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

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The U.S. Environmental Protection Agency has developed a categorical logistic regression approach for regressing ordered response categories on one or more factors due to toxicant exposures. This approach can also be used in analyzing chemical warfare agent toxicity. Towards this end, three previous mammalian studies (involving acute inhalation exposures to G-type nerve agents) were reviewed and analyzed. For all three studies, slightly more than one standard deviation separated an effective concentration (EC_{XX}) for severe effects from a lethal concentration (LC_{XX}) for XX% affected. Such knowledge can be used to better estimate threshold lethality and characterize the dose-response curves.

I. 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; emergency response procedures; *etc*. Modeling and simulation (M&S) plays an important role towards this end. For these models, toxicity needs to be 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] 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).

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A better approach is to investigate multiple endpoints in the same study, as was done by Cresthull, *et al*.[11] who reported both severe effects and lethality as a function of C and T. A separate probit analysis was performed 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. Important information was ignored which could better characterize the steepness of the DR curve.

One solution to this problem is to employ categorical (or ordinal) logistic regression.[12] The U.S. Environmental Protection Agency (EPA) is currently developing this method for its own applications (such as supporting the Benchmark Dose (BMD) model).[13-16] 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[4,5,7,8,11] 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[17] of this type of knowledge were then explored.

II. TOXICITY STUDIES REVIEWED

1. OVERVIEW. The three studies reviewed for this work were Cresthull, *et al*. (1957),[11] Mioduszewski, *et al*. (2001 and 2002),[4,5] and Anthony, *et al*. (2003).[7,8] All 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.

TABLE 1: Summary of CW Nerve Agent Studies Reviewed

Name of Study	Year(s) Conducted	Agent(s) Studied	Species Used	Total Number of Subjects	Gender	Subjects by Agent	Subjects per Test Group	Number of Runs	Vapor Concentrations (mg/m^3)	Exposure Times (minutes)	Primary Endpoint(s)
Cresthull, et al. (1957)	1953 to 1954	GA, GB and GF	Rhesus Monkey	152	Mostly Female	GA (56) GB (52) GF (44)	4	36	GA: 18.1 to 81 GB: 6.6 to 29.1 GF: 7.3 to 59	2 and 10	Incap. & Lethality (1 d)
Mioduszewski, et al. (2001 & 2002)	1998 to 2000 (two phases)	GB	Sprague-Dawley Rat	700	Males (350) Females (350)	All GB	10 or 20	43	2.0 to 54.4	5, 10, 30, 60, 90, 240 and 360	Lethality (1 & 14 d)
Anthony, et al. (2002)	2001 to 2002	GF and GB	Sprague-Dawley Rat	500	Males (240) Females (260)	GF (320) GB (180)	5, 10 or 20	38	GB: 3.5 to 35.9 GF: 2.0 to 41.9	10, 60 and 240	Lethality (1 & 14 d)

In all three studies, the animals were exposed (whole body) in dynamic airflow inhalation chambers.[18] 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. 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).[12] 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]

2. 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.

3. 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 (Cresthull, *et al*.).

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

III. STATISTICAL THEORY

Probit analysis was the method used by Cresthull, *et al*.,[11] 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.[12] 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.[2,12,21] 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.

1. 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.[1,5,19,20]

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) $ECT_{50} = \text{antilog}(\eta)$

(2) $m = 1 / \sigma$

(3) $Z = \dfrac{\{\log (CT) - \log(ECT_{50})\}}{\sigma}$

where η is the median of log(effective dosage), σ^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.

TABLE 3. Rat GB IH Quantal Data (Mioduszewski, *et al*.)

T (min)	C (mg/m³)	CT (mg-min/m³)	Male Number per Category M	S	L	C (mg/m³)	CT (mg-min/m³)	Female Number per Category 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

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.[1,3,5,20] 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,[1,21] and for vapor toxicity studies Equation (4) is fitted:[1]

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

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

4

vapor exposure usually refers to the slope on vapor concentration ($m = k_C$) instead of the slope on time. The greater the 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*.).[22] The effect of such heterogeneity will be to reduce the probit slope. Also, probit analysis on its own can only characterize the DR-P curve for a particular endpoint.

