Impact of Non-constant Concentration Exposure on Lethality of Inhaled Hydrogen Cyanide

Lisa M. Sweeney,*† Douglas R. Sommerville,‡ and Stephen R. Channel‡‡

*Henry M. Jackson Foundation for the Advancement of Military Medicine, Naval Medical Research Unit Dayton, Wright-Patterson AFB, OH 45433; †US Army, Edgewood Chemical Biological Center, Aberdeen Proving Ground, MD 21010; and ‡Leidos, Linton, IN 47441

To whom correspondence should be addressed. Fax: (937) 904-9412. E-mail: LMS29@cwru.edu.

Received October 3, 2013; accepted November 22, 2013

The ten Berge model, also known as the toxic load model, is an empirical approach in hazard assessment modeling for estimating the relationship between the inhalation toxicity of a chemical and the exposure duration. The toxic load (TL) is normally expressed as a function of vapor concentration (C) and duration (t), with TL equaling C × t being a typical form. Hypothetically, any combination of concentration and time that yields the same “toxic load” will give a constant biological response. These formulas have been developed and tested using controlled, constant concentration animal studies, but the validity of applying these assumptions to time-varying concentration profiles has not been tested. Experiments were designed to test the validity of the model under conditions of non-constant acute exposure. Male Sprague-Dawley rats inhaled constant or pulsed concentrations of hydrogen cyanide (HCN) generated in a nose-only exposure system for 5, 15, or 30 min. The observed lethality of HCN for the 11 different C versus t profiles was used to evaluate the ability of the model to adequately describe the lethality of HCN under the conditions of non-constant inhalation exposure. The model was found to be applicable under the tested conditions, with the exception of the median lethality of very brief, high concentration, discontinuous exposures.

Key Words: hydrogen cyanide; nose-only inhalation; acute lethality; non-constant concentrations; pulsed exposures; toxic load model; ten Berge model.

An accurate understanding of the potential acute vapor/gas lethality of chemical warfare agents and toxic industrial chemicals is needed for a variety of applications including military operational risk management and civilian consequence management. The hazard posed by inhalation of chemical warfare agents or toxic industrial chemicals (from either accidental or deliberate releases) is a function of the manner of the dispersion and transport of the compound to the exposed personnel, the intensity and duration of that exposure, and the toxicological properties of the given chemical. Typically, laboratory animals are exposed for a specified duration to a constant concentration of the compound of concern, effects are observed, and these results guide estimates of human lethality. In contrast, real-world human exposures typically involve concentrations that vary with time. One of the key assumptions in the human risk estimation is that all human exposures that yield the same cumulative exposure, regardless of the concentration–time profile, will produce the same outcome. The validity of this assumption has not been experimentally tested.

Various modeling approaches are applied to address the need for predictive acute toxicity parameters that allow for casualty estimation and planning (Rhomberg, 2009). Traditionally, the exposure magnitude was empirically quantified by taking the assumed average concentration (C) multiplied by the total exposure duration (t) to derive the cumulative “dosage” (C × t). This approach (ie, Haber’s rule) was originally developed by Haber to characterize the acute inhalation toxicity of chemical warfare agents during the World War I period (Flury, 1921; Haber, 1924; Witschi, 1999). First observed by Flury (1921) with hydrogen cyanide (HCN) lethality, it is now well understood that the LC50 (product of concentration and exposure duration producing 50% lethality) for a chemical can vary as a function of duration (Mannan, 2005). The ten Berge model, also known as the toxic load (TL) model, was developed to empirically account for this time dependence, with the TL (or C × t) replacing dosage (C × t). A new parameter, the toxic

Disclaimer The views expressed in this article are those of the authors and do not necessarily reflect the official policy or position of the Department of Defense, nor the U.S. Government.

The experiments reported herein were conducted in compliance with the Animal Welfare Act and in accordance with the principles set forth in the “Guide for the Care and Use of Laboratory Animals,” Institute of Laboratory Animal Resources, National Research Council, National Academy Press, 1996.

The authors are either an employee or contract employees of the U.S. Government. This work was prepared as part of their official duties. Title 17 U.S.C. §105 provides that ‘Copyright protection under this title is not available for any work of the United States Government.’ Title 17 U.S.C. §101 defines a U.S. Government work as a work prepared by a military service member or employee of the U.S. Government as part of that person’s official duties.

