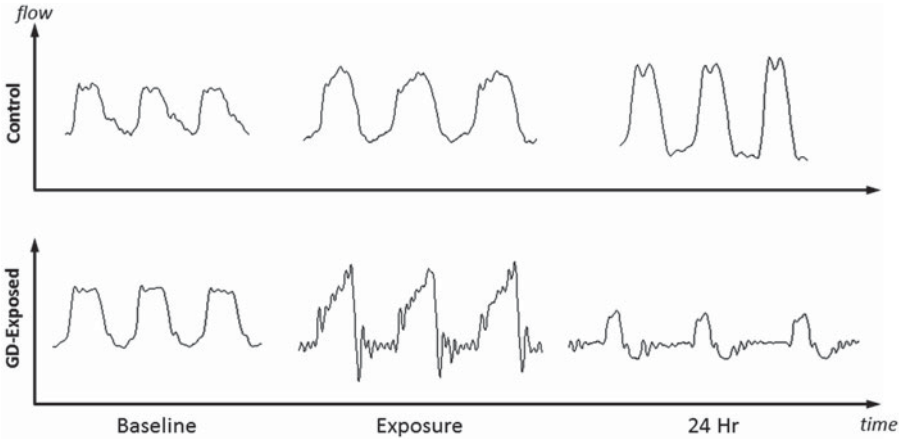

Figure 3. Plethysmography signals following exposure to soman or vehicle controls. Representative segment of several breaths before, during and 24 h after exposure using a head-out plethysmography chamber in control and soman-exposed (600 mg × min/m^3) animals. The *x*-axis is defined as time (seconds) and the *y*-axis is flow (milliliters per minute).

24 h post-exposure in animals exposed to 600 mg × min/m^3 of soman in comparison to the control group (Figure 6A). At 24 h, PEF increased in all soman-exposed animals, with a significant increase in animals exposed to 600 mg × min/m^3 of soman (Figure 6B). No significant increases in PIF and PEF were observed in soman-exposed animals in comparison to control animals during agent exposure.

Discussion

These results indicate that inhalational exposure to soman vapor in our head-out inhalational model causes significant changes in various parameters of respiratory dynamics at 24 h post-exposure. We previously reported the respiratory toxicity, lung injury and a preliminary assessment of inhaled bronchodilators and steroid therapy following inhalational exposure to soman (Perkins et al., 2013). A majority of the observed changes in respiratory dynamics recorded in conscious male rats by head-out plethysmography were dependent on the agent concentration and time, in that we observed more prominent alterations in respiratory dynamics in animals 24 h post-exposure to 600 mg × min/m^3 of soman. Similar to this study, higher doses of the organophosphate paraoxon resulted in significant alterations in various respiratory dynamic parameters when compared to lower doses (Villa et al., 2007). In addition to the evaluation of respiratory dynamic parameters, various clinical observations were recorded (data not included). Animals exposed to 600 mg × min/m^3 of soman exhibited more signs of cholinergic intoxication, such as licking, chewing, salivation, lacrimation, facial clonus, straub tail and increases in defecation and urination, in comparison to animals exposed to 520 and 560 mg × min/m^3 of soman, as previously reported (Perkins et al., 2013). Only animals exposed to 560 and 600 mg × min/m^3 of soman exhibited convulsions, suggesting a direct effect on the central nervous system (CNS). Several studies have indicated that inhalational exposure to CWNAs induces a combination of neurological and respiratory toxicities (Gupta et al., 1987; Phalen, 1976; Taysse et al., 2006). Severe and sustained convulsions were observed in one rat exposed to 560 mg × min/m^3 and in two rats exposed to 600 mg × min/m^3 of soman until euthanasia at 24 h post-exposure. Surviving rats that exhibited severe convulsions had prominent alterations in the evaluated respiratory dynamics parameters. The control, vehicle-exposed group showed no observed signs of intoxication or alteration in respiratory dynamics. Exposure to the vehicle control PFH during the inhalation exposure period did result in changes in the various parameters in comparison to the pre-exposure period in which animals were only exposed to room air. PFH has been used for partial liquid ventilation and has been shown to improve lung function and to partially alleviate acute respiratory distress syndrome (Bleyl et al., 2002; Hirschl et al., 1996). Control animals exposed to PFH in this and previous studies showed no gross abnormalities or signs of toxicity (Perkins et al., 2013).

