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Acute toxicity when concentration varies with time: A case study with carbon monoxide inhalation by rats

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ABSTRACT

Exposure to time-varying concentrations of toxic compounds is the norm in both occupational settings and daily human life, but little has been done to investigate the impact of variations in concentration on toxic outcomes; this case study with carbon monoxide helps fill that gap. Median acute lethality of 10-, 20-, 40-, and 60-min continuous exposures of rats to carbon monoxide was well described by the toxic load model ($k = C^n \times t$; k is constant, C = test concentration, n = toxic load exponent, and t = exposure duration) with n = 1.74. Dose response-relationships for 1-h exposures including a recovery period between 10- or 20-min pulses showed greater similarity (in both median lethality and steepness of dose-response curve) to continuous exposures with equivalent pulse duration and concentration, rather than a 60-min exposure with equivalent time-weighted average concentrations or toxic load. When pulses were of unequal concentration (3:1 ratio), only the high concentration pulse contributed to lethality. These findings show that fluctuations or interruptions in exposure over a short time scale (60 min or less) can have a substantial impact on outcomes (when n > 1), and thus high-resolution monitoring data are needed to aid interpretation of resulting outcomes.

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1. Introduction

Exposure to time-varying concentrations of toxic compounds is the norm in both occupational settings and daily human life, in contrast to the almost exclusive use of constant concentration exposures in toxicity assessment. Surprisingly little has been done to systematically investigate the impact of variations in concentration vs. time on toxic outcomes using experimental approaches, though some theoretical aspects have received attention (Kaplan, 2009; Saltzman, 1996; Rhomberg, 2009; Pauluhn, 2015).

The toxic load model (ten Berge equation, that is, $k = C^n \times t$, where k is constant, C is the test concentration, n is the chemical-specific toxic load exponent, and t is the exposure duration) (ten Berge et al., 1986; Ride, 1995; Sommerville et al., 2006) is used to estimate consequences from exposure of humans to toxic materials. Examples include prediction of casualties in situations where civilians are exposed to toxic industrial chemicals, or military personnel are exposed to chemical warfare agents (Sommerville et al., 2010; Sommerville, 2016; Department of Defense, 2005; National Research Council, 2001; Young et al., 2009). The toxic load models may use results of animal studies to make the estimates. Animal inhalation studies are typically conducted so that animals are exposed to a known, steady concentration of the compound of concern for a defined period of time. The validity of the toxic load models for extrapolation from constant exposures to time-varying exposures is being tested by comparing outcomes from rat studies with standard (constant) profiles with outcomes from studies where rats are exposed to time varying concentrations. In a previous series of experiments using hydrogen cyanide (HCN), we concluded that as long as continuous exposures lasted 5 min or longer, the toxic load model was consistent with the outcomes of variable-concentration exposures with and without

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gaps (brief [30% of total duration] periods with no exposure) (Sweeney et al., 2014, 2015). No definite conclusions could be made about the toxic load model concerning shorter exposures (less than 5 min) due to probable breath-holding on the part of the rats. In both the previous and current efforts in this laboratory, the aims were not so much to understand a specific test chemical due to concerns about that chemical. Rather, the aim was to better understand how toxicity related to constant-concentration exposures can be used to predict the consequences of the myriad possible non-constant exposures experienced in the real world. In the current investigation, efforts were made to assess the concordance between previous findings and those for a different compound (carbon monoxide; CO), and to test the importance of the duration of the gap. To that end, acute lethality studies were conducted where male Sprague Dawley rats were exposed to CO by inhalation with various concentration vs. time profiles, including longer gaps (33–67% of total duration), as compared to the previous HCN studies (30% of total duration). It is expected that the results of this study of CO will be relevant to the modeling of other industrial chemicals and chemical warfare agents.

