# RELATIONSHIP BETWEEN THE DOSE-RESPONSE CURVES FOR LETHALITY AND SEVERE EFFECTS FOR CHEMICAL WARFARE NERVE AGENTS

DOUGLAS R. SOMMERVILLE
U.S. Army Edgewood Chemical Biological Center
5183 Blackhawk Road, ATTN: AMSSB-RRT-IM (Bldg. E5951)
Aberdeen Proving Ground, MD 21010-5424
douglas.sommerville@us.army.mil

In recent years, the U.S. Environmental Protection Agency has developed a categorical (or ordinal) logistic regression approach for regressing ordered categories of toxic responses (*e.g.*, no effects, non-adverse effects, other effects of increasing severity, *etc*.) on one or more factors (*e.g.* dose, exposure time, type of agent, *etc*.) due to a chemical agent exposure. The advantage of such an approach is that two types of dose-response curves (severity of effect and percent of individuals versus dose) are fitted simultaneously. For chemical warfare (CW) agent toxicity, ordinal logistic regression provides a means to statistically demonstrate and quantify the steepness of both types of dose-response curves for acute exposures to organophosphate-type CW agents (or nerve agents). Experimental animal data from three acute inhalation studies were reviewed and analyzed separately using a probit link-function: (1) monkeys exposed to GA (tabun), GB (sarin) or GF (cyclosarin); (2) rats exposed to GB; and (3) rats exposed to GB or GF. For each study, both vapor concentration and exposure time were varied. Clinical signs and mortality were recorded for all three studies. From these signs three categorical responses were defined: death, severe effects and less than severe effects. An animal was categorized as having severe effects if it exhibited at least one of the following signs (yet did not die within 24 hours post-exposure): convulsions, gasping, collapse or prostration. The regression analysis indicated that for all three studies slightly more than one standard deviation (1.0 to 1.4) separated an effective concentration ($EC_{XX}$) for severe effects from a lethal concentration ($LC_{XX}$) for XX% affected. This method provides a better way of estimating threshold lethality, because for the data analyzed, threshold lethality (approximately a $LC_{01}$ or $LC_{05}$) is the equivalent to about an $EC_{16}$ (for severe effects). The 16% effect level can be estimated with greater confidence from experimental quantal data via probit analysis than the 1% effect level. Thus, questionable extrapolation of the dose-mortality curve down to the 1% level can be avoided by using the dose-severe effect curve in its place.

## INTRODUCTION

Human toxicity estimates for chemical warfare (CW) agents are required to properly evaluate agent-related health hazards under a variety of situations: military deployment operations; handling, storage and destruction of CW agents; and emergency response procedures. Modeling and simulation (M&S) plays an important role towards this end. For use in such models, it is required that toxicity is expressed as a function of exposure parameters (dosage and exposure duration). Knowledge is also needed of the dose-response (DR) curves for the population at risk: severity of effect (DR-S) and percent of affected individuals (DR-P) as a function of the dose.

To address these needs, data for CW organophosphate (or nerve) agents have been traditionally analyzed via the probit analysis (or binary logistic regression)[1-3] of the quantal (or binary) data taken for a particular toxicological endpoint (*e.g.*. alive or dead, presence or absence of miosis, *etc.*) as a function of one or more factors (dosage, vapor concentration, exposure duration, *etc*.). It has been standard practice to define the resulting mortality-response relationships in terms of a linear time-integrated concentration (*i.e.*, vapor concentration (C) multiplied by the exposure time (T), or CT for short–a dosage).[4] Two important parameters are produced by probit analysis that characterize the DR-P curve for any particular toxicant: median dosages (either median effective ($ECT_{50}$) or lethal ($LCT_{50}$)) and the probit slope. Both Mioduszewski *et al.*[4,6] and Anthony *et al.*[7,8] employed this method. However, on its own probit analysis can only

characterize the DR-P curve for a particular endpoint. Knowledge about the other dose-response curve, DR-S, for nerve agents is also very important, especially since it is known to be very steep.[9]

However, defining the DR-S curve requires additional measures. The simplest approach has been to compare the reported literature values of $ECT_{50}$'s* and probit slopes calculated via probit analysis for a range of endpoints.[10,11] However, the accuracy of calculated $ECT_{50}$ ratios for different endpoints is reduced when the values in the ratio come from separate studies (*i.e.* comparing the $ECT_{50}$ (miosis) from Study A to the $ECT_{50}$ (convulsions) from Study B).

A better approach is to investigate multiple endpoints in the same study, as was done by Cresthull, *et al.*[12] who reported both severe effects and lethality as a function of C and T. A probit analysis was performed separately on each endpoint. The estimated $ECT_{50}$'s (incapacitation) and $LCT_{50}$'s were compared to estimate the steepness of the DR-S curve. Unfortunately, regressing toxicological responses using a binary format implicitly assumes that the responses are independent of each other, which is not the case here. The result is that important information is ignored which could better characterize the steepness of the DR curve.

One solution to this problem is to employ categorical (or ordinal) logistic regression.[13] The U.S. Environmental Protection Agency (EPA) is currently developing this method for its own applications (such as supporting the Benchmark Dose (BMD) model).[14-17] Instead of the binary response used in probit analysis, categorical logistic regression uses ordered categories of toxic responses (*e.g.*, no effects , non-adverse effects, other effects of increasing severity, *etc*.), which are regressed as a function of one or more factors (*e.g.* dose, exposure time, type of agent, *etc*.). The advantage of this approach is that the two types of DR curves (DR-P and DR-S) are fitted simultaneously. Thus, for CW nerve agents, ordinal logistic regression provides a means to statistically demonstrate and better quantify the steepness of both types of curves for acute inhalation (IH) exposures. Data from three CW nerve agent studies[5-8,12] were reviewed and re-analyzed using ordinal regression. The purpose of the analysis was to determine the relationship between the DR-P curves for lethality and severe effects resulting from IH exposures to G-type nerve agents. Potential risk assessment applications[18] of this type of knowledge were then explored.

## TOXICITY STUDIES REVIEWED

**Overview**. The three studies reviewed for this work were Cresthull, *et al.* (1957),[12] Mioduszewski, *et al.* (2001 and 2002),[4-6] and Anthony, *et al*. (2002),[8] all of which were conducted at what is now the Edgewood Chemical Biological Center (ECBC) A brief summary of the studies is presented in Table 1. The Toxicology Team, ECBC, maintains raw data and other materials associated with these studies.