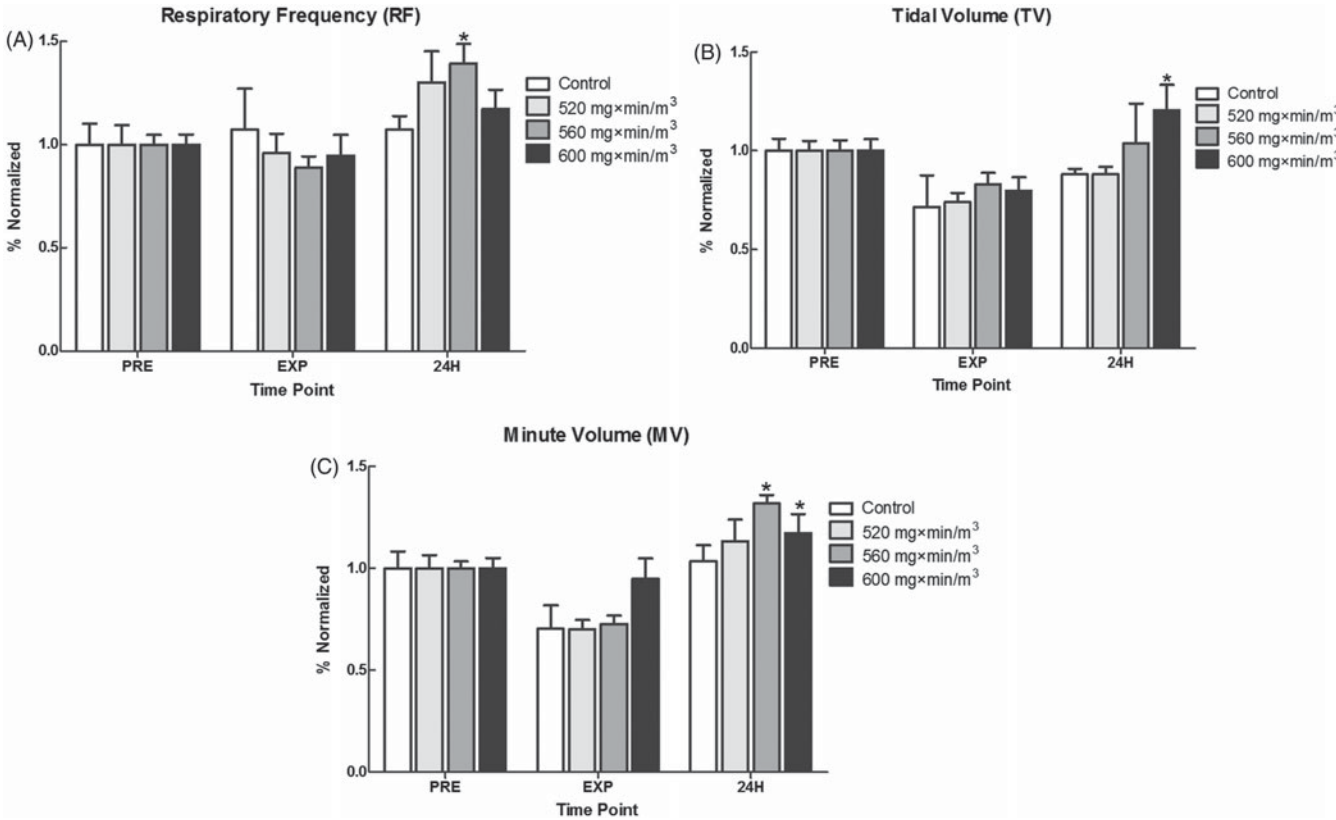

Figure 4. RF, TV and MV following exposure to soman. The RF, TV and MV were measured in animals ($n = 6$) before, during and 24 h after exposure to soman. (A) RF. There was a significant increase in RF at 24 h post-exposure in animals exposed to 560 mg × min/m^3 of soman. (B) TV. At 24 h there was a significant increase in TV in animal exposed to 600 mg × min/m^3 of soman. (C) MV. Exposure to 560 and 600 mg × min/m^3 resulted in significant increases in MV at 24 h post-exposure in comparison to controls. Asterisk indicates statistical significance ($p < 0.05$ when compared to control) between control and exposure groups.

