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Evaluating the validity and applicable domain of the toxic load model: Impact of concentration vs. time profile on inhalation lethality of hydrogen cyanide

Lisa M. Sweeney [a,*], Douglas R. Sommerville [b], Stephen R. Channel [c], Brian C. Sharits [d], Nathan M. Gargas [d], Chester P. Gut Jr. [d]

[a] Henry M. Jackson Foundation for the Advancement of Military Medicine, Naval Medical Research Unit Dayton, Wright Patterson Air Force Base, OH 45433, United States
[b] U.S. Army Edgewood Chemical Biological Center, Aberdeen Proving Ground, MD 21010, United States
[c] Leidos, Linton, IN 47441, United States
[d] CAMRIS, Naval Medical Research Unit Dayton, Wright Patterson Air Force Base, OH 45433, United States

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ABSTRACT

The ten Berge model (or "toxic load" model) is often used to estimate the acute toxicity for varying combinations of inhaled concentration and duration. Expressed as $C^n \times t$ = toxic load (TL), TLs are assumed constant for various combinations of concentration (C) and time (t). Experimental data in a recent acute inhalation study of rats exposed to time-varying concentrations of hydrogen cyanide (HCN) supported the validity of the toxic load model except under very brief, discontinuous, high concentration exposures. In the present investigation, experiments were conducted to extend the evaluation of the applicable domain of the model for acute lethality of HCN in the rat (cumulative exposure range of 2900–11,000 ppm min). The lethality of HCN over very short (<5 min) durations of high concentrations did not conform to the toxic load model. A value of $n = 1.57$ was determined for uninterrupted exposures $\geqslant$5 min. For 30-min exposures, the presence or absence of a gap between two exposure pulses of different concentrations, the relative duration, relative height, and the ordering of the pulses (low then high, vs. high then low) did not appear to have a meaningful impact on the toxic load required for median lethality.

1. Introduction

To address the need to estimate the acute toxicity of vapors and gases of potentially exposed individuals for varying combinations of concentration and duration, the ten Berge model (ten Berge and van Heemst, 1983; ten Berge et al., 1986), also known as the toxic load model (Ride, 1995; Sommerville et al., 2006), is often used. The ten Berge/toxic load model is expressed as $C^n \times t = k$, where "n", the toxic load exponent, and "k", the toxic load, are constant for various combinations of concentration (C) and time (t). These parameters, n and k, are typically derived from toxicity studies where animals were exposed to different concentrations of test chemicals for specified exposure durations. When $n = 1$, this equation simplifies to $C \times t = k$ and is known as Haber's Rule (Haber, 1924; Witschi, 1999). The toxic load model is used in the U.S. for military operational risk assessments (Department of Defense, 2005; Sommerville et al., 2010) that inform strategic planning for response actions and in civilian applications such as the development of Acute Exposure Guidelines (National Research Council, 2001). Tabulated values for n and k are available for a wide range of chemicals and endpoints from multiple sources (Health and Safety Executive, 2015; Mannan, 2005).

A theoretical basis for the toxic load model and its extension from the constant-concentration exposures typically found in the laboratory to the time-varying exposures encountered in a typical release scenario has had limited development until relatively recently (Rhomberg, 2009; Kaplan, 2009; Pauluhn, 2015), perhaps in part due to the paucity of relevant experimental data that could be used to test such theories. Because no experimental studies had systematically investigated acute toxicity under nonconstant concentration vs. time profiles, a case study was conducted using hydrogen cyanide (HCN) as the test chemical and acute lethality in rats as the endpoint (Sweeney et al., 2014). In that study, rats were exposed to either constant concentrations of HCN or

* Corresponding author. Fax: +1 937 904 9412.
E-mail addresses: lisa.sweeney.3.ctr@us.af.mil, lmsweeney@aol.com (L.M. Sweeney).

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experienced "pulsed" exposures consisting of two different concentrations of HCN (2:1 or 5:1 ratio), with or without a "gap" between pulses (30% of the total exposure duration), with a total duration (exposure plus gap, if applicable) of 5 or 30 min. Most of the tested scenarios (6/8) were found to conform to the toxicity expected based on the toxic load model; the two exceptions were very brief, high concentration, discontinuous exposures (exposures with "gaps") where higher-than expected HCN concentrations were required to produce equivalent lethality. If the recovery time afforded by the gap was protective in a short (5-min) exposure, it would be expected to be protective in a longer exposure as well (30 min) but was not. We speculated that during very brief pulses (1.75 min), some rats were able to reduce their systemic exposure via breath-holding, an adaptation that could not be sustained during longer exposure durations. Despite the extensive application of the toxic load/ten Berge model, the findings of Sweeney et al. (2014) provide the first known experimental support for the model under non-constant concentration exposure conditions. The applicability of these findings to other chemicals is unknown, but is most likely to hold for other compounds that act by similar modes of action or on similar time scales.

In the present investigation, additional experiments were designed and conducted to extend the evaluation of the applicable domain of the toxic load model for the case of acute lethality of HCN in the rat. Additional concentration vs. time profiles were evaluated to clarify the toxicity of brief exposures and to determine if the order (high-then-low, vs. low-then-high) or the relative durations of the pulses has an impact on lethality.