Published by Oxford University Press on behalf of the Society of Toxicology 2013.
This work is written by (a) US Government employee(s) and is in the public domain in the US.
load exponent \( (n) \), is introduced, which is an empirically fitted coefficient that is chemical, toxicity endpoint, and exposure scenario specific. This model has been applied extensively to toxicity estimates for industrial compounds by ten Berge et al. (ten Berge and van Heemst, 1983; ten Berge et al., 1986). When \( n = 1 \), this model is reduced to “Haber’s Law or Rule”. For many toxic industrial chemicals and chemical warfare agents it has been determined that \( n \approx 1 \) (Mannan, 2005). In these cases, the LC\(_{50}\) increases as the duration increases (i.e., a larger mass of chemical will need to be inhaled to produce the same toxicity when an individual’s exposure is spread out over a longer duration, all other factors being equal). The U.S. Department of Defense (DOD) currently uses the toxic load model to estimate the time-dependency of human (military) toxicity of chemical and biological agents (Department of Defense [DOD], 2005; Sommerville et al., 2010). For civilian applications, it is used by the U.S. Environmental Protection Agency (U.S. EPA) in the development of their Acute Exposure Guideline Levels (AEGLS) (National Research Council, 2001), as well as by other non-U.S. agencies (Fairhurst and Turner, 1993; Franks et al., 1996; Tissot and Pichard, 2002; RIVM, 2005).

It has not been experimentally established whether the model in its present form is adequate for simulating real-world scenarios encountered by personnel exposed to chemical warfare agents and toxic industrial chemicals (Ride, 1995; Yee, 1996; Sommerville et al., 2006; Platt et al., 2011). The lack of prior justification for the ten Berge/toxic load model, theoretical or otherwise, and its extension to time varying exposures have been noted previously (Rhomberg, 2009; Kaplan, 2009). Despite exhaustive search, there are currently no known acute inhalation animal studies that have systematically investigated non-constant concentration–time profiles. Typical whole-body exposure chambers could not adequately generate such profiles. However, it is now possible with current technology to generate such profiles using nose-only exposure systems. This study sought to demonstrate the quantitative relationship between non-constant concentration–time profiles (reflective of reality) and standard fixed concentration–time profiles that have been the historical norm in laboratory toxicity studies.

**MATERIALS AND METHODS**

**Test chemical selection.** A toxic load exponent which demonstrably differs from 1 is expected to enhance the likelihood that deviations from a constant-concentration exposure will yield results that differ from the current forms of the model. The value of \( n \) can vary widely among endpoints. Bushnell (1997) found that, for accuracy of signal detection by trained rats exposed to trichloroethylene, the results were best described with \( n = 2.16 \), but for response time, a value of \( n = 7.11 \) was determined. Therefore, it was deemed necessary to select a test chemical, species, and endpoint for this study for which the toxic load exponent was already well-characterized and different from 1.

HCN was selected based on its well-characterized toxicity from constant-concentration studies, the ease in which the vapor can be generated, and its relatively large toxic load exponent value for lethality (with estimates ranging from 1.85 [DOD, 2005] to 2.6 [National Research Council, 2002]). The mechanism of action for HCN’s effects involves prevention of oxygen utilization in tissues, including the brain, as a result of the inhibition of cytochrome oxidase via reversible binding to the iron-containing heme group. HCN may be rapidly metabolized, predominantly by rhodanese in the liver and skeletal muscle mitochondria, by transfer of sulfur from thiosulfate to cyanide, thereby forming thiocyanate, which is eliminated in the urine (National Research Council, 2002, 2008).

**Animals.** All experiments involving animals were conducted in accordance with the Institute of Laboratory Animal Resources Guide for the Care and Use of Laboratory Animals and were approved by the Wright-Patterson Air Force Base Institutional Animal Care and Use Committee and the Air Force Surgeon General’s office. A total of 670 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 singly housed, and food and water were made available ad libitum. Following 10-day quarantine and acclimation, rats were randomized by weight into their respective exposure groups (10 per exposure). Animals were ordered in batches to maintain similar age (between 53 and 63 days old) and weight (between 213.8 and 325.1 g) at exposure.

**HCN exposure.** Animals were exposed one time via nose-only inhalation (described in detail 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 5–30 minute exposure). Tube acclimation on the exposure day involved placing each of the animals in an open nose-only tube on a laboratory counter top for 30 minutes prior to the initiation of HCN exposures. Time of death and appearance of severe effects were monitored and recorded during exposure, with surviving animals returned to the Vivarium for an immediate 1 hour post-exposure period followed by an additional 23 hours of post-exposure observation. After the 24-hour observation period, surviving rats were euthanized following the approved protocol.