2. ORDINAL LOGISTIC REGRESSION. Conceptually, ordinal regression simply involves the division of ordered multi-level categorical responses into a series of cumulative binary responses.[22] 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]

TABLE 4. Rat GF and GB IH Quantal Data (Anthony, *et al.*)

Gender	T (min)	GF C (mg/m³)	CT (mg-min/m³)	M	S	L	GB C (mg/m³)	CT (mg-min/m³)	M	S	L
Female	10	17.2	172	9	1	0	18.0	180	0	10	0
		21.5	215	7	3	0	21.6	216	4	5	1
		23.3	233	10	0	0	22.7	227	0	8	2
		23.9	239	2	3	5	23.8	238	0	3	7
		25.2	252	2	2	6	24.8	248	0	3	7
		26.9	269	1	3	6	26.6	216	0	0	10
		31.1	311	0	0	10					
Male	10	17.2	172	10	0	0	22.7	227	8	2	0
		21.5	215	10	0	0	26.7	267	1	8	1
		31.1	311	1	8	1	28.7	287	0	6	4
		34.4	344	5	3	2	32.8	328	0	5	5
		41.9	419	0	1	9	35.9	287	0	2	8
Female	60	4.9	294	6	2	2	5.6	336	0	4	1
		5.7	342	4	4	2	6.1	366	0	4	6
		5.9	354	0	1	9	6.6	396	0	0	5
		6.4	384	0	0	10					
		7.2	432	0	0	10					
Male	60	4.9	294	10	0	0	6.6	396	1	4	0
		5.7	342	5	4	1	7.0	420	1	5	4
		6.4	384	0	6	4	7.5	450	0	1	4
		7.2	432	1	2	7					
		7.8	468	0	0	10					
Female	240	2.0	480	7	2	1	3.5	840	0	5	5
		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	240	2.0	480	3	4	3	4.3	1032	8	2	0
		2.0	480	4	6	0	5.6	1344	0	3	7
		2.2	528	0	8	2					
		2.5	600	1	3	6					
		3.3	792	0	1	9					

(5) $\quad Y_N\{0|1,2\} = k_{\{0|1,2\}} + k_C \log C + k_T \log T + k_i \,(\text{other factors})$

(6) $\quad Y_N\{0,1|2\} = k_{\{0,1|2\}} + k_C \log C + k_T \log T + k_i \,(\text{other factors})$

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 intercepts, $k_{0|1,2}$ and $k_{0,1|2}$, are for the normits of the cumulative probabilities of an effect exceeding in severity the low {0} and medium {1} responses, respectively.[22]

When using Eqns. (5) and (6), it is implicitly assumed that the values of the individual 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).[12,21] An iterative-reweighted least squares algorithm is used to obtain maximum likelihood parameter estimates.[21,23]

3. 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 (R_η), Eqns. (5) and (6) can rearranged to produce:

$$(7) \qquad \log_{10}\left[\frac{ECT_{50}}{LCT_{50}}\right] = R_\eta = \kappa / k_C \qquad\qquad (8) \qquad \kappa = \left[k_{\{0,1|2\}} - k_{\{0|1,2\}}\right] = \left[k_{severe} - k_{lethal}\right]$$

where κ is the distance in normits between the percent affected levels of the severe and lethality DR curves. For instance, when κ 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 κ equals two, then ECT_{84} equals a LCT_{16}.

Confidence limits on estimates for both R_η and κ can be calculated. Barry (1978)[24] gives the standard error of a ratio, (a / b), which is based upon the propagation of error formula for a ratio:

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

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) $\pm$ (2)(std err). The following relations from Mood, *et al.* (1974)[25] were also used to get the necessary information for determining the limits for both R_η and κ:

$$(10) \qquad \text{var(a} \pm \text{b)} = \text{var}(a) + \text{var}(b) \pm (2)\text{cov(a, b)} \qquad\qquad (11) \qquad \text{cov}(a \pm b, c) = \text{cov}(a, c) \pm \text{cov}(b, c)$$

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

IV. DATA ANALYSIS

An ordinal logistic regression program (a component of MINTAB® Version 13) was used to perform the calculations. The three datasets (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)):

$$(12) \qquad Y_N\{severe\} = k_{severe} + k_C \log C + \sum_i^N k_i G_i \qquad\qquad (13) \qquad Y_N\{lethal\} = k_{lethal} + k_C \log C + \sum_i^N k_i G_i$$

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), R_η and κ for the whole dataset, as well as

6

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, R_η and κ 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, R_η and κ were equal in value for the whole dataset.