2. Materials and methods

2.1. Selection of test chemical

The current experiments and analyses build on previous efforts described in Sweeney et al. (2014). The aim of both the current and previous work was to test the validity of the toxic load model by means of a case study or series of case studies rather than being driven by a desire to understand the test chemical itself. HCN was selected as the test chemical largely due to the necessity to select a chemical, species (rat), and endpoint (lethality) for which the toxic load exponent was known to differ from 1 (Department of Defense, 2005; National Research Council, 2002). The ability to readily and consistently generate the targeted vapor concentrations was considered advantageous from the standpoint of experimental logistics. HCN also demonstrates consistent toxicity among species, binding to cytochrome oxidase and thereby reversibly preventing oxygen utilization in sensitive tissues such as the brain (National Research Council, 2002, 2008).

2.2. Overview of experimental design

Laboratory rats were exposed to an atmosphere containing HCN using a nose-only exposure system. A variety of $C \times t$ profiles were generated in order to discern the impact (or lack thereof) of the following factors on HCN lethality: constant concentration exposure vs. variable concentration exposure (i.e., two pulses with different concentrations), the ordering of the height of the two pulses, the relative widths of the pulses, the presence or absence of a gap between the two pulses, and the total duration of the test (exposure durations plus gap). The height ordering was reversed from what was tested in a prior series of exposures (high concentration followed by low concentration in Sweeney et al., 2014). Conflicting findings on the importance of a "gap" were previously identified in 5-min vs. 30-min exposures, so an intermediate exposure duration (10-min) was tested in this series. Relative pulse duration was also varied to test the toxic load model under an additional parameter and to create more realistic $C \times t$ profiles.

Three baseline (conventional) profiles as well as 8 non-constant (nonconventional) exposure profiles were chosen to further investigate the applicable domain of the toxic load model, with respect to acute HCN lethality in rats. A total of 60 trials were conducted (10 rats per trial). The baseline profiles consisted of exposures of 2.33, 10, or 30 min in duration to a constant concentration of HCN. The non-constant test profiles were 10 or 30 min in duration, with either two pulses of equal duration or two pulses at a duration ratio of 1:2. Pulse 2 concentrations were set at a fixed multiple of the initial concentration (5-fold higher). Gaps between pulses were either 0 min (no gap) or 30% of the total duration (i.e., 3 min or 9 min). The $C \times t$ profiles for this series are depicted in Fig. 1.

As in Sweeney et al. (2014), the study design (Fig. 1) consisted of baseline exposures (no change in concentration over time) (Profiles 1, 6, and 11) and the investigation of three tested factors affecting the shape of the $C \times t$ profile using a factorial design (Profiles 2–5 and 7–10). The current Profiles 8 and 10 provide mirror images to exposures conducted in Phase 1 (Phase 1 Profiles 8 and 10, respectively), facilitating a direct comparison of low–high vs. high–low ordering on pulse height. For each profile, with the exception of Profile 6, at least 4 exposure concentrations were tested (see Appendix A), which included trials approximating the median lethal concentration (LC_{50}) plus additional concentrations selected to provide coverage of a dose–response range, ideally with response rates neither 0% nor 100%.

2.3. Animal exposures and monitoring

The animal protocol was approved by the Wright–Patterson Air Force Base (WPAFB) Institutional Animal Care and Use Committee and the Air Force Surgeon General's office. A total of 600 male Sprague–Dawley (*Rattus norvegicus*) rats [Crl:CD(SD) BR rats], 5–6 weeks old, were purchased from Charles River Laboratories (Wilmington, MA). Rats were maintained in an animal facility approved by the Association for Assessment and Accreditation of Laboratory Animal Care International, pair housed prior to exposure, and provided husbandry in accordance with the National Research Council's *Guide for the Care and Use of Laboratory Animals*. Food and water were made available for all animals *ad libitum* during periods of non-exposure. Rats were quarantined and acclimated to the facility for 10 days. During quarantine and acclimation periods the rats were pair housed. Following release from quarantine, all animals were weighed. This weight was used to sort the rats to their prospective exposure group (10 rats per exposure). For a given shipment (lot number) of animals, the heaviest were assigned to the first exposure group, followed by the next heaviest animals to the second exposure group, and so on so that differences in weight among groups tested over a time span of up to 4 days would be minimized. The lightest animals from a given shipment were assigned to the final exposure groups. When more than one exposure was planned for a single study day, the animals were redistributed evenly by weight among the two or three exposures for that day. Due to the span of time over which the exposures were to be carried out, animals were ordered in batches (each batch corresponded to 1 week of testing) so that the animals were similar in age and weight at exposure. The sorting process and multiple batches yielded consistent body weights throughout the study.

Animals were exposed 1 time via nose-only inhalation (described below). Acclimation to the nose-only tubes was not done prior to the exposure day, due to the short duration of the exposures (a single 2.33–30 min exposure). Tube acclimation on the exposure day involved placing each of the animals in an open