**Exposure conditions and apparatus.** A mix of HCN with 21% oxygen, balance nitrogen from a cylinder was diluted by clean breathing air to attain the desired concentration. The dilution and clean control air for the exposure system was supplied by an air compressor filtered for oils, organics, and particulates by a compressed breathing air purification system (Model No.: RP050, MST, Inc., Hicksville, OH). Cylinders of HCN/oxygen/nitrogen mix exposure gas were obtained from Weiler Welding (Moraine, OH); all of the gas cylinders were certified to within ±2% by the gravimetric method of analysis by the producers (Custom Gas Solutions, Durham, NC, and the American Gas Group, Toledo, OH).

Animals were exposed using a single 12-position Cannon style nose-only exposure unit (constructed in house). The exposure atmosphere flowed at approximately 0.5 l/min per open port through a central, inner plenum and out through the delivery nozzles that directed the HCN gas mix into the breathing zone of each animal. A total of 6 l/min was the target supply air flow rate for the nose-only exposure unit. The nose-only exposure system was fitted with connections for a differential pressure gauge to monitor static pressure. The outer plenum of the nose-only exposure system carried the animals’ exhaled breath and excess test atmosphere into the exhaust system. The nose-only exposure unit operated as a push-pull system where the air supply was positive and the exhaust flow was negative. The air supply was set at the target flow rate and the exhaust was adjusted to maintain a static pressure (Magnehelic Gauge, Dwyer Instrument Co., Michigan City, IN) in the range of −0.05 to −0.10 inches of water for the exposures. Open nose-only tubes with a plastic butt pusher were used for animal containment during the exposures.

Two parallel dilution systems were used (Fig. 1). Each dilution system was capable of being turned on or off independently from the other with a trio of 3-way electric solenoid valves (Model No.: SV31, Circle Valve Technologies, Inc., Harleysville, PA). This configuration gave the ability to produce a clean air gap in the exposure. In each system, HCN study gas and the clean breathing air from the breathing air system were regulated by a pair of electronic mass flow controllers (Model No.: HFC-202, Teledyne Hastings Instruments, Hampton, VA) which were controlled by a Power Pod power supply (Model No.: THPS-400, Teledyne Hastings Instruments, Hampton, VA). Once the HCN and breathing air flows passed through the mass flow controllers,
they were mixed and then flowed through the solenoid valves that directed the gases to either the nose-only tower or a bypass tube which dumped into the system exhaust. Vacuum for the nose-only tower was supplied by an air-operated vacuum generator (Model No.: TD260M, Air-Vac Engineering, Seymour, CT).

**Environmental parameters.** Temperature and relative humidity were measured by a certified Rotronic temperature/relative humidity monitor (Model No.: HF53W, ROTRONIC AG, Switzerland) and logged with LabVIEW data collection software (National Instruments Corp., Austin, TX). Static pressure was measured by a certified BAPI static pressure sensor (Model No.: ZPS-05, Building Automation Products, Inc., Gays Mills, WI). Temperature, humidity, and static pressure were monitored in the vented hood containing the nose-only exposure unit. Pre-study testing showed there was little difference in the temperature readings based on location (hood area or nose-only port). To the extent possible, the temperature was maintained between 64°F and 79°F (18°C and 26°C) and the relative humidity was maintained between 30% and 70%.

**Exposure monitoring.** The concentration of the HCN gas was measured with a Nicolet 380 Fourier Transform Infrared Spectrometer (FT-IR) (Thermo Fisher Scientific Inc., Waltham, MA) tuned to a peak at 3339.5 cm⁻¹. The FT-IR sampled at 500 ml/min from the intake tube leading to the nose-only exposure unit. The validity of sampling from this spot was verified by sampling from the intake, exhaust, and a port on the nose-only exposure unit at both 250 and 500 ml/min to confirm uniformity of readings. The uniformity of the HCN atmospheres at individual exposure ports was previously verified by recording the concentration at a minimum of 5 different exposure ports without animals to show the similarity at a single port and between individual ports. Redundant sampling secondary confirmation of exposure concentrations from the intake to the nose-only exposure unit and the bypassed intake gas stream before it dumped into the exhaust was performed using slower-response Interscan Analyzers (Model No.: RM28, Interscan Corporation, Chatsworth, CA). LabVIEW software was used to monitor concentration, cylinder gas flow, and clean air dilution flow once per second during exposure as well as for the 5 minutes before and after exposure. Exposure gas concentration data was collected via the FT-IR using Omnic Software (Thermo Fisher Scientific, Inc., Waltham, MA). Achieved concentrations for a given trial were reported as the average of the last 30% of the duration of the pulse; these values were used to compute dosimetry for the exposed rats.

