

# Impact of Non-Constant Concentration Exposures on Lethality of Inhaled Hydrogen Cyanide

Douglas R. Sommerville, PE\*, Stephen R. Channel, DVM,MS,DABT\*\*, Lisa M. Sweeney, Ph.D., DABT, CHMM\*\*\*

(\* ) U.S. Army Edgewood Chemical Biological Center, (\*\* ) Leidos, Inc., (\*\*\*) Naval Medical Research Unit--Dayton

## Abstract

The toxic load (TL) model is an empirical approach in hazard assessment modeling for estimating the relationship between a chemical's inhalation (IH) toxicity and the exposure duration. The TL is normally expressed as a function of vapor concentration (C), duration (t) and a constant--or C<sup>n</sup>. Hypothetically, any combination of C and t that yields the same TL 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—the first dataset of its kind. Over two separate studies, male Sprague-Dawley rats inhaled constant or pulsed concentrations of hydrogen cyanide (HCN) generated in a nose-only exposure system for durations ranging from 2.33 to 30 min. The observed lethality of HCN for the 21 different C versus t profiles was used to evaluate the TL model's ability to adequately describe the HCN lethality under the conditions of non-constant IH 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. The implication of these results directly extends to the substantial effort from both the Department of Defense 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 in response to public health emergencies (e.g., potential terrorist attacks).

## Objective and Introduction

**Objective:** Conduct inhalation exposures that can be used to compare relative inhalation toxicity of constant and non-constant concentration-time (C-t) exposure profiles. Assess traditional quantitative toxicity parameters, e.g. toxic load (TL), developed from traditional laboratory constant C-t studies against the non-constant C-t profiles more applicable in real world exposures to hazardous chemicals.

**Introduction:** All current chemical fate, transport and dispersion models for chemical warfare agents base hazard prediction output on a mathematical assumption: toxicity parameters developed from non-fluctuating C-t profiles studies in laboratory animal species are relevant to real world, in which highly variable exposure conditions are faced by humans. The Toxic Load (TL) (or ten Berge) model is one of the more common approaches for predicting toxicity as function of duration. Determining how to properly integrate [C<sup>n</sup>(t)] dt for TL model will require toxicity data using fluctuating C-t profiles. No known data of this type existed prior to the present effort. Typical chambers/exposure systems are not well suited for such profiles.

## TL Model—How Best to Integrate?

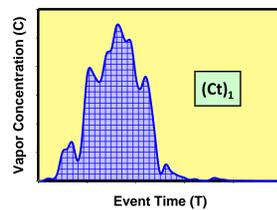
### Calculation of Administered Dosages/TL

$$\text{Dosage (Ct)} = \int_{t=0}^{t=T} [C(t)] dt$$

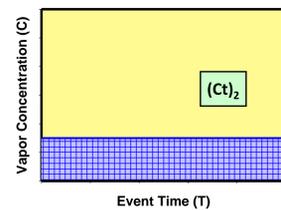
If n = 1, TL becomes the traditional dosage term

$$\text{Toxic Load (TL)} = \int_{t=0}^{t=T} [C(t)]^n dt$$

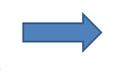
How long of a time step (dt) should be used? Changes in dt will produce differing TL values



Equivalent areas under the curve (Ct)<sub>1</sub> = (Ct)<sub>2</sub>  
(C<sup>n</sup>t)<sub>1</sub> does not necessarily equal (C<sup>n</sup>t)<sub>2</sub>



What dt value is most appropriate for predicting toxicity? Currently, transport & dispersion models use dt = t



(C<sup>n</sup>t)<sub>1</sub> = (C<sup>n</sup>t)<sub>2</sub>

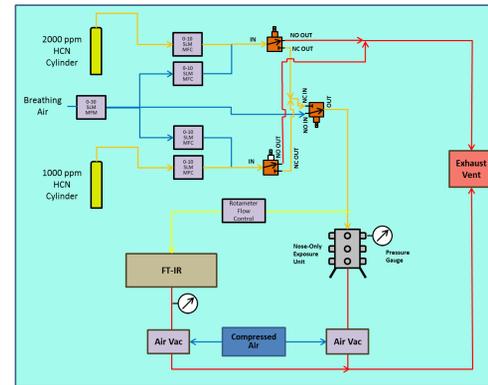
(C<sup>n</sup>t)<sub>1</sub> ≠ (C<sup>n</sup>t)<sub>2</sub>

Data from previous animal experimental studies are not suitable for answering above question.