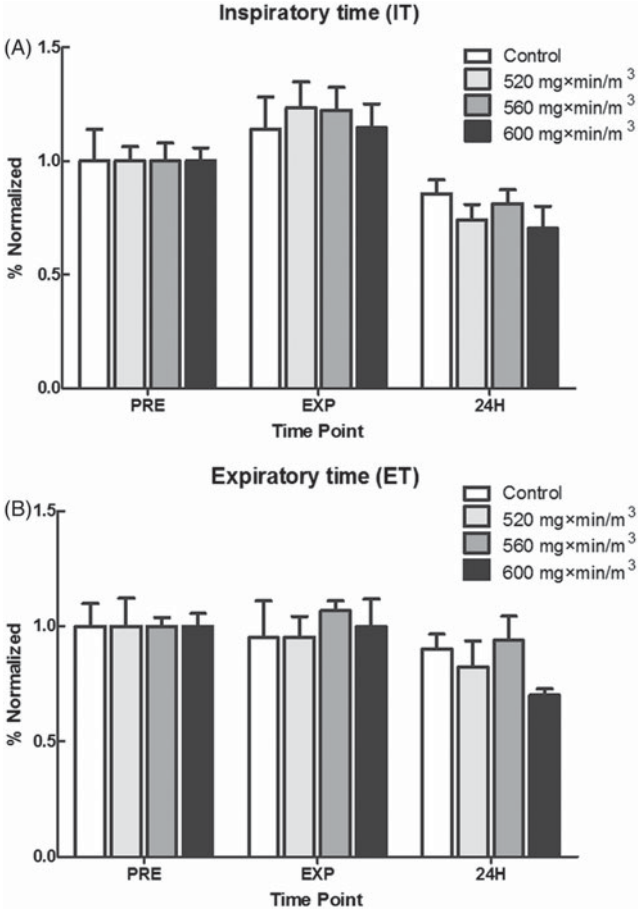

Figure 5. Effects of soman inhalation on IT and ET. (A) IT. There were no significant changes during and 24 h after exposure to soman in comparison to the control group. (B) ET. There were no significant changes during and 24 h after exposure to soman in comparison to the control group. Although the IT of animals exposed to 600 mg × min/m^3 of soman decreased in comparison to controls, it was statistically insignificant. Control ($n = 6$), 520 mg × min/m^3 ($n = 6$), 560 mg × min/m^3 ($n = 6$) and 600 mg × min/m^3 soman ($n = 6$). Asterisk indicates statistical significance ($p < 0.05$ when compared to control) between control and exposure groups.