In all three studies, the animals were exposed (whole body) in dynamic airflow inhalation chambers.[19] For Cresthull, *et al*., the agent vapor concentrations were allowed to reach equilibrium at the target value for the run; after which, the animals were quickly introduced into (and removed from) the chamber *via* a sliding animal carriage. The exposure duration was, thus, the time between introduction and removal.

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* The definition of an effective dosage ($ECT_{XX}$) includes aspects from both types of dose-response curves. An $ECT_{XX}$ for Effect A is the dosage needed to produce either Effect A or an effect of greater severity (from the same route of exposure) in XX% of the subjects exposed to that dosage. Thus, cumulative measures are found for both the effect severity curve (an effect of equal or greater severity) and the percent affected (XX%) curve.

In the other two studies, the animals were placed into the chamber prior to the introduction of agent vapor. Then, the chamber was quickly brought to equilibrium at the target vapor concentration. The concentration was kept constant once equilibrium had been reached. The concentration-time profile generated was described by MacFarland (1987).[13] His definition of exposure duration was the one used in these studies--the interval from the start of the flow of agent into the chamber to the time-point when the agent flow is stopped. Following exposure, the chamber was purged with air for 10 minutes, and the animals were then removed from the chamber. None of the animals were restrained during an exposure run. Both Cresthull *et al*. (1957) and Mioduszewski, *et al*. (2001 and 2002) have been previously used in the development of acute exposure guideline level values for CW nerve agents.[10,11]

**Table 1**
*Summary of CW Nerve Agent Studies Reviewed*

| Name of Study | Cresthull, *et al*. (1957) | Mioduszewski, *et al*. (2001 and 2002) | Anthony, *et al*. (2002) |
|---|---|---|---|
| Year(s) Conducted | 1953 to 1954 | 1998 to 2000 in two separate phases | 2001 to 2002 |
| Agent(s) Investigated | GA, GB and GF | GB | GF and GB |
| Species Used | Rhesus Monkey | Sprague-Dawley Rat | Sprague-Dawley Rat |
| Total Number of Subjects | 152 | 700 | 500 |
| Gender | Mostly Female | Equal number of Males and Females | Males (240)<br>Females (260) |
| Breakdown by Agent of Number of Subjects | GA (56), GB (52), GF (44) | All GB | GF (320), GB (180) |
| Number of Subjects per Exposure Group | 4 | 10 or 20 | 5, 10 or 20 |
| Number of Runs | 36 | 43 | 38 |
| Vapor Concentrations (mg/m3) | GA: 18.1 to 81<br>GB: 6.6 to 29.1<br>GF: 7.3 to 59 | GB: 2.0 to 54.4 | GB: 3.5 to 35.9<br>GF: 2.0 to 41.9 |
| Exposure Times (minutes) | 2 and 10 | Phase I: 10, 30, 90, 240<br>Phase II: 5, 60, 360 | 10, 60 and 240 |
| Primary Endpoint(s) of Interest | Incapacitation and Lethality (1 day) | Lethality (1 and 14 days) | Lethality (1 and 14 days) |

**Definition of Severe Effects and Lethality**. The ordered ternary responses defined for the present work (lethality (L), severe effects (S) and less than severe effects (M)) were defined from the clinical signs and mortality, which were recorded in the three studies. Mortality within 24 hours post exposure was counted as a lethal effect. An animal was categorized as having severe effects if it exhibited convulsions, gasping, collapsed or prostration (yet did not die within 24 hours post exposure). Mortality occurring between one and 14 days post exposure was treated as a severe effect response. In Cresthull, *et al.*, incapacitation was defined as collapse or convulsions.

**Experimental Quantal Data**. The experimental quantal data from Cresthull, *et al*., Mioduszewski, *et al*., and Anthony, *et al.* are presented (using a discrete format) in Tables 2 to 4. For example, in Table 2 for T = 10 minutes and C = 18.1 mg/m $^3$ GA, there are two animals with no effects severe or above, one animal having at least severe effects

and no mortality, and one animal that died or in shorthand—[2, 1, 1].* In the discrete format, the sum of the values in a table row equals the total number of individuals exposed using a particular set of test parameters (agent, C, T and gender), which for the present example equals four (or 2+1+1). The original notebooks were also reviewed to gather additional information on the categorical response distributions.

**Table 2**
*Monkey G-Type IH Quantal Data from Cresthull, et al.*

| Agent | T (min) | C ($mg/m^3$) | CT ($mg\text{-}min/m^3$) | Number per Category | | |
|---|---|---|---|---|---|---|
| | | | | M | S | L |
| GA | 2 | 33.3 | 67 | 4 | 0 | 0 |
| | | 50.8 | 102 | 2 | 2 | 0 |
| | | 54.3 | 109 | 0 | 4 | 0 |
| | | 62.0 | 124 | 0 | 1 | 3 |
| | | 62.3 | 125 | 0 | 3 | 1 |
| | | 65.0 | 130 | 0 | 2 | 2 |
| | | 68.5 | 137 | 2 | 2 | 0 |
| | | 71.0 | 142 | 0 | 0 | 4 |
| | | 81.0 | 162 | 1 | 0 | 3 |
| | 10 | 18.1 | 181 | 2 | 1 | 1 |
| | | 18.8 | 188 | 0 | 2 | 2 |
| | | 21.7 | 217 | 0 | 0 | 4 |
| | | 23.0 | 230 | 1 | 0 | 3 |
| | | 24.6 | 246 | 0 | 0 | 4 |
| GB | 2 | 8.8 | 18 | 4 | 0 | 0 |
| | | 13.7 | 27 | 1 | 0 | 3 |
| | | 16.4 | 33 | 2 | 1 | 1 |
| | | 17.0 | 34 | 4 | 0 | 0 |
| | | 18.6 | 37 | 1 | 0 | 3 |
| | | 19.7 | 39 | 2 | 1 | 1 |
| | | 23.5 | 47 | 1 | 1 | 2 |
| | | 29.1 | 58 | 0 | 1 | 3 |
| | 10 | 6.6 | 66 | 1 | 2 | 1 |
| | | 8.1 | 81 | 0 | 1 | 3 |
| | | 8.2 | 82 | 0 | 2 | 2 |
| | | 10.0 | 100 | 0 | 0 | 4 |
| GF | 2 | 31.0 | 62 | 2 | 1 | 1 |
| | | 42.0 | 84 | 1 | 1 | 2 |
| | | 44.0 | 88 | 0 | 1 | 3 |
| | | 48.0 | 96 | 0 | 0 | 4 |
| | | 59.0 | 118 | 0 | 0 | 4 |
| | 10 | 7.3 | 73 | 3 | 1 | 0 |
| | | 10.0 | 100 | 2 | 1 | 1 |
| | | 12.2 | 122 | 0 | 2 | 2 |
| | | 13.0 | 130 | 0 | 2 | 2 |
| | | 15.5 | 155 | 0 | 2 | 2 |
| | | 19.9 | 199 | 0 | 0 | 4 |

*Note: Shaded row was not used in the final analysis after having been identified as a statistical outlier in the initial analysis.*

* This is in contrast to a common toxicology convention of displaying quantal data in a cumulative format, where the number of animals having an effect of equal or greater severity are included in an effect category. In which case, the above example [2, 1, 1] would be written instead as [4, 2, 1].