**Experimental design.** A variety of C × t profiles (Fig. 2) were generated in order to discern the potential impact of the following factors on HCN lethality: constant concentration exposure vs. variable concentration exposure (ie, two pulses with different concentrations), the relative heights of the two pulses (concentration associated with pulse 1 divided by the concentration associated with pulse 2), the presence versus absence of a gap between the two pulses (ie, discontinuous vs. continuous exposure), and the total duration of the test (exposure durations plus gap). Three baseline (conventional) profiles as well as 8 non-constant
FIG. 2. Graphical depiction of C versus t profiles of median lethal toxic exposures for inhaled HCN in male Sprague-Dawley rats. LC_{t,50} determined by benchmark dose analysis of 5–8 different exposures of 10 rats per profile (Table 3).
(nonconventional) exposure profiles were chosen to validate or invalidate the toxic load model. For each profile, at least 5 runs (with different initial concentrations) were conducted, with 10 rats per run. The baseline profiles consisted of exposures of 5, 15, or 30 minutes in duration to a constant concentration of HCN, in order to rigorously identify the toxic load exponent in the equation \( C \times t \). The non-constant test profiles were 5 or 30 minutes in duration, with two pulses of equal duration (with concentrations in a ratio of 5:1 or 2:1), with a gap between pulses of either 0 minutes (no gap) or 30% of the total duration (ie, 1.5 minutes or 9 minutes). The pulse ratios were selected to provide one case where the second pulse would be expected to only minimally contribute to the additional body burden of toxicant (5:1 ratio), and another case with a more substantial contribution to the body burden (2:1 ratio). For each for the 11 profiles, at least 5 exposure concentrations were tested, 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%. The exposure apparatus was able to maintain excellent fidelity to the exposure profiles as demonstrated in Figure 3. The variation in concentrations between the random sampling of ports on the nose-only exposure unit was <1%.

**Determination of median lethal concentrations.** The lethality results for each profile were evaluated using U.S. EPA’s Benchmark Dose Software (BMDS, U.S. EPA, version 2.2.0) with the BMDS Wizard (ICF International, Fairfax, VA, Version 1.9.1). The suite of models evaluated consisted of the Logistic, Loglogistic, Probit, LogProbit, Weibull, Quantal-Linear, Multistage, Gamma, and Dichotomous-Hill models. A background response rate of zero was assumed appropriate for acute lethality, and model parameters were constrained to meet this limitation. Dose was expressed as \( C \times t \) (in ppm x min). \((C \times t) \) was calculated for each pulse; the product from each pulse was summed to derive the dose for the trial). The data for each profile were evaluated using the Cochrane Armitage trend test to establish the existence of a statistically significant dose-response relationship. Outputs from the dose-response analysis included a graphical presentation of the dose-response relationship (including 95% Wald confidence limits on the fraction affected), an estimate of the goodness-of-fit, the best estimates of the dose producing a 20% or 50% response (benchmark doses [BMD] of LC\(_{20}\) or LC\(_{50}\)), the 97.5th percent lower confidence limit on the BMD, and standardized residual errors (SRE). BMDS software does not provide an upper confidence limit, so the upper confidence limit was estimated by assuming that the uncertainty distribution is symmetrical (upper confidence limit = 2 x BMD – lower confidence limit). The range between the 97.5th percent lower and upper confidence limits was therefore assumed to encompass the 95% confidence limits on the LC\(_{20}\) or LC\(_{50}\). The goodness-of-fit was characterized by a p-value. In the event of a p < 0.10 (a poor fit, per U.S. EPA, 2012) for the best-fit model, the datum with the highest standardized residual error (typically >2, per U.S. EPA, 2012 guidance) was eliminated and the analysis repeated with the reduced data set. If necessary, a second datum with the highest remaining residual error was eliminated to see if the data could be reduced to a data set yielding an acceptable p-value. The analysis was interpreted as identifying an outlier if the resulting LC\(_{20}\) confidence limits of the reduced data set were narrower than for the full data set.