In addition to calculating values for k_C, R_η and κ for each dataset, Eqns. (9) to (11) were used (with the variance-covariance matrix of the model fit returned by MINTAB®) to estimate the errors associated with these values (as well as for those associated with individual group ECT_{50} and LCT_{50} values).

V. RESULTS

The data analysis results are shown in Tables 5 to 10. Tables 5 to 7 have the individual group ECT_{50} and LCT_{50} estimates, while Tables 8 to 10 present the estimated probit slope (k_C), κ and R_η values for each dataset, respectively. When available, previously reported values are shown for comparison.

TABLE 5. Monkey G-Type IH ECT_{50} (Severe) & LCT_{50} Values from Present Study & Cresthull, *et al.*

		Severe Effects				Lethality			
		Estimates from Ordinal Logistic Regression		Cresthull, et al (1957) (24 hours Post-Exposure)		Estimates from Ordinal Logistic Regression		Cresthull, et al (1957) (24 hours Post-Exposure)	
Agent	T (min)	ECT_{50} (mg-min/m^3)	95% Fiducial Limits	ECT_{50} (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
GA		102	90 to 115	102	none reported	131	118 to 146	135	123 to 152
GB	2	36	31 to 40	30	none reported	46	40 to 53	42	29 to 60
GF		58	49 to 70	62	none reported	76	65 to 88	75	63 to 87
GA		145	121 to 173	<180	none reported	187	161 to 217	187	164 to 221
GB	10	56	46 to 67	<66	none reported	72	61 to 85	74	62 to 87
GF		96	82 to 112	100	none reported	124	108 to 143	130	112 to 151

VI. DISCUSSION

1. 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 7). 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.* (2003), respectively.

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

Mioduszewski, *et al.* and Anthony, *et al.* did not report values for ECT_{50} (severe). The ECT_{50} values in Tables 6 and 7 from the present analysis are the first such reported values for these datasets.

2. PROBIT SLOPES (k_C). For each dataset, the k_C estimates from the ordinal regression are in agreement with those originally (see Table 8). 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.

    3. DISTANCE (NORMIT) BETWEEN SEVERE AND LETHALITY DOSE-RESPONSE CURVES. The distance (κ) (see Eqn. (8)) is found to range from 1.02 to 1.44 normits for the three datasets reviewed (see Table 9). The average of κ values equals 1.30. Values for κ 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 11). 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).

    Based on the estimated variances of the individual κ values, there is a significant difference (with 99% confidence) between the

TABLE 6. Rat GB IH ECT_{50} (Severe) and LCT_{50} Values from Present Study and Mioduszewski, *et al.*

Gender	T (min)	ECT_{50} (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
		Estimates Derived from Ordinal Logistic Regression				Mioduszewski, et al (2001) (24 hours Post-Exposure)	
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

TABLE 7. Rat GF and GB IH ECT_{50} (Severe) and LCT_{50} Values from Present Study and Anthony, *et al.*

Gender	T (min)	ECT_{50} (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
		Estimates Derived from Ordinal Logistic Regression				Anthony, et al (2001) (24 hours Post-Exposure)	
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
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
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	10	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
Female	60	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
Female	240	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

8

TABLE 8. Probit Slope (Concentration) Estimates for G-Type Agents IH Exposures.

Dataset	Estimates from Ordinal Logistic Regression		Original Median and Range Reported (24 hour post-exposure)	
	Probit Slope (k_C)	95% Confidence 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: 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. ECT$_{50}$/LCT$_{50}$ Ratio (R_η) Estimates for G-Type Nerve Agents IH Exposures

Dataset	Estimates from Ordinal Logistic Regression		Original Median and Range Reported (24 hour post-exposure)	
	R_η $10^\wedge(k/k_C)$	95% Conf. Limits	R_η $10^\wedge(k/k_C)$	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		

TABLE 9. Estimates for Distance (κ) Between Severe (S) and Lethality (L) Dose-Response Curves for G-Type Nerve Agents IH Exposures.