**Table 3**

*Rat GB IH Quantal Data from Mioduszewski, et al.*

| | Male | | | | | Female | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| T | C | CT | Number per Category | | | C | CT | Number per Category | | |
| (min) | ($mg/m^3$) | ($mg\text{-}min/m^3$) | M | S | L | ($mg/m^3$) | ($mg\text{-}min/m^3$) | M | S | L |
| 5 | 36.3 | 182 | 1 | 7 | 2 | 25.6 | 128 | 5 | 3 | 2 |
| | 44.0 | 220 | 3 | 4 | 3 | 28.2 | 141 | 9 | 1 | 0 |
| | 48.1 | 241 | 0 | 6 | 4 | 31.5 | 158 | 1 | 3 | 6 |
| | 51.4 | 257 | 1 | 2 | 7 | 36.3 | 182 | 1 | 3 | 6 |
| | 54.4 | 272 | 0 | 3 | 7 | 44.0 | 220 | 0 | 1 | 9 |
| 10 | 15.3 | 153 | 9 | 1 | 0 | 9.6 | 96 | 10 | 0 | 0 |
| | 18.7 | 187 | 7 | 2 | 1 | 12.0 | 120 | 9 | 1 | 0 |
| | 21.8 | 218 | 0 | 6 | 4 | 15.3 | 153 | 1 | 7 | 2 |
| | 27.1 | 271 | 0 | 2 | 8 | 18.7 | 187 | 2 | 4 | 4 |
| | 34.3 | 343 | 0 | 0 | 10 | 21.8 | 218 | 0 | 1 | 9 |
| 30 | 6.0 | 180 | 10 | 0 | 0 | 6.0 | 180 | 6 | 4 | 0 |
| | 7.4 | 222 | 8 | 2 | 0 | 7.4 | 222 | 0 | 9 | 1 |
| | 9.0 | 270 | 1 | 6 | 3 | 8.5 | 255 | 0 | 5 | 5 |
| | 10.3 | 309 | 0 | 0 | 10 | 9.0 | 270 | 0 | 3 | 7 |
| | 12.1 | 363 | 0 | 0 | 10 | 12.1 | 363 | 0 | 1 | 9 |
| 60 | 6.0 | 360 | 2 | 5 | 3 | 5.9 | 354 | 1 | 7 | 2 |
| | 6.4 | 384 | 3 | 5 | 2 | 6.0 | 360 | 0 | 4 | 6 |
| | 7.0 | 420 | 1 | 8 | 1 | 6.4 | 384 | 1 | 6 | 3 |
| | 7.6 | 456 | 1 | 3 | 6 | 7.0 | 420 | 0 | 5 | 5 |
| | 8.1 | 486 | 3 | 1 | 6 | 7.6 | 456 | 0 | 0 | 10 |
| 90 | 4.0 | 360 | 6 | 4 | 0 | 4.0 | 360 | 0 | 8 | 2 |
| | 4.1 | 369 | 9 | 1 | 0 | 4.1 | 369 | 4 | 5 | 1 |
| | 4.5 | 405 | 1 | 6 | 3 | 4.5 | 405 | 0 | 4 | 6 |
| | 4.9 | 441 | 0 | 6 | 4 | 4.9 | 441 | 2 | 1 | 7 |
| | 5.5 | 495 | 0 | 2 | 8 | 5.5 | 495 | 0 | 0 | 10 |
| 240 | 2.1 | 504 | 10 | 0 | 0 | 2.1 | 504 | 10 | 0 | 0 |
| | 2.7 | 648 | 9 | 0 | 1 | 2.7 | 648 | 0 | 6 | 4 |
| | 3.3 | 792 | 7 | 2 | 1 | 3.3 | 792 | 0 | 4 | 6 |
| | 4.2 | 1008 | 0 | 6 | 4 | 4.2 | 1008 | 0 | 2 | 8 |
| | 4.4 | 1056 | 0 | 4 | 6 | 4.4 | 1056 | 0 | 0 | 10 |
| 360 | 2.3 | 828 | 5 | 3 | 2 | 2.3 | 828 | 5 | 4 | 1 |
| | 2.7 | 972 | 2 | 6 | 2 | 2.4 | 864 | 0 | 10 | 0 |
| | 2.8 | 1008 | 2 | 7 | 1 | 2.7 | 972 | 2 | 4 | 4 |
| | 3.0 | 1080 | 0 | 2 | 8 | 2.8 | 1008 | 0 | 5 | 5 |
| | 3.5 | 1260 | 0 | 1 | 9 | 3.0 | 1080 | 0 | 1 | 9 |