**Determination of the toxic load exponent.** An estimate of the TL exponent (“n” in \( C \times t \)) for HCN was derived as follows. The TL equation, \( C \times t = k \) (where k is a constant for a specific response level), is applied to the median lethal concentration, so \( (C_{50}) = t = \frac{TL_{50}}{k} \). Taking the logarithm (base 10) of this equation and rearranging,

\[
\log(t) = \log(TL_{50}) - n \log(C_{50})
\]

The logarithms of the \( C_{50} \) estimates and durations for the three constant-concentration profiles (Profiles 1, 6, and 11; Fig. 2) were used as inputs to a linear regression to derive \( \log(TL_{50}) \) (the intercept) and n (the slope, multiplied by –1).

**Comparisons of lethal toxic loads based on concentration versus time profiles.** Two general expressions for the TL received during exposure to an airborne chemical (used for risk assessment applications), as a function of time, are:

\[
TL = \int_0^T C(t) \, dt \quad \text{or} \quad TL = \sum_{j=1}^{n} C_j \cdot T_j
\]

where:
- \( C(t) \) is the instantaneous agent concentration as a function of time (ppm or mg/m\(^2\)),
- \( \tau \) is the total exposure duration (minutes),
- \( n \) is the toxic load exponent (dimensionless),
- \( C \) is the mean concentration over interval \( \tau \) (ppm or mg/m\(^2\)),
- \( \tau \) is the integration interval (minutes),
- \( p \) is the number of integration intervals (dimensionless)

TLs were computed both for an assumed perfect pulsed exposure using the second form of Equation (2) using piecewise concentrations (\( TL = C_1 \times t_1 + C_2 \times t_2 + C_3 \times t_3 \)), the 97.5th percent lower and upper confidence limits was therefore estimated by assuming that the uncertainty distribution is symmetrical (zero) and the duration-averaged concentration (\( TL = \sum_{j=1}^{p} (C_j \times t_j) / p \)) where \( C_{avg} = \sum_{j=1}^{p} (C_j \times t_j) / p \).

Statistical analyses of TL\(_{50}\) and TL\(_{20}\) estimates were conducted using SigmaPlot (version 12.5); a p-value of <.05 was used to establish statistically-significant differences. Groups to be compared were tested for normality using the Shapiro–Wilk test. Normally distributed data were tested for equality of variance: if variances were found to be equal, the group comparison was made using the 2-tailed t-test. If the groups failed the Shapiro–Wilk test (ie, data were determined not to be normally distributed) or found to have unequal variance, they were compared using the Mann–Whitney rank sum test for the comparison of medians. Comparisons were made between the following groups based on the design criteria: baseline versus pulsed (profiles 1, 6, and 11 vs. profiles 2–5 and 7–10); short duration versus long duration (profiles 1–5 vs. profiles 6–10); continuous versus discontinuous (profiles 1–3, 6–8, and 11 vs. profiles 4, 5, 9, and 10); small versus large pulse height difference (profiles 2, 4, 7, and 9 vs. profiles 3, 5, 8, and 10). An additional comparison between the discontinuous, short duration exposures (profiles 4 and 5) and all other exposures (profiles 1–3 and 6–11) was prompted by inspection of the data.

**RESULTS**

**Effects of Inhaled HCN**

Outcome data for all profiles are summarized in Table 1 (clinical signs) and Table 2 (lethality); the test concentrations and 24-hr lethality outcomes for each trial are presented in Supplementary Data; individual animal data have also been reported elsewhere (Sweeney et al., 2013). All clinical signs in survivors reversed rapidly (ie, within minutes) upon cessation of exposure with no visible effects noted in survivors 1 hr after completion of exposure. Very strong, statistically-significant dose-response trends for lethality were identified for all 11 profiles, as indicated by the Cochrane-Armitage test (p no greater than .0043). The benchmark dose modeling results are summarized Table 3.