## Materials and Methods

### Exposure System

- Hydrogen cyanide in 21% O<sub>2</sub>, balance N<sub>2</sub>
  - HCN chosen because it is well studied & characterized
- Two separate gas generation systems
- Mass flow controllers: meter gas & dilution air
- Mix together prior to entering chamber
- Solenoid valves to start and stop flows
- Monitor using FT infrared spectroscopy
- Enables generation of pulsed C-t profiles
  - Goal is to study TL model and not HCN toxicity



### Probit-Style Experiments (Finney (1971))

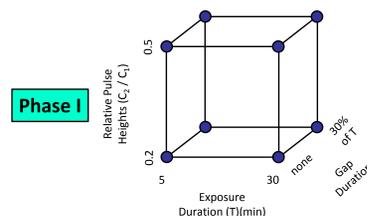
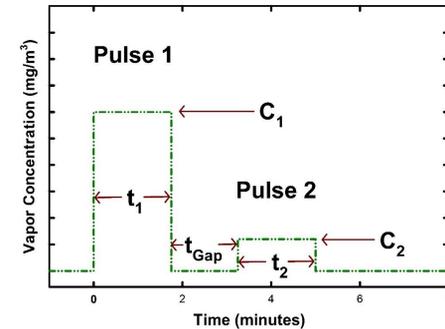
- 5-8 groups of 10 subjects per exposure profile
- Median effective Ct calculated for each profile
  - 24 hour post-exposure observational period used
  - US EPA Benchmark Dose Software used for calculations

## Experimental Design

- For both phases
  - Full 2<sup>3</sup> factorial design (8 pulsed profiles)
    - Exposure and Gap Durations are common factors in both phases
  - Baseline profiles (3 constant durations)
    - Use to establish toxic load exponent (n)
  - Calculated LC<sub>50</sub> & two LTL<sub>50</sub><sup>s</sup> for each of the 11 profiles

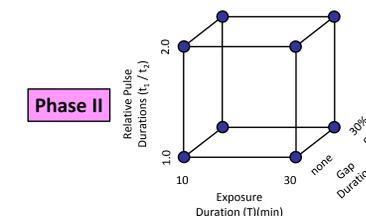
C<sub>1</sub>, C<sub>2</sub> = vapor concentration with agent stream for Pulses 1 & 2  
C<sub>avg</sub> = average concentration (including both peaks & gaps)

T = total duration—defined as from start of Pulse 1 to end of Pulse 2  
t<sub>1</sub>, t<sub>2</sub>, t<sub>gap</sub> = durations of Pulses 1 & 2 and Gap



For all factorial profiles (t<sub>1</sub>/t<sub>2</sub>) = 1 and C<sub>1</sub> > C<sub>2</sub>