**Table 4**

*Rat GB and GF IH Quantal Data from Anthony, et al.*

| Agent | Gender | T (min) | C ($mg/m^3$) | CT ($mg\text{-}min/m^3$) | Number per Category | | |
|---|---|---|---|---|---|---|---|
| | | | | | M | S | L |
| GF | Female | 10 | 17.2 | 172 | 9 | 1 | 0 |
| | | | 21.5 | 215 | 7 | 3 | 0 |
| | | | 23.3 | 233 | 10 | 0 | 0 |
| | | | 23.9 | 239 | 2 | 3 | 5 |
| | | | 25.2 | 252 | 2 | 2 | 6 |
| | | | 26.9 | 269 | 1 | 3 | 6 |
| | | | 31.1 | 311 | 0 | 0 | 10 |
| | Male | | 17.2 | 172 | 10 | 0 | 0 |
| | | | 21.5 | 215 | 10 | 0 | 0 |
| | | | 31.1 | 311 | 1 | 8 | 1 |
| | | | 34.4 | 344 | 5 | 3 | 2 |
| | | | 41.9 | 419 | 0 | 1 | 9 |
| | Female | 60 | 4.9 | 294 | 6 | 2 | 2 |
| | | | 5.7 | 342 | 4 | 4 | 2 |
| | | | 5.9 | 354 | 0 | 1 | 9 |
| | | | 6.4 | 384 | 0 | 0 | 10 |
| | | | 7.2 | 432 | 0 | 0 | 10 |
| | Male | | 4.9 | 294 | 10 | 0 | 0 |
| | | | 5.7 | 342 | 5 | 4 | 1 |
| | | | 6.4 | 384 | 0 | 6 | 4 |
| | | | 7.2 | 432 | 1 | 2 | 7 |
| | | | 7.8 | 468 | 0 | 0 | 10 |
| | Female | 240 | 2.0 | 480 | 7 | 2 | 1 |
| | | | 2.0 | 480 | 1 | 8 | 1 |
| | | | 2.2 | 528 | 0 | 3 | 7 |
| | | | 2.5 | 600 | 0 | 2 | 8 |
| | | | 3.3 | 792 | 0 | 0 | 10 |
| | Male | | 2.0 | 480 | 3 | 4 | 3 |
| | | | 2.0 | 480 | 4 | 6 | 0 |
| | | | 2.2 | 528 | 0 | 8 | 2 |
| | | | 2.5 | 600 | 1 | 3 | 6 |
| | | | 3.3 | 792 | 0 | 1 | 9 |
| GB | Female | 10 | 18.0 | 180 | 0 | 10 | 0 |
| | | | 21.6 | 216 | 4 | 5 | 1 |
| | | | 22.7 | 227 | 0 | 8 | 2 |
| | | | 23.8 | 238 | 0 | 3 | 7 |
| | | | 24.8 | 248 | 0 | 3 | 7 |
| | | | 26.6 | 216 | 0 | 0 | 10 |
| | Male | | 22.7 | 227 | 8 | 2 | 0 |
| | | | 26.7 | 267 | 1 | 8 | 1 |
| | | | 28.7 | 287 | 0 | 6 | 4 |
| | | | 32.8 | 328 | 0 | 5 | 5 |
| | | | 35.9 | 287 | 0 | 2 | 8 |
| | Female | 60 | 5.6 | 336 | 0 | 4 | 1 |
| | | | 6.1 | 366 | 0 | 4 | 6 |
| | | | 6.6 | 396 | 0 | 0 | 5 |
| | Male | | 6.6 | 396 | 1 | 4 | 0 |
| | | | 7.0 | 420 | 1 | 5 | 4 |
| | | | 7.5 | 450 | 0 | 1 | 4 |
| | Female | 240 | 3.5 | 840 | 0 | 5 | 5 |
| | Male | | 4.3 | 1032 | 8 | 2 | 0 |
| | | | 5.6 | 1344 | 0 | 3 | 7 |

## STATISTICAL THEORY

Probit analysis was the method used by Cresthull, *et al*.,[12] Mioduszewski, *et al*.,[4-6] and Anthony, *et al*[7,8] for the analysis of their data. A brief review of probit analysis is presented herein, followed by a review of its extension for use with ordered categorical responses with three or more levels (or ordinal logistic regression), whose application towards CB nerve agents is the subject of this work.

To perform either a binary or ordinal logistic regression, a link-function is used to connect the random and systematic components of the regression model.[13] This is accomplished by transforming the probability of an effect or response to a linear scale. Several probability distributions are commonly used for this transformation: probit, logit and complementary log-log.[2,13,22] Historically, CB nerve agent toxicology has used a probit link-function, which is implicit in the use of probit analysis. For ease of comparison, ordinal regression with a probit link-function is used in this work. Thus, the following discussions implicitly assume the use of a probit link-function.

**Probit Analysis**. For each individual, there is a dose or dosage* that is just sufficient to produce a specified biological response. These just-sufficient dosages are called effective dosages to distinguish them from administered dosages. The distribution of effective dosages for a homogeneous population is usually lognormal.[1,5,20,21]

Although statisticians typically describe the lognormal distribution of effective dosages by the mean and variance of log(effective dosage), toxicologists usually describe the distribution by the median effective dosage, $ECT_{50}$, and the probit (or Bliss) slope, $m$:

$$(1) \qquad ECT_{50} = \text{antilog}(\eta) \qquad (2) \qquad m = 1/\sigma$$

$$(3) \qquad Z = \frac{\{\log(CT) - \log(ECT_{50})\}}{s}$$

where $\eta$ is the median of log(effective dosage), $\sigma^2$ is the variance of the distribution, and $Z$ is the standard normal random variable. The $ECT_{50}$ is used in a cumulative fashion by toxicologists: 50% of the exposed individuals will exhibit a specified biological response of equal or greater severity for the same exposure route. Effective dosages for response levels other than 50% can be calculated using Eqn. (3) with known values for $ECT_{50}$ and $m$, and using the $Z$ value corresponding to the cumulative probability of interest (*e.g.* $Z$ equals 0 for a 50% response). Toxicologists traditionally use base 10 logarithms to calculate the probit (Bliss) slope.[1,3,5,21] This convention is used herein.

Although the normal distribution is continuous, quantal (binary) data are used to estimate the distribution parameters ($ECT_{50}$ and $m$).[1] Probit analysis and maximum likelihood estimation (MLE) are used to estimate these parameters from data.[1,22] The following equation is fitted via probit analysis/MLE for vapor toxicity studies:[1]

$$(4) \qquad Y_N = (Y_P - 5) = k_0 + k_C \log C + k_T \log T + k_i \,(\text{other factors})$$

where $Y_N$ is a normit, $Y_P$ is a probit, and the $k$'s are fitted coefficients. The constants $k_C$ and $k_T$ are the probit slopes for concentration and time, respectively. Often, experiments are conducted with exposure time held constant, which reduces Eqn. (4) to the traditional probit equation.[1] Thus, the probit slope for a vapor exposure usually refers

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* The terms dose and dosage are often used interchangeably, but they do have different definitions. Dose is the total amount of a substance that is administered, while dosage is an amount administered relative to some other quantity (*e.g.*, body mass, body surface, and/or time).[20] For inhalation exposures, dosage is the term used.[20]

to the slope on vapor concentration ($m = k_C$) instead of the slope on time. The greater the probit slope, the smaller the variance is in the distribution of individual susceptibilities.

When fitting Eqn. (4), all variability in the data will contribute to the estimate for *m*, be it from variance due to individual susceptibilities, batch effects, experimental error, *etc*. Probit analysis performed on a compilation of data from many sources will not produce an accurate measure of variance among individuals due to the heterogeneity introduced by differences among the studies (*e.g.*, experiment procedures, type of animals used, *etc*.).[23] The effect of such heterogeneity will be to reduce the probit slope. Also, as was previously noted, probit analysis on its own can only characterize the DR-P curve for a particular endpoint.