2. Materials and methods

2.1. Selection of test chemical

As noted above, the current experiments and analyses build off of previous efforts in which HCN was used as the test chemical (Sweeney et al., 2014, 2015). The current effort was a departure from the previous efforts in that a different test chemical was used. By using a different chemical, limits on how widely applicable the previous findings could potentially be discerned, or the findings reinforced. The selection of CO as a test chemical was motivated by many of the same considerations that led to the selection of HCN for our previous case studies. Specifically, it was expedient to select a chemical, species (rat), and endpoint (lethality) for which the toxic load exponent (n) was known to differ from 1. The best estimate of n for CO lethality in rats was 1.73 based on analysis of available data for 5–60 min exposures of Sprague Dawley rats to CO (Darmer et al., 1972; Lapin, 1981; Hartzell et al., 1985). Various subsets of the data (e.g., only freely moving animals, only head-only exposures; exposures at a single laboratory) yielded toxic load exponents ranging from 1.4 to 3.9, lending confidence that the toxic load exponent in a lethality study would exceed 1. While both HCN and CO are considered asphyxiant toxicants, with their toxicity under simultaneous exposure likely to be additive (National Research Council, 2008) their mechanisms of action are believed to differ. The toxicity of CO is ascribed primarily to its binding to blood (preventing the binding and transport of oxygen to tissues), whereas HCN does not have an affinity for hemoglobin, but rather inhibits oxygen utilization via interactions with mitochondrial cytochrome oxidase. Furthermore, while both substances are rapidly cleared when exposure ceases, the means by which this occurs differs between compounds. While CO clearance is dependent on exhalation, HCN is cleared via metabolism to thiocyanate, which is then excreted in urine (Hartzell et al., 1985; National Research Council, 2008).

Additional considerations for the selection of CO as a test chemical were the ability to readily and consistently achieve the target exposure concentrations and animal welfare concerns. The fast-acting nature of CO (deaths were expected to occur during or shortly after exposure, per Lapin, 1981, Levin et al., 1987) would be anticipated to minimize unnecessary pain/distress for the exposed rats.

2.2. Overview of experimental design

Laboratory rats were exposed to a breathing atmosphere containing CO using a custom-built nose-only exposure system (described below). Male Sprague Dawley rats were selected as the test strain and species due to the availability of the database of acute CO studies covering a range of exposure durations in this sex and strain (e.g., Darmer et al., 1972; Lapin, 1981; Hartzell et al., 1985). The size of the database was expected to reduce the likelihood of needing to conduct multiple range-finding exposures to attain the desired level of mortality, and thus minimize animal use.

The animals were exposed under concentration-time scenarios selected to allow the following factors to be assessed: constant concentration exposure vs. variable concentration exposure (i.e., two pulses), the ordering of the height of two pulses or unequal concentration, intermittent vs. continuous exposure, and the duration of the gap between the two pulses. Four baseline (conventional, constant) profiles and six non-constant profiles were chosen to permit the assessment of the factors. The baseline profiles of 10, 20, 40, or 60 min exposure to a constant concentration of CO (Profiles 1–4) were selected to cover the range from the shortest pulse duration to the total exposure duration in the non-constant profiles. Non-constant profiles lasted one hour and had two pulses of equal duration, with or without a gap. Total exposure duration and gap durations were selected based on the reported CO elimination half-life (as carboxyhemoglobin) of 23 min in rats (Andersen et al., 1991). While recognizing that half-lives may depend on concentration, the degree to which steady-state was attained prior to cessation of exposure, ventilation rate, and other factors, the 20- and 40- min gaps covered 33–67% of the exposure duration and would allow for clearance of ~50% or 75% of the accumulated body burden prior to the second pulse. The rats exposed under these profiles would therefore exhibit partial, but incomplete, recovery (from a pharmacokinetic standpoint) prior to the second pulse. Profiles 5 and 6 consisted of two 30-min pulses with a 3-fold difference in concentration; in Profile 5, the high-concentration pulse occurred first, followed immediately by the low concentration pulse, while in Profile 6, the order was reversed. Profiles 7 and 8 were also mirror images, consisting of two 10-min pulses with a 40-min gap; as in Profiles 5 and 6, the pulse concentration ratio was 3:1, with the high concentration first in Profile 7 and last in Profile 8. Profiles 9 and 10 consisted of two 20-min pulses with a 20-min gap. In Profile 9, the initial pulse had a concentration 3-fold higher than the second pulse, whereas in Profile 10, the pulses were of equal concentration. Each profile was run multiple times at various concentrations (Profiles 1–4) or pairs of concentrations (5–10) in order to characterize the full dose-response range (discussed in greater detail below, in Section 2.7). The C vs. t time courses for the computed median lethal concentrations (LC_{50}) associated with these profiles are depicted in Fig. 1.

2.3. Animal exposures and monitoring

The animal protocol was approved by Wright-Patterson Air Force Base's Institutional Animal Care and Use Committee and by the United States Air Force Surgeon General's office, in compliance with all applicable Federal regulations governing the protection of animals in research. Six hundred male Sprague Dawley (*Rattus norvegicus*) rats (obtained from Charles River Laboratories, Raleigh, NC) were used to obtain a comprehensive understanding of the lethality of CO using various C vs. t profiles. Animals were delivered at 35–42 days old to allow adequate time for quarantine prior to exposure. Due to the time span over which the exposures were conducted, animals were ordered in batches so all animals were less than 10 days of age from each other at time of exposure.