Dataset	Species	Estimates from Ordinal Logistic Regression		
		Distance (S to L) (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. Comparison of Equivalent ECT$_{XX}$ and LCT$_{YY}$ Levels for G-type 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

monkey κ value of Cresthull, *et al*. and the two rat κ values of Mioduszewski, *et al*. and Anthony, *et al*. This suggests that the existence of a species effect on κ values for G-type agent IH toxicity, particularly since the two separate rat studies produced identical κ values. However, additional work is needed before any definitive conclusions can be reached.

In addition to using ordinal logistic regression to estimate κ from quantal data sets, it is also possible to use Eqn. (7) to estimate κ from historical studies where no raw quantal data is provided. Only estimates for R_η and k_C are needed, and it is not necessary to use estimates from the same study.

4. RATIO OF ECT$_{50}$ AND LCT$_{50}$ VALUES (R_η). R_η is found to range from 0.77 to 0.83 for the three datasets reviewed (see Table 10 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.

5. 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 R_η (as mentioned previously). However, there is a species effect on both κ (smaller for the monkey than for the rat) (see Table 9) and k_C (smaller for the monkey than for the rat) (see Table 8). Thus, there is no change in R_η values, since changes in both κ 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 κ value), than the rats in Mioduszewski, *et al*. and Anthony, *et al*.

6. DEFINING THRESHOLD LETHALITY. Historically, it has been difficult to define the threshold lethality.[17] The operational community needs threshold lethality estimates for modeling purposes, exposure criteria, risk assessment, *etc*. Level 3 of the Acute Exposure Guideline Levels (AEGL-3) is an example of a threshold lethality estimate.[26] 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}).[10,17] 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.

Instead, 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 11 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.

VII. CONCLUSIONS

Estimation of the relationship between the lethality and severe effects DR curves for IH exposures to G-type nerve agents has been accomplished via the use of ordinal logistic regression on data from three previous animal studies. Knowledge of the mathematical relationship between the two curves provides a better way 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 current approach).

For IH 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.

ENDNOTES

* The definition of an effective dosage (ECT_{XX}) has aspects from both types of DR 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. Thus, cumulative measures are found for both DR-S (an effect of equal or greater severity) and DR-P (XX%) curves.

** 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 is included in an effect category. Thus, the above example [2, 1, 1] would be written instead as [4, 2, 1].

*** 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).[19] For IH exposures, dosage is the term used.[19]

REFERENCES

[1] Finney, DJ, **Probit Analysis**. Third Edition, Cambridge University Press, Cambridge, 1971.

[2] Hosmer, DW and Lemeshow, S, **Applied Logistic Regression**. John Wiley & Sons, New York, 1989.

[3] Bliss, CI, *The Determinations of the Dosage-Mortality Curve from Small Numbers*, **Quarterly Journal of Pharmacology**. **2**: 192-216, 1938.

[4] Mioduszewski, RJ, *et al*., *Interaction of Exposure Concentration and Duration in Determining Acute Toxic Effects of Sarin Vapor in Rats*, **Toxicological Sciences**. **66**: 176-184, 2002.

[5] Mioduszewski, RJ, *et al.*, *ECBC Low Level Operational Toxicity Program: Phase I—Inhalation Toxicity of Sarin Vapor in Rats as a Function of Exposure Concentration and Duration*, **ECBC-TR-183**. US Army ECBC, APG, MD, August 2001.   AD# A394372.

[6] Mioduszewski, RJ, *et al.*, *Low Level Sarin Vapor Exposure in Rats: Effect of Exposure Concentration and Duration on Pupil Size*, **ECBC-TR-235**. US Army ECBC, APG, MD, May 2002.   AD# A402869.

[7] Anthony, SJ, *et al.*, *Inhalation Toxicity of GF Vapor in Rats as a Function of Exposure Concentration and Duration and Its Potency Comparison to GB*, presented at **Biosciences 2002 Medical Defense Review**, US Army MRICD, Hunt Valley, MD, 2-7 June 2002.

[8] Anthony, SJ, *et al.*, *Inhalation Toxicity of GF Vapor in Rats as a Function of Exposure Concentration and Duration and Its Potency Comparison to GB*, **ECBC-TR-335**. US Army ECBC, APG, MD, November 2003.