**Ordinal Logistic Regression**. Conceptually, ordinal regression simply involves the division of ordered multi-level categorical responses into a series of cumulative binary responses.[23] In the case of ternary data, with ordered discrete response levels of low {0}, medium {1} and high {2}, the following ordered binary combinations are produced: {0} vs. {1 and 2}; and {0 and 1} vs. {2}. Thus, one way to express the model is to apply Eqn. (4) to each binary combination:[1]

$$(5) \qquad Y_N\left\{0\middle|1,2\right\} = k_{\{0|1,2\}} + k_{\mathrm{C}} \log \mathrm{C} + k_{\mathrm{T}} \log \mathrm{T} + k_i \,(\text{other factors})$$

$$(6) \qquad Y_N\left\{0,1\middle|2\right\} = k_{\{0,1|2\}} + k_{\mathrm{C}} \log \mathrm{C} + k_{\mathrm{T}} \log \mathrm{T} + k_i \,(\text{other factors})$$

where $Y_N\{0\,|\,1,2\}$ and $Y_N\{0,1\,|\,2\}$ are the normits for the binary responses of {0} vs. {1 and 2}; and {0 and 1} vs. {2}, respectively. The constants, $k_{0\,|\,1,2}$ and $k_{0,\,1\,|\,2}$, are the intercepts for the normits of the cumulative probabilities of an effect exceeding in severity the low {0} and medium {1} responses, respectively.[23]

When using Eqns. (5) and (6), it is implicitly assumed that the values of the individual various probit slopes (*i.e*. $k_C$, $k_T$ , $k_i$, *etc*.) are constant (*e.g.* $k_C$, (in Eqn. (5)) equals $k_C$ (in Eqn. (6))). Otherwise there would be conditions where Eqns. (5) and (6) would intersect, a probabilistic impossibility for ordered responses.[1] As with probit analysis, MLE is used to provide fits for Eqns. (5) and (6).[13,22] An iterative-reweighted least squares algorithm is used to obtain maximum likelihood parameter estimates.[22,23]

**Dose-Percent Response Curves (Severe and Lethality)**. For the present study, Eqns. (5) and (6) are used to solve for $ECT_{50}$ (severe) and $LCT_{50}$, respectively. To calculate the $ECT_{50}$ / $LCT_{50}$ ratio, Eqns. (5) and (6) can rearranged to produce:

$$(7) \qquad \log_{10}\left[\frac{\mathrm{ECT}_{50}}{\mathrm{LCT}_{50}}\right] = ? \;/\; k_C$$

$$(8) \qquad \kappa = \left[k_{\{0,1|2\}} - k_{\{0|1,2\}}\right] = \left[k_{severe} - k_{lethal}\right]$$

where $\kappa$ is the distance in normits between the percent affected levels of the severe and lethality DR curves. For instance, when $\kappa$ equals one, the $ECT_{50}$ equals a $LCT_{16}$ (since the 50 and 16% cumulative effect levels from a standard normal distribution are separated by one standard deviation), or if $\kappa$ equals two, then $ECT_{84}$ equals a $LCT_{16}$.

Confidence limits on estimates for both $\{\kappa / k_C\}$ and $\kappa$ can be calculated. The standard error of a ratio, (*a* / *b*), is given by Barry (1978),[25] which is based upon the propagation of error formula for a ratio:

(9) $$\text{std err of } \left(\frac{a}{b}\right) = \left(\frac{a}{b}\right)\sqrt{\left(\frac{\text{var}(a)}{a^2}\right) + \left(\frac{\text{var}(b)}{b^2}\right) - (2)\left(\frac{\text{cov}(a,b)}{ab}\right)}$$

where var($a$), var($b$), and cov($a$,$b$) are the variance of the quantities, $a$ and $b$, and their covariance, respectively. The 95% confidence limits for the ratio will equal ($a$ / $b$) ± (2)(std err). The following relations from Mood, *et al.* (1974)[26] were also used to get the necessary information for determining the limits for both $\{\kappa / k_C\}$ and $\kappa$:

(10) $$\text{var}(a \pm b) = \text{var}(a) + \text{var}(b) \pm (2)\text{cov}(a, b)$$

(11) $$\text{cov}(a \pm b, c) = \text{cov}(a, c) \pm \text{cov}(b, c)$$

where cov($a \pm b$, $c$) is the covariance of the quantity, ($a \pm b$), with a third quantity, $c$.

## DATA ANALYSIS

An ordinal logistic regression program (a component of MINTAB® Version 13) was used to perform the calculations. The three datasets (in Tables 2 to 4) were analyzed separately. The ternary data consisted of the number of subjects having less than severe effects (M), severe effects (S), and lethality (L), as previously defined.

Only one continuous predictor, logC, was used in the present analysis. The other available continuous predictor, T, was treated as a categorical factor instead, since the emphasis was on the estimating the relationship between severe and lethal DR-P curves. Complications were avoided by not trying to directly model the non-linear dependence of toxicity on logT. Both Mioduszewski, *et al.*[4-6] and Anthony, *et al.*[7,8] have found that log($LCT_{50}$) versus logT was non-linear for G-agent IH toxicity.

In addition to logC, full factorial designs were used in each of the three studies to investigate the effect of two or more of the following factors: agent type, exposure duration (T) and gender. Cresthull, *et al*. investigated agent type (3 levels) and exposure duration (2 levels), for a total of 6 groupings. Mioduszewski, *et al*. studied gender (2 levels) and exposure duration (7 levels), for a total of 14 groupings. Anthony, *et al.* explored all three predictors, using a total of 12 groupings [agent type (2 levels), gender (2 levels), and exposure duration (3 levels)].

For the present analysis, the following model was used in the ordinal regression programs (modifications of Eqns. (5) and (6)):

(12) $$Y_N\{severe\} = k_{severe} + k_C \log C + \sum_i^N k_i G_i$$

(13) $$Y_N\{lethal\} = k_{lethal} + k_C \log C + \sum_i^N k_i G_i$$

where $G_i$ equals one when modeling the $i$-th group (from the total number ($N$) of groups from the full factorial) of a dataset and zero for all other groups, and the $k_i$'s are fitted coefficients. This approach produces only one value each for the probit slope ($k_C$), $\{\kappa / k_C\}$ and $\kappa$ for the whole dataset, as well as individual $ECT_{50}$ and $LCT_{50}$ values for each group. By dividing a dataset into smaller independent subsets (for separate analyzes using MINTAB®), it is possible to obtain multiple values for $k_C$, $\{\kappa / k_C\}$ and $\kappa$ as a function of the various factors within a dataset. However, it was found for each parameter that the individual subset values were not significantly different (statistically) from other subset values within the larger dataset. Thus, it was assumed that $k_C$, $\{\kappa / k_C\}$ and $\kappa$ were constant in value for the whole dataset.