**Determination of the Toxic Load Exponent**

The LC\(_{50}\)s of the three “baseline” profiles with constant-concentration exposures (profiles 1, 6, 11; LC\(_{50}\) = LC\(_{50}\)/t) were used to determine the toxic load exponent (“n” in \( C \times t \)) and the TL\(_{50}\) (LC\(_{50}\) x t) via linear regression of \( \log(t) \) vs. \( \log(LC_{50}) \) (Fig. 4). The \( r^2 \) of the regression was .996, n was determined to be 1.66, and TL\(_{50}\) was 312 x 10\(^{3}\) ppm mg/m\(^2\)-minutes. The TL exponent value of 1.66 determined for this study was compared
to values derived in other analyses of HCN lethality data. ten Berge et al. (1986) derived an estimate of $n = 1.64$ from the rat lethality data of Barcroft (1931) and NRC (2002) derived an estimate of $n = 2.6$ from the 5 to 60 minute rat exposure data of Lapin (1981). To compare predictions based on the current “$n$” to the values in the literature, the 15-minute LC$_{50}$ was assumed “known” and the LC$_{50}$ estimates for 5–30 minute exposures were estimated by extrapolation with the literature.
values of “n” (dashed lines in Fig. 4). Predictions made with the ten Berge et al. (1986) value of n cannot be distinguished from the current analysis, while 5- and 30-minute LC50 predictions made with the NRC (2002) n value are outside the 95% confidence limits from benchmark dose analysis of the current study. Similarly, for the LC20, the r2 of the regression was .996, n was determined to be 1.66, and TL20 was 206 × 103 ppm1.66-minutes.

Comparisons of Median Lethal Toxic Loads Based on Concentration Versus Time Profiles

Lethal TLs were computed using two methods, a piecewise-method (using the discrete concentrations for each pulse of the exposure) and a duration-averaging method (using the time-weighted average concentration for the entire exposure duration, including “gaps”). The TLs and their 95% confidence limits are depicted graphically (Figs. 5 and 6). When calculated by the piecewise method, the TL50s for profiles 4 and 5 clearly exceed the TL50 and the bounds on the TL50 derived from the baseline profiles (1, 6, and 11) (Fig. 5a). When the TL50 is calculated using the duration-averaging approach, the separation between profiles 4 and 5 and the remaining profiles is diminished, but still evident from visual inspection. The relative sizes of the confidence ranges for the TL50,8 were larger than for the TL50 because the study was designed to yield the most information on the median lethal exposures. Nonetheless, the TL20 comparisons (Fig. 6) do not, for the most part, support the contention that lethality differs substantially among the profiles. No statistically significant differences were found for 5 minute versus 30 minute exposures or based on variation in the pulse height ratio (2:1 vs. 5:1 concentration ratio between first and second pulses). Lethal TL were significantly different (higher) in exposures that were both short (5 minutes) and discontinuous as compared to the other concentration vs. time profiles for both the TL50 and TL20, when calculated using the piecewise approach, but only for the TL50 when computed using a duration-averaged approach (Table 4).

**DISCUSSION**

The primary objective of this study was to develop data sets that could be used for the assessment of the ten Berge/
Sweeney et al., who found that the LC for the 5-minute exposures, but not the 30-min
s similar (Table 3). This response may have been limited to the highest
, then C
s. In profiles 4 and
1, that the TL
50 or LCt
-s for 30-, 60-, and 240-minute expo
s (that is, 5 minutes or 30 minutes), in general, the shape of the
toxic load model (C
 × t) under conditions of non-constant exposure. A key consideration in the selection of the test compound was that the TL exponent, n, was not 1, because if
 = 1 in C
 × t, +C
 × t, then C
 × t +C
 × t does not differ from C
 × t. Based on the three baseline profiles, the TL exponent was determined to be 1.66, validating the choice of chemical.

One relatively simple way of assessing the predictivity of the model was to compare LCt
-s from profiles with the same total duration, but different profile shapes (eg, one vs. two pulses, presence or absence of a gap). For exposures of the same length (that is, 5 minutes or 30 minutes), in general, the shape of the profile (Fig. 2) does not appear to have had a significant impact
TABLE 3
Calculated Lethality of Inhaled HCN in male Sprague-Dawley Rats