[9] Reutter, S, *Hazards of Chemical Weapons Release during War: New Perspectives*, **Environmental Health Perspectives**. **107**(12): 985-990, December 1999.

[10] Watson, A, *et al.*, *Nerve Agents, GA, GB, GD, GF, and VX: Acute Exposure Guideline Levels*, Appendix 1 in Clark, K, ed., **Acute Exposure Guideline Levels for Selected Airborne Chemicals. Vol. 3**. National Research Council, Subcommittee on Acute Exposure Guideline Levels (Bakshi, KS, Chair), The National Academies Press, Washington, DC, pp. 15-300, 2003.

[11] Cresthull, P, *et al.*, *Inhalation Effects (Incapacitation and Mortality) for Monkeys Exposed to GA, GB, and GF Vapors*, **CWLR 2179**. US Army Chemical Warfare Laboratories, Army Chemical Center, Edgewood Arsenal, MD, 16 September 1957. AD# 145581.

[12] Agresti, A, **Categorical Data Analysis**. John Wiley & Sons, New York, 1990.

[13] Allen, BC, *et al.*, *Categorical Regression for Dose-Response Modeling of Toxicity Data and It's Application to RfD/C Development*, **Department of Defense Workshop**. Sponsored by the US EPA, National Center for Environmental Assessment, Cincinnati, OH. Held 27 April 1998, Wright-Patterson Air Force Base, Dayton, OH.

[14] US EPA, *The Use of the Benchmark Dose Approach in Health Risk Assessment*, **EPA/630/R-94/007**. US EPA, Office of Research and Development, Washington, DC, 1995.

[15] Guth, DJ, *et al.*, *Categorical Regression Analysis of Acute Exposure to Tetrachloroethylene*, **Risk Analysis**. **17**(3): 321-332, 1997.

[16] Dourson, ML, *et al.*, *Novel Methods for the Estimation of Acceptable Daily Intake*, **Toxicology and Industrial Health**. **1**(4): 23-41, 1985.

[17] Sommerville, DR, *A Novel Idea on How to Define Threshold Lethality for Nerve Agent Inhalation Toxicity*, **Third Annual Decontamination Commodity Area Conference**. Salt Lake City, UT, sponsored by the Joint Service Material Group's Commodity Area Manager for Decontamination, 23-26 May 2000.

[18] MacFarland, HN, *Designs and Operational Characteristics of Inhalation Exposure Equipment*, in Salem, H, ed. **Inhalation Toxicology**, Marcel Dekker, New York. pp. 93-120, 1987.

[19] Salem, H, *Principles of Inhalation Toxicology*, in Salem, H, ed. **Inhalation Toxicology**. Marcel Dekker, Inc., New York, pp. 1-33, 1987.

[20] Crosier, RB, and DR Sommerville, *Relationship Between Toxicity Values for the Military Population and Toxicity Values for the General Population*, **ECBC-TR-224**. US Army ECBC, APG, MD, UNCLASSIFIED, March 2002. AD# A400214.

[21] **MINITAB^{TM} Statistical Software**, Release 13.32. MINITAB Inc., 3081 Enterprise Drive, State College, PA 16801-3008, website: /**www.minitab.com**/. 2002.

[22] Franks, AP, *et al.*, *The Relationship Between Risk of Death and Risk of Dangerous Dose for Toxic Substances*, **Journal of Hazardous Materials**. **51**: 11-34, 1996.

[23] McCullagh, P, and Nelder, J, **Generalized Linear Models**, Chapman and Hall, NY, 1992.

[24] Barry, BA, **Errors in Practical Measurement Science, Engineering and Technology**. John Wiley & Sons, Inc., NY, 1978.

[25] Mood, AM, *et al.*, **Introduction to the Theory of Statistics**. Third Edition, McGraw-Hill, NY, 1974.

[26] Crossgrove, RE, ed., **Standing Operating Procedures for Developing Acute Exposure Guideline Levels for Hazardous Chemicals**. National Research Council, Committee on Toxicology, Subcommittee on Acute Exposure Guideline Levels (Krewski, D, Chair), National Academy Press, Washington, DC, 2001.