In addition to calculating values for $k_C$, $\{\kappa / k_C\}$ and $\kappa$ for each dataset, Eqns. (9) to (11) were used (in conjunction with the variance-covariance matrix of the model fit returned by MINTAB®) to estimate the errors associated with these values. Also, error estimates for individual group $ECT_{50}$ and $LCT_{50}$ values were made in the same fashion.

## RESULTS

The results of the data analysis are presented in Tables 5 to 11. Tables 5 to 8 contain the estimates for individual group $ECT_{50}$ and $LCT_{50}$ values, while Tables 9 to 11 present the estimated probit slope ($k_C$), $\{\kappa / k_C\}$ and $\kappa$ values for each dataset. When available, values previously reported by the researchers are shown for comparison.

**Table 5**
*Monkey G-Type IH $ECT_{50}$ (Severe) Values from Ordinal Logistic Regression and Cresthull, et al.*

| | | Estimates from Ordinal Logistic Regression | | Cresthull, et al (1957) (24 hours Post-Exposure) | |
|---|---|---|---|---|---|
| Agent | T (min) | $ECT_{50}$ (Severe) (mg-min/m$^3$) | 95% Fiducial Limits | $ECT_{50}$ (Severe) (mg-min/m$^3$) | 95% Fiducial Limits |
| GA | 2 | 102 | 90 to 115 | 102 | none reported |
| GB | | 36 | 31 to 40 | 30 | none reported |
| GF | | 58 | 49 to 70 | 62 | none reported |
| GA | 10 | 145 | 121 to 173 | <180 | none reported |
| GB | | 56 | 46 to 67 | <66 | none reported |
| GF | | 96 | 82 to 112 | 100 | none reported |

**Table 6**
*Monkey G-Type IH $LCT_{50}$ Values from Ordinal Logistic Regression and Cresthull, et al.*

| | | Estimates from Ordinal Logistic Regression | | Cresthull, et al (1957) (24 hours Post-Exposure) | |
|---|---|---|---|---|---|
| Agent | T (min) | $LCT_{50}$ (mg-min/m$^3$) | 95% Fiducial Limits | $LCT_{50}$ (mg-min/m$^3$) | 95% Fiducial Limits |
| GA | 2 | 131 | 118 to 146 | 135 | 123 to 152 |
| GB | | 46 | 40 to 53 | 42 | 29 to 60 |
| GF | | 76 | 65 to 88 | 75 | 63 to 87 |
| GA | 10 | 187 | 161 to 217 | 187 | 164 to 221 |
| GB | | 72 | 61 to 85 | 74 | 62 to 87 |
| GF | | 124 | 108 to 143 | 130 | 112 to 151 |

**Table 7**

*Rat GB IH $ECT_{50}$ (Severe) and $LCT_{50}$ Values from Ordinal Logistic Regression and*

| | | Estimates Derived from Ordinal Logistic Regression | | | | Mioduszewski, et al (2001) (24 hours Post-Exposure) | |
|---|---|---|---|---|---|---|---|
| Gender | T (min) | $ECT_{50}$ (Severe) (mg-min/$m^3$) | 95% Fiducial Limits | $LCT_{50}$ (mg-min/$m^3$) | 95% Fiducial Limits | $LCT_{50}$ (mg-min/$m^3$) | 95% Fiducial Limits |
| Female | 5 | 136 | 128 to 145 | 173 | 163 to 184 | 166 | 151 to 186 |
| Male | | 184 | 173 to 196 | 234 | 220 to 248 | 240 | 211 to 287 |
| Female | 10 | 144 | 134 to 183 | 183 | 171 to 196 | 184 | 167 to 205 |
| Male | | 185 | 173 to 198 | 235 | 220 to 252 | 231 | 211 to 255 |
| Female | 30 | 196 | 183 to 209 | 249 | 233 to 265 | 263 | 241 to 292 |
| Male | | 225 | 211 to 240 | 286 | 268 to 305 | undefined | undefined |
| Female | 60 | 300 | 281 to 320 | 381 | 360 to 404 | 387 | 357 to 417 |
| Male | | 354 | 334 to 375 | 450 | 425 to 476 | 459 | 412 to 472 |
| Female | 90 | 319 | 300 to 340 | 406 | 383 to 430 | 404 | 385 to 426 |
| Male | | 366 | 346 to 388 | 466 | 440 to 493 | 448 | 427 to 482 |
| Female | 240 | 589 | 547 to 633 | 748 | 697 to 803 | 741 | 654 to 825 |
| Male | | 801 | 749 to 857 | 1018 | 952 to 1090 | 1040 | 917 to 1466 |
| Female | 360 | 780 | 735 to 827 | 991 | 938 to 1048 | 987 | 946 to 1039 |
| Male | | 830 | 781 to 882 | 1055 | 996 to 1117 | 1048 | 973 to 1150 |

*Mioduszewski, et al.*

**Table 8**

*Rat GB and GF IH $ECT_{50}$ (Severe) and $LCT_{50}$ Values from Ordinal Logistic Regression and Anthony, et al.*

| | | | Estimates Derived from Ordinal Logistic Regression | | | | Anthony, et al (2001) (24 hours Post-Exposure) | |
|---|---|---|---|---|---|---|---|---|
| Agent | Gender | T (min) | $ECT_{50}$ (Severe) (mg-min/$m^3$) | 95% Fiducial Limits | $LCT_{50}$ (mg-min/$m^3$) | 95% Fiducial Limits | $LCT_{50}$ (mg-min/$m^3$) | 95% Fiducial Limits |
| GF | Female | 10 | 222 | 213 to 231 | 267 | 256 to 278 | 253 | 244 to 266 |
| | Male | | 305 | 288 to 324 | 367 | 347 to 389 | 371 | 344 to 405 |
| GB | Female | | 187 | 179 to 197 | 226 | 216 to 235 | 235 | 228 to 243 |
| | Male | | 253 | 236 to 271 | 304 | 283 to 326 | 316 | 297 to 348 |
| GF | Female | 60 | 286 | 271 to 302 | 344 | 328 to 361 | 334 | 317 to 349 |
| | Male | | 335 | 319 to 352 | 403 | 384 to 423 | 396 | 376 to 416 |
| GB | Female | | 288 | 266 to 311 | 346 | 322 to 372 | 355 | 332 to 376 |
| | Male | | 359 | 335 to 384 | 432 | 405 to 461 | 433 | 409 to 464 |
| GF | Female | 240 | 447 | 425 to 471 | 539 | 513 to 565 | 533 | 506 to 566 |
| | Male | | 470 | 448 to 494 | 566 | 540 to 594 | 595 | 550 to 677 |
| GB | Female | | 686 | 623 to 757 | 826 | 753 to 907 | 840 | 766 to 922 |
| | Male | | 1090 | 1016 to 1169 | 1312 | 1222 to 1408 | 1296 | 1152 to 1486 |