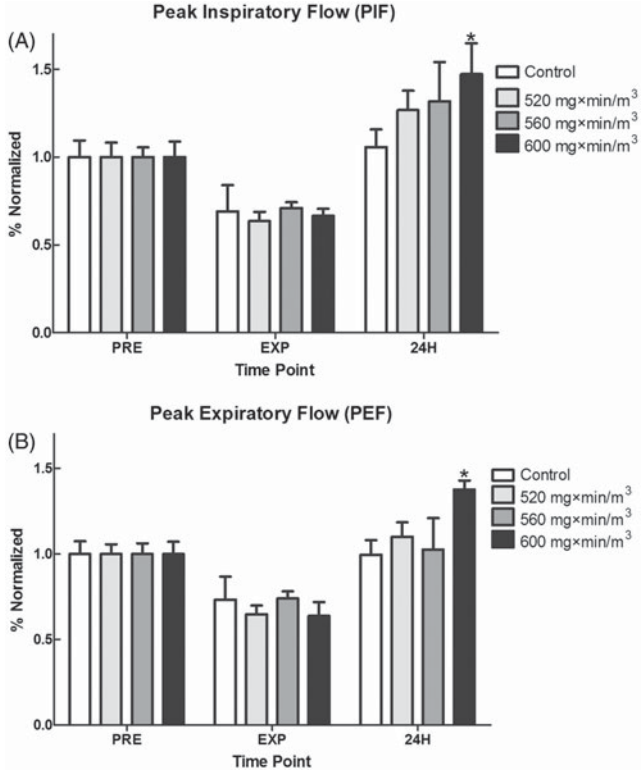

Figure 6. PIF and PEF following exposure to soman. (A) PIF. There was a significant increase in PIF in animals exposed to 600 mg × min/m^3 of soman in comparison to the control group. (B) PEF. At 24 h the PEF increased in all somanexposed animals, with a significant increase in animals exposed to 600 mg × min/m^3 of soman. Asterisk indicates statistical significance ($p < 0.05$ when compared to control) between control and exposure groups.

Rats exposed to 600 mg × min/m^3 of soman experienced significant body weight loss, an indication of acute CWNA-induced toxicity. Prominent body weight loss at 24 h post-exposure has also been observed in anesthetized guinea pigs exposed via endotracheal aerosolization to 561 and 841 mg × min/m^3 of soman (Perkins et al., 2010). Surviving animals exposed to 9750, 10 950, 12 200 and 14 625 mg × min/m^3 of diisopropyl fluorophosphate, or DFP, averaged 9.4% body weight loss (Wong et al., 2013). Dose-dependent, significant increases in wet:dry ratio are an indication of edema and may be a result of increased permeability and air-blood barrier disruption. A possible limitation in the evaluation of wet:dry weight edema is that the recorded edema may be a direct effect of the induction of copious secretion in the respiratory tract and lung injury. This trend was observed at 24 h post-exposure in animals exposed to 600 mg × min/m^3 of soman, and similar increases in edema have been reported in other soman exposure experiments and in those experiments using VX, *O*-ethyl S-(2-(diisopropylamino)ethyl) methylphosphonothioate and organophosphates (Peng et al., 2014; Perkins et al., 2010; Wong et al., 2013; Wright et al., 2006). The edema observed in all soman-exposed animals may directly affect the respiratory parameters evaluated in this study. Inhalation exposure to the organophosphate parathion resulted in airway obstruction and pulmonary edema in guinea pigs, which the author suggested was due to increased thickening of the tracheobronchial walls (Segura et al., 1999). Pulmonary edema, as a result of increases in transcapillary filtration and permeability of the pulmonary microvascular endothelial barriers of the lungs, has been reported in acute and chronic respiratory toxicity (Parker & Townsley, 2004). Additional studies need to be conducted to determine if inhalational exposure to soman results in chronic pulmonary edema, which would require an alternative therapeutic regimen. Administration of therapies that open up the airways and relax the smooth muscle tissues of the respiratory tract may improve respiratory function and decrease or lessen the onset of respiratory toxicity and lung injury. Acute organophosphate poisoning in rodents has been reported to involve sequential CNS respiratory disturbance-induced apnea and impairment of pulmonary gas exchange with prominent airway secretions (Gaspari & Paydarfar, 2007). Therapeutic countermeasures that reduce the production of copious secretions in the oral nasal cavity may open the airway and increase oxygen exchange, improve respiration, and enhance survival following exposure to CWNAs. Respiratory failure and neuropathology are the mechanisms of CWNA toxicity, but the extent of their contribution to mortality is unclear (Taysse et al., 2006). Possibly, a portion of the soman vapor

directly enters the brain through the olfactory neuroepithelium lining of the nasal cavity, inducing neurotoxicity and resulting in alterations in respiratory function. The ability of small molecules to bypass the blood-brain barrier and directly enter the brain through the nasal cavity and olfactory neuroepithelium has been previously observed in rats (Chow et al., 1999; Einer-Jensen & Larsen, 2000). AChE inhibition by CWNAs in the various regions of the brain, especially the pons and medulla oblongata, resulted in respiratory disturbances (Bajgar et al., 2004; Kassa and Bajgar, 1998; Shih et al., 2005). Preliminary evaluation of the histological changes in various brain regions following inhalational exposure to soman vapor using this exposure model suggests that exposure to $600\,mg \times min/m^3$ induces severe brain injury and that this model can be a useful tool for evaluating treatment regimens following CWNA-induced neurotoxicity.