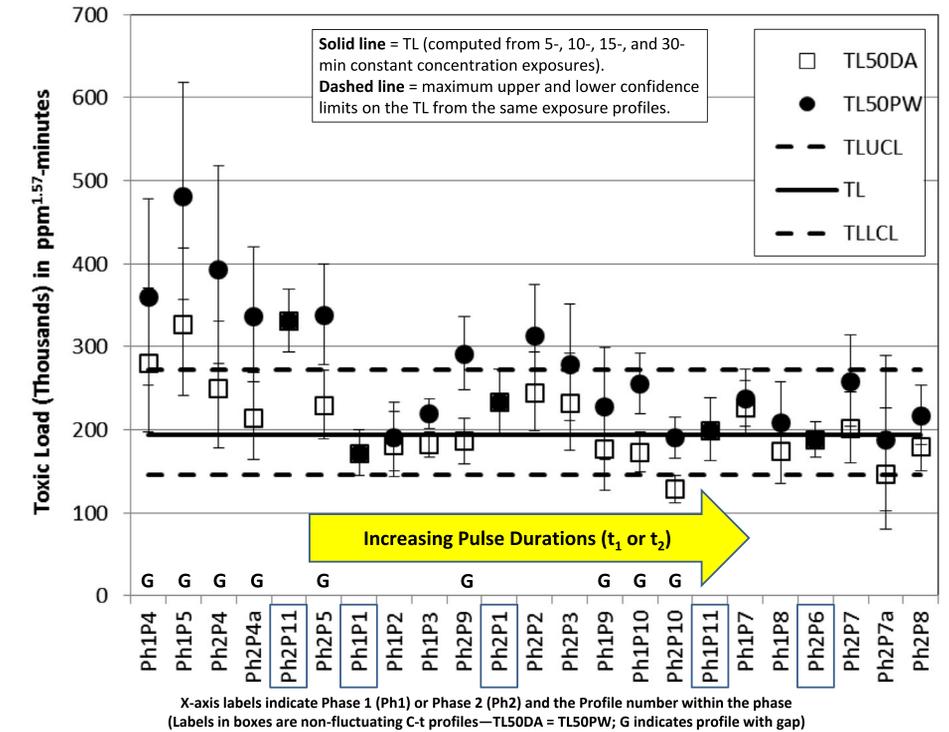
Profile Number	Conc Pulse 1	Total Duration (min.)	Pulse 1 Duration (min.)	Gap Duration (min.)	Conc Pulse 2	Pulse 2 Duration (min.)	Profile Type
1	Ci	5	5	NA	NA	NA	Baseline
2	Ci	5	2.5	0	0.5*Ci	2.5	Factorial
3	Ci	5	2.5	0	0.2*Ci	2.5	Factorial
4	Ci	5	1.75	1.5	0.5*Ci	1.75	Factorial
5	Ci	5	1.75	1.5	0.2*Ci	1.75	Factorial
6	Ci	30	30	NA	NA	NA	Baseline
7	Ci	30	15	0	0.5*Ci	15	Factorial
8	Ci	30	15	0	0.2*Ci	15	Factorial
9	Ci	30	10.5	9	0.5*Ci	10.5	Factorial
10	Ci	30	10.5	9	0.2*Ci	10.5	Factorial
11	Ci	15	15	NA	NA	NA	Baseline



For all factorial profiles (C<sub>1</sub>/C<sub>2</sub>) = 0.2 and t<sub>1</sub> ≥ t<sub>2</sub>

Profile Number	Conc Pulse 1	Total Duration (min.)	Pulse 1 Duration (min.)	Gap Duration (min.)	Ratio T1/T2	Pulse 2 Duration (min.)	Profile Type
1	Ci	10	10	NA	NA	NA	Baseline
2	Ci	10	6.67	0	2	3.33	Factorial
3	Ci	10	5	0	1	5	Factorial
4	Ci	10	4.67	3	2	2.33	Factorial
5	Ci	10	3.5	3	1	3.5	Factorial
6	Ci	30	30	NA	NA	NA	Baseline
7	Ci	30	20	0	2	10	Factorial
8	Ci	30	15	0	1	15	Factorial
9	Ci	30	14	9	2	7	Factorial
10	Ci	30	10.5	9	1	10.5	Factorial
11	Ci	2.33	2.33	NA	NA	NA	Baseline

## Results



### Total TL Definitions

#### Duration Average (DA)

$$TL_{DA} = (C_{avg})^n \times t$$

$$dt = t$$

#### Piece-wise (PW)

$$TL_{PW} = (C_1)^n \times t_1 + (C_2)^n \times t_2$$

$$dt = t_1$$

- Large differences observed between predicted and computed TLs for very short durations and high concentrations
  - May be due to physiological compensation (i.e. breath-holding)
- Both TL definitions had values outside of the CLs of the TL for the non-fluctuating C-t profiles (n = 1.57)
  - TL50DA—3 out of 16 values
  - TL50PW—7 out of 16 values. This definition does more poorly with shorter pulses

- At longer durations, both TL definitions are within the CLs.
- Factors with no statistical significance
  - Pulse height order and height ratio
  - Shorter vs. longer durations
- Factors with limited support for statistical significance
  - Constant vs. pulse profiles
  - Pulse duration ratio
  - No gap vs. gap

## Conclusion

TL50DA (duration average) is an adequate TL definition for constant C-t profiles and simple fluctuating C-t profiles for exposures not involving physiological compensation.

**Animal Care and Use:** The experiments using this system 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 Animals Resources, National Research Council, National Academy Press, 1996. The experiments were conducted by the Naval Medical Research Unit-Dayton at their facility in Wright-Patterson Air Force Base, Dayton, OH.

### References:

- (Ph1) Sweeney et al. (2014) Toxicol. Sci. 138:205-216.
- (Ph2) Sweeney et al. (2015) Regul. Toxicol. Pharmacol. 71:571-584.
- Finney DJ, *Probit Analysis*; The University Press: Cambridge, UK, 1971.

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