**Table 9**

*Probit Slope(Concentration) Estimates for G-Type Nerve Agents IH Exposures from Ordinal Logistic Regression and Original Researchers.*

| | Estimates from Ordinal Logistic Regression | | Median and Range Reported by Original Researchers (24 hour post-exposure) | |
|---|---|---|---|---|
| Dataset | Probit Slope ($k_C$) | 95% Conf. Limits | Probit Slope ($k_C$) | Range of Values |
| Cresthull, et al (1957) | 9.1 | 6.4 to 11.9 | 11.0 | 6.6 to 15.4 |
| Mioduszewski, et al. (2001) | 13.9 | 12.3 to 15.5 | 13.2 | 8 to 24.4 |
| Anthony, et al. (2002) | 18.0 | 15.4 to 20.5 | 23.5 | 13.3 to 31.2 |

*Note: For shaded blocks above, Cresthull, et al. arrived at essentially one probit slope value for their entire dataset, along with an estimate for the standard error. Thus, instead of a range of values, the 95% confidence limits calculated from their standard error are shown in the table.*

**Table 10**

*Estimates for Distance (k) Between Severe (S) and Lethality (L) Dose-Response Curves for G-Type Nerve Agents IH Exposures from Ordinal Logistic Regression*

| | | Estimates from Ordinal Logistic Regression | | |
|---|---|---|---|---|
| Dataset | Species | S to L Distance (k) (normits) | Variance (S to L Dist) | 95% Conf. Limits |
| Cresthull, et al (1957) | Monkey | 1.02 | 0.0225 | 0.72 to 1.32 |
| Mioduszewski, et al. (2001) | Rat | 1.44 | 0.0069 | 1.28 to 1.61 |
| Anthony, et al. (2002) | Rat | 1.44 | 0.0100 | 1.24 to 1.65 |

**Table 11**

*$ECT_{50}/LCT_{50}$ Ratio Estimates for G-Type Nerve Agents IH Exposures from Ordinal Logistic Regression and Original Researchers*

| | Estimates from Ordinal Logistic Regression | | Median and Range Reported by Original Researchers (24 hour post-exposure) | |
|---|---|---|---|---|
| Dataset | ($ECT_{50}/LCT_{50}$) $10^{(k / k_C)}$ | 95% Conf. Limits | ($ECT_{50}/LCT_{50}$) $10^{(k / k_C)}$ | Range of Values |
| Cresthull, et al (1957) | 0.77 | 0.70 to 0.85 | 0.80 | 0.71 to 0.96 |
| Mioduszewski, et al. (2001) | 0.79 | 0.76 to 0.81 | | |
| Anthony, et al. (2002) | 0.83 | 0.81 to 0.85 | | |

## DISCUSSION

**Group $ECT_{50}$ and $LCT_{50}$ Estimates.** The estimates for median effective dosages for severe effects and lethality from ordinal logistic regression are in agreement with those reported by the original researchers for the datasets that were reviewed (see Tables 5 to 8). The means of the absolute percent differences (see Eqn. (14) below) were

found to equal 4.9, 2.1 and 2.6%, for the datasets from Cresthull, *et al.* (1957), Mioduszewski, *et al*. (2001) and Anthony, *et al*. (2002), respectively.

$$(14) \qquad \text{abs \% diff} = (100)\left|\frac{XCT_{50}\text{ (original)} - XCT_{50}\text{ (ordinal)}}{XCT_{50}\text{ (original)}}\right|$$

In the cases of Mioduszewski, *et al*. (2001) and Anthony, *et al*. (2002), values for $ECT_{50}$ (severe) were not reported, thus the $ECT_{50}$ (severe) values in Tables 7 and 8 from the ordinal regression analysis are the first such reported values for these datasets.

**Probit Slopes ($k_C$).** For each dataset, the probit slope (concentration) estimates from the ordinal regression are in agreement with those reported by the original researchers (see Table 9). These results confirm previous findings on the steepness of the DR-P curves for G-type nerve agents.[9,10] For the ordinal regression $k_C$ values, the differences between the $k_C$ values from the three datasets are statistically significant. The larger $k_C$ values (less individual variability) from the two rat studies (Mioduszewski, *et al*. and Anthony, *et al*.) (*vs.* the monkey study) is probably due to the genetically defined laboratory rats as compared to the monkeys used by Cresthull *et al.* However, other reasons for differences between the rat and monkey studies (batch effects, experimental error, *etc*.) cannot be entirely ruled out. Within the two studies investigating two or more agents (Cresthull, *et al*. and Anthony, *et al*.), the difference in probit slopes between the agent subsets are not statistically different; so, it is unlikely that the changes in probit slopes are due to differences between the agents.

**Distance (Normit) Between Severe and Lethality Dose-Response Curves.** The distance ($\kappa$) (see Eqn. (8)) is found to range from 1.02 to 1.44 normits for the three datasets reviewed (see Table 10). The average of $\kappa$ values equals 1.30. Values for $\kappa$ from these datasets were not previously reported.

Using $\kappa = 1.30$ for G-type nerve agent IH exposures, it is found that an $ECT_{16}$ (severe) approximately equals the $LCT_{01}$. Going both further up and down the dose-percent response curves, other equivalencies can be calculated (see Table 12). The steepness of the DR-S curve is readily demonstrated by the fact that the dosage causing incapacitation (or greater effect) in 84% of exposed individuals will also kill about half (45.4%) of those within the incapacitated (or greater) group. Furthermore, trying to use a G-type nerve agent to achieve complete incapacitation with minimal fatalities among a target group is an impossibility, since there will be an 85% lethality rate among the 99 out of 100 incapacitated subjects at an $ECT_{99}$ (severe).