Alterations in respiratory parameters measured at 24 h after inhalational soman exposure varied between the dose groups. Animals exposed to $520\,mg \times min/m^3$ of soman showed no significant alterations in any of the collected respiratory parameters, while animals exposed to $560\,mg \times min/m^3$ of soman only showed significant increases in RF and MV. However, prominent alterations in MV, TV, PIF and PEF were observed in animals exposed to $600\,mg \times min/m^3$ of soman, strongly suggesting that high doses of soman are required to produce significant changes in respiratory function at 24 h post-exposure. It has been reported that lower doses of soman act primarily through CNS respiratory effects and that higher doses involve both central and peripheral nervous system respiratory effects (Worek & Szinicz, 1993). Similar increases in RF, TV, MV, PIF and PEF were observed in anesthetized guinea pigs exposed to lethal doses of soman by microinstillation (Perkins et al., 2011). Inhalational exposure to the non-volatile CWNA VX also resulted in significant variations in several respiratory dynamic parameters in guinea pigs at 24 h post-exposure; these returned to normal within 7 days (Rezk et al., 2007). Alterations in respiratory dynamics observed in both volatile and non-volatile CWNAs further suggest the effect that cholinergic-induced intoxication has on the respiratory system. Subcutaneous administration of $2 \times LD50$ soman increased MV, TV and RF at early time points in guinea pigs (Taysse et al., 2006). Further studies on the respiratory dynamics following inhalational exposure to soman that focus on measurements at additional time points may provide useful information on the respiratory toxicity associated with inhalational exposure to CWNAs.

We previously reported that inhalational exposure to lethal doses of the organophosphate DFP resulted in significant alterations in MV, TV, ET, IT, PEF and PIF during the exposure period (Wong et al., 2013). However, at 24 h post-exposure, inhalational exposure to $12\,200\,mg \times min/m^3$ of DFP only resulted in significant alterations in PEF. All of the respiratory parameters were first normalized to their pre-exposure values and then to their body weight, as a result of the documented effect of body weight on respiratory parameters (Alexander et al., 2008; Bide et al., 2000; Guyton, 1947). As compared to soman, during inhalational exposure to DFP, a higher intensity in the classical signs of organophosphate-induced toxicity was observed, resulting in more acute alterations in respiratory dynamics during that time period. However, the lack of sustained alterations in respiratory dynamics at 24 h in DFP-exposed animals, compared to soman-exposed animals at 24 h post-exposure, indicates a strong agent-dependent effect on both the intensity and persistence of toxicity. Collectively, these studies further suggest that organophosphates or pesticides, such as DFP, may not be suitable alternatives for evaluating CWNA-induced toxicity as there are statistically and physiologically significant differences in the intensity and time-dependent development of respiratory toxicity.

The objective of this study was to utilize a recently developed head-out vapor inhalation exposure system to evaluate changes in respiratory dynamics following inhalational exposure to the CWNA soman. Loss of body weight, an indication of toxicity, and pulmonary edema, determined by wet/dry ratio, and real-time collection of various respiratory dynamic parameters within a head-out plethysmography chamber were used to demonstrate toxicity and alterations in respiratory dynamics following inhalational exposure to soman. Additionally, no pre- or post-exposure treatments such as atropine and/or oximes were administered, thus providing a more realistic examination of respiratory dynamics following soman-induced respiratory toxicity. The evaluation of multiple respiratory dynamic parameters has been considered to be extremely useful in determining the physiological impact that a toxicant has on the respiratory system (Costa, 1985). The collective evaluation of respiratory dynamics has also been used to determine the underlying pathology associated with inhalational exposure to various toxic chemicals (Costa et al., 1986; Hoymann, 2007; Mauderly, 1984). In summary, we have shown useful data that characterize the toxicological effects of inhalational exposure to soman on the respiratory dynamics of conscious rats. Extensive assessment of the various toxicological effects following exposure to CWNA will be fundamental to the scientific challenge of determining possible biomarkers of toxicity and mitigating toxic effects through therapeutic countermeasures.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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