| Exposure Duration | Profile | LCt
50 (95 percent confidence interval) (ppm-min.) | LCt
20 (95 percent confidence interval) (ppm-min.) | Model | Model Fit, \( p \)-Value |
|-------------------|---------|---------------------------------------------|---------------------------------------------|-------|---------------------|
| 5-minutes         | 1
\(^{a}\) | 3871 (3476–4267) | 3096 (2423–3769) | LogProbit | 0.87 |
| 2
\(^{a}\) | 4005 (3457–4553) | 3294 (2123–4465) | LogProbit | 0.55 |
| 3
\(^{a}\) | 4018 (3806–4230) | 3649 (3288–4011) | LogProbit | 0.76 |
| 4                | 5280 (4233–6328) | 3242 (1855–4629) | LogProbit | 0.58 |
| 5                | 5826 (4813–6838) | 3540 (2513–4568) | LogProbit | 0.58 |
| 30 minutes        | 6                | 7841 (7180–8501) | 6376 (5648–7105) | Gamma | 0.72 |
| 7                | 8850 (8042–9659) | 7197 (5504–8890) | Gamma | 0.38 |
| 8
\(^{a}\) | 7463 (6375–8551) | 5262 (3482–7042) | Weibull | 0.50 |
| 9                | 7558 (6128–8988) | 6146 (2830–9463) | Gamma | 0.40 |
| 10
\(^{a}\) | 7445 (6770–8119) | 6054 (4733–7375) | Gamma | 0.21 |
| 15 minutes        | 11               | 6340 (5569–7111) | 4552 (3331–5772) | Log-Probit | 0.70 |

Profiles 1, 6 and 11 are constant concentration exposures for 5, 30 and 15 minutes, respectively (see Figure 2 for depiction of profiles).

\(^{a}\)One trial dropped from analysis.

\(^{b}\)Two trials dropped from analysis.

FIG. 4. Comparison of experimental data (symbols plus 95% confidence intervals) and best fit line for determination of the toxic load exponent (n) for median lethality (solid line), and predictions based on the current 15-minute LC
 and n values of ten Berge et al. (1986) (dotted line) and NRC (2002) (dashed line) (C expressed in ppm, t in minutes), log base 10.
than the other 9 profiles. The interpretation of the TL\textsubscript{20} findings cannot be done with as much confidence as for the TL\textsubscript{50}s, due to the study design, which emphasized the median lethality. Nevertheless, a difference between the short, discontinuous profiles and other profiles is clearly evident for the LC\textsubscript{50}s, but not the LC\textsubscript{20}s. It is this finding that prompts us to conclude that the higher LC\textsubscript{t,50}s in profiles 4 and 5 are due to concentration-dependent effects on the rats. Assuming that this phenomenon accounts for the apparent “outliers,” the remaining results support the hypothesis that time-averaged concentrations of HCN, in combination with dose-response relationships derived from traditional constant-concentration exposures, can provide adequate predictions of HCN toxicity under conditions of time-varying exposure.

An unexpected finding in this study was that the median lethal concentrations for the “baseline” profiles (constant-concentration exposures) determined in this laboratory were substantially higher than expected (approximately double the expected LC\textsubscript{20}s) based on the previous studies conducted with the same sex and strain (Lapin, 1981; Vernot \textit{et al}., 1977) (Table 5). No reason for the difference in lethality was readily apparent, but it may relate to genetic drift. To avoid the influence of genetic drift in cancer analyses, it has been recommended that only data from the last 3–7 years be used as historical controls (Baldrick, 2005). The stability of historical control incidence of spontaneous neoplasms has been assessed for three strains of laboratory rats, and while tumor drift was not found to be common, it “occurred far more often in outbred rat strains (Wistar and Sprague-Dawley) than in the inbred rat strain” (Tennekes \textit{et al}., 2004). Prior to the 1990s, Charles River Laboratories had 23 separate production colonies in 8 different countries (White and Lee, 1998). Thus, it is possible that the Sprague-Dawley rats used by Lapin (1981) and Vernot \textit{et al}.(1977), prior to the restructuring and repopulation of the colonies, had genetic differences from those used in the work reported here. Use of a nose-only exposure system (rather than whole-body or head-only) may also have contributed to differences in lethality compared to the earlier literature. Regardless of the source of the differences, the new data by themselves are uniquely suited to the goal of identifying the impact (if any) of the characteristics of the C versus t profile (pulses, gaps in exposure, etc.) on the frequency an observed endpoint (lethality).

In summary, an inhalation system was developed to create exposure profiles where concentration varied over time. This system proved to be a versatile inhalation exposure system with the ability to control the testing conditions. In the current study, data were generated to test the validity of the ten Berge/toxic load model for extrapolation from constant exposures to time-varying exposures. The validity of applying formulas developed using controlled, constant concentration animal studies to time-varying exposures had not previously been tested due to
a lack of appropriate data sets. For this data set, the first of its kind, the model was found to be applicable under the tested conditions, with the exception of the median lethality of very brief, high concentration, discontinuous exposures. The implication of these results directly extends to the substantial effort from both the DOD and the Department of Homeland Security Chemical Security Analysis Center to develop TL parameter estimates for high priority toxic industrial chemicals. Those agencies are required by their mandates to estimate casualties from possible hostile use of toxic industrial chemicals against military and/or civilian targets. The predictive (vs. protective) parameter values, invariably based on traditional constant concentration/time laboratory animal studies, form the basis for planning response actions and logistical supply decisions, such as management of the US Strategic National Stockpile, a federally owned and managed repository of medications, medical