**Table 12**

*Comparison of Equivalent $ECT_{XX}$ and $LCT_{YY}$ Levels for G-type Nerve Agent IH Exposures*

| $Y_N$ Severe (normits) | $Y_N$ Lethal (normits) | XX% Severe (or greater) | YY% Lethal | Ratio YY% to XX% |
|---|---|---|---|---|
| -2.00 | -3.3 | 2.3 | 0.0 | 2.1 |
| -1.00 | -2.3 | 15.9 | 1.1 | 6.8 |
| 0.00 | -1.3 | 50.0 | 9.7 | 19.4 |
| 1.00 | -0.3 | 84.1 | 38.2 | 45.4 |
| 2.00 | 0.7 | 97.7 | 75.8 | 77.6 |
| 2.31 | 1.01 | 99.0 | 84.4 | 85.3 |

Based on the estimated variances of the individual $\kappa$ values, there is a significant difference (with 99% confidence) between the monkey $\kappa$ value of Cresthull, *et al*. and the two rat $\kappa$ values of Mioduszewski, *et al*. and Anthony, *et al*. This suggests that the existence of a species effect on $\kappa$ values for G-type agent IH toxicity, particularly since the two separate rat studies produced identical $\kappa$ values. However, additional work is needed before any definitive conclusions can be reached.

In addition to using ordinal logistic regression to estimate $\kappa$ from quantal data sets, it is also possible to use Eqn. (7) to estimate $\kappa$ from historical studies where no raw quantal data is provided. All that is needed are estimates for $ECT_{50}/LCT_{50}$ and $k_C$, and it is not a requirement that the parameter estimates be taken from the same study.

**Ratio of $ECT_{50}$ and $LCT_{50}$ Values.** The $ECT_{50}/LCT_{50}$ ratio is found to range from 0.77 to 0.83 for the three datasets reviewed (see Table 11 and Eqn. (7)). Based on the estimated 95% confidence limits of the individual ratio values, there is no significant difference between the values from the three datasets. The average of the ratio values equals 0.80. Only Cresthull, *et al*. reported an estimate for the ratio, 0.80, which is in agreement with the ordinal regression ratio value of 0.77 for this dataset.

**Steepness of Dose-Response Curves.** The $ECT_{50}/LCT_{50}$ ratio represents a comparison between the steepness of the two DR curves (DR-P and DR-S) (see Eqn. (7)). There is no statistically significant species effect on $ECT_{50}/LCT_{50}$ (as mentioned previously). However, there is a species effect on both $\kappa$ (smaller for the monkey than for the rat) (see Table 10) and $k_C$ (smaller for the monkey than for the rat) (see Table 9). Thus, there is no change in $ECT_{50}/LCT_{50}$ values, since changes in both $\kappa$ and $k_C$ have roughly the same dependence on species. In practical terms, this means that the monkeys in Cresthull, *et al*., had more individual variability (lower $k_C$ value), but a steeper DR-S curve (lower $\kappa$ value), than the rats in Mioduszewski, *et al*. and Anthony, *et al.*

**Defining Threshold Lethality.** Historically, defining the threshold lethality for a nerve agent has been a difficult task.[18] The operational community needs threshold lethality estimates for purposes of modeling, exposure criteria, risk assessment, *etc*. Level 3 of the Acute Exposure Guideline Levels (AEGL-3) is an example of a threshold lethality exposure estimate.[27] In practical terms, a threshold lethality dosage is commonly defined as the dosage that will cause mortality in about 1% of the exposed individuals (a $LCT_{01}$).[10,11,18] Unfortunately, probit analysis is not suitable for accurate extrapolation from the 50% down to the 1% effect level.[1] Extrapolations beyond the 16% to 84% range are not recommended, as demonstrated by the widening fiducial limits in the example probit analysis plot shown in Figure 1. The shape of the probit plots (both fit and fiducial limits) is typical of what is expected:[1] large random errors are involved in estimating the two key values needed for the extrapolation, the $LCT_{50}$ and $k_C$.

The use of ordinal logistic regression provides a better approach to the problem of defining threshold lethality. For G-type agent IH exposures, the results from Table 12 demonstrate that an $ECT_{16}$ (severe) is equivalent to an $LCT_{01}$. Thus, instead of the questionable extrapolation from the median lethal dosage down to the 1%, the more statistically defensible extrapolation from the median effective (severe) dosage down to the 16% level can be performed instead. Thus, the concerns of the toxicologist about the limitations of probit analysis in estimating threshold lethality are satisfactorily addressed.

## CONCLUSIONS

Estimation of the relationship between the DR curves for lethality and severe effects has been accomplished for inhalation exposures to G-type nerve agents via the use of ordinal logistic regression on data from three previously conducted animal studies.

Knowledge of the mathematical relationship between the two curves provides a better means to define threshold lethality dosage by using the dose-severe effect curve in its place. The use of ordinal logistic regression is statistically and toxicologically defensible for this application, thereby addressing concerns with the known limitations of probit analysis (the previously used approach).

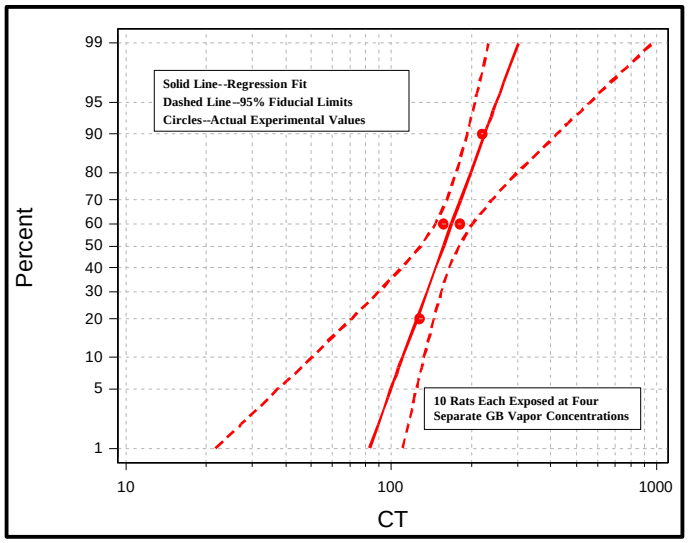


**Figure 1**

*Percent Cumulative Probability as a Function of Dosage for Female Sprague-Dawley—Five Minute GB IH Exposure from Mioduszewski, et al. (2001)*

For inhalation exposures to G-type agents, it was found that an $ECT_{16}$ (severe) is equivalent to a $LCT_{01}$ (a distance of 1.27 standard deviations). At the 16% level for severe effects, it is not improbable that an occasional death will occur among any small group of untreated victims with severe effects (convulsions, *etc*.)—exactly what is meant by threshold lethality. By defining threshold lethality using a sub-lethal endpoint, a safe and conservative approach is achieved, with a higher degree of statistical confidence.

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