**TABLE 4**

<table>
<thead>
<tr>
<th>Design Characteristics (Profiles)</th>
<th>( \text{TL}_{50} )</th>
<th>( \text{TL}_{20} )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Piecewise</td>
<td>Duration-Averaged</td>
</tr>
<tr>
<td>Baseline vs. pulsed (1, 6, and 11 vs. 2–5 and 7–10)</td>
<td>Yes\textsuperscript{b}</td>
<td>No\textsuperscript{b}</td>
</tr>
<tr>
<td>Duration (1–5 vs. 6–10)</td>
<td>No\textsuperscript{c}</td>
<td>No\textsuperscript{c}</td>
</tr>
<tr>
<td>Continuous vs. discontinuous (1–3, 6–8, and 11 vs. 4, 5, 9, and 10)</td>
<td>Yes\textsuperscript{d}</td>
<td>No\textsuperscript{d}</td>
</tr>
<tr>
<td>Pulse height ratio (2, 4, 7, and 9 vs. 3, 5, 8, and 10)</td>
<td>No\textsuperscript{e}</td>
<td>No\textsuperscript{e}</td>
</tr>
<tr>
<td>Short, discontinuous exposures vs. all others (4 and 5 vs. 1–3 and 6–11)</td>
<td>Yes\textsuperscript{d}</td>
<td>Yes\textsuperscript{c}</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Profiles depicted in Figure 2.
\textsuperscript{b}Failed Shapiro-Wilk test for normality; group comparison based on Mann-Whitney Rank Sum test.
\textsuperscript{c}Passed Shapiro-Wilk test for normality and equal variance test; group comparison based on t-test.
\textsuperscript{d}Passed Shapiro-Wilk test for normality but failed equal variance test; group comparison based on Mann-Whitney Rank Sum test.
TABLE 5
Estimates of the median lethal inhaled concentration of HCN in male Sprague-Dawley rats

<table>
<thead>
<tr>
<th>Duration</th>
<th>LC₅₀ (ppm)</th>
<th>Exposure System</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 min.</td>
<td>774</td>
<td>Nose-only</td>
<td>This study</td>
</tr>
<tr>
<td></td>
<td>369</td>
<td>Whole-body</td>
<td>Lapin (1981)</td>
</tr>
<tr>
<td></td>
<td>398</td>
<td>Head-only</td>
<td>Lapin (1981)</td>
</tr>
<tr>
<td></td>
<td>484</td>
<td>Not stated</td>
<td>Vernot et al. (1977)</td>
</tr>
<tr>
<td>15 min.</td>
<td>423</td>
<td>Nose-only</td>
<td>This study</td>
</tr>
<tr>
<td></td>
<td>196</td>
<td>Whole-body</td>
<td>Lapin (1981)</td>
</tr>
<tr>
<td></td>
<td>163</td>
<td>Head-only</td>
<td>Lapin (1981)</td>
</tr>
<tr>
<td>30 min.</td>
<td>261</td>
<td>Nose-only</td>
<td>This study</td>
</tr>
<tr>
<td></td>
<td>173</td>
<td>Whole-body</td>
<td>Lapin (1981)</td>
</tr>
<tr>
<td></td>
<td>85</td>
<td>Head-only</td>
<td>Lapin (1981)</td>
</tr>
</tbody>
</table>


ACKNOWLEDGMENTS

The authors would like to thank the following staff at Naval Medical Research Unit Dayton: Brian Wong for contributions to the design of the exposure system; Brian Sharits, Arden James, and Doug Baver for exposure system construction and operation; and Nathan Gargas, Tracy Doyle, and Michelle Okolica for animal handling and observation. The authors would also like to thank Jerry Glasow of the Defense Threat Reduction Agency, John Cockayne of Leidos, and Kyong Park, US Army Edgewood Chemical Biological Center, for their technical advice and assistance.

REFERENCES


Funding

This work was supported by the Defense Threat Reduction Agency. Dr Sweeney’s efforts were conducted under Work Unit Number H1107.

Supplementary data are available online at http://toxsci.oxfordjournals.org/.


