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Estimation of Human Toxicity from Animal Inhalation Toxicity Data:

2 (Abridged). GB Toxicity Reassessed Using Newer Techniques for Estimation of Human Toxicity

R.W. Bide, S.J. Armour and E. Yee
Defence R&D Canada – Suffield

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Abstract

Estimated human inhalation toxicity values for GB were calculated using a new three dimensional, non-linear dose response (toxicity) model combined with re-evaluated allometric equations relating animal and human respiration. Historical animal studies of GB toxicity containing both exposure and fractional animal response data were used to test the new process. The final data set contained 6621 animals, 762 groups, 37 studies and 7 species. The toxicity of GB for each species was empirically related to exposure concentration (***C***; mg/m^3) and exposure time (***T***; min) through the surface function

$$\boldsymbol{Y} = b_0 + b_1 \text{Log}_{10}\boldsymbol{C} + b_2 \text{Log}_{10}\boldsymbol{T} \qquad \text{or} \qquad \boldsymbol{Y} = b_0 + b_2 \text{Log}_{10}\boldsymbol{C}^{\boldsymbol{n}}\boldsymbol{T}$$

where ***Y*** is the PROBIT, b_0, b_1 and b_2 are constants and ***n*** is the "toxic load exponent". Between exposure times 0.17 and 30 min, the average value for ***n*** in 7 species was 1.35 ± 0.15. The near parallel toxic load equations for each species and the linear relationship between minute volume/ body weight ratio and the inhalation toxicity (LCt_{50}) for GB were used to create a pseudo-human data set and then an exposure time/toxicity surface for the human. The calculated ***n*** for the human was 1.38 ± 0.01. The pseudo-human data had much more variability at low exposure times. Raising the lower exposure limit to one min did not change the LCt_{50} but did result in lower variability. Raising the lower value to 2 min was counterproductive. Based on the toxic load model for 1 - 30 min exposures, the ***n*** value was 1.40 and the human GB toxicities (LCt_{01}, LCt_{05}, LCt_{50} and LCt_{95}) for 70 kg humans breathing 15 L/min were estimated to be 11, 16, 36 and 83; 18, 25, 57 and 132; 24, 34, 79 and 182 $mg.min/m^3$ for 2, 10 and 30 min exposures, respectively. These values are recommended for general use for the total human population. The empirical relationships employed in the calculations may not be valid for exposure times >30 min.

Resumé

Les valeurs humaines de toxicité par inhalation ont été calculées, pour l'extrait à l'éther, en utilisant un nouveau modèle (de toxicité), tridimensionnel et non linéaire de réaction à une dose, combinés à des équations allométriques réévaluées qui font la relation entre la respiration humaine et la respiration animale. Les études historiques de toxicité du sarin chez les animaux contenant les données de la réaction des animaux à la fois avec exposition et fractionnée ont été utilisées pour tester le nouveau procédé. L'ensemble des données finales contenait 6621 animaux, 762 groupes, 38 études et 7 espèces. La toxicité du sarin par inhalation pour chaque espèce était empiriquement liée à la concentration de l'exposition (***C*** ; mg/m^3) et à la durée de l'exposition (*T*; min) par la fonction de surface**.**

$$\boldsymbol{Y} = b_0 + b_1 \text{Log}_{10}\boldsymbol{C} + b_2 \text{Log}_{10}\boldsymbol{T} \qquad \text{ou} \qquad \boldsymbol{Y} = b_0 + b_2 \text{Log}_{10}\boldsymbol{C}^{\boldsymbol{n}}\boldsymbol{T}$$

Quand ***Y*** est le PROBIT, b_0, b_1 et b_2 sont les constantes et ***n*** est «l'exposant de la charge toxique ». Pour des durées d'exposition allant de 0,17 à 30 min, la valeur moyenne de ***n*** était de 1,35 ± 0,15 chez les 7 espèces. Les équations quasi parallèles de la charge toxique pour les 7 espèces et la relation linéaire entre le rapport volume d'air aspiré par minute/poids du corps et la toxicité de l'inhalation (CLt_{50}) pour le sarin ont été utilisées pour créer des ensembles de données pseudo humaines et puis un rapport durée d'exposition / surface de toxicité pour les humains. Le ***n*** calculé pour les données humaines allant de 0,17 à 30 min était de 1,38 ± 0,01. Les données pseudo humaines étaient beaucoup plus variables à des durées plus faibles d'exposition. L'augmentation à une minute de la valeur la plus basse d'exposition n'a pas changé la CLt_{50} mais a résulté en une plus faible variabilité. Il s'est avéré contre-productif d'augmenter la valeur la plus basse à 2 minutes. En se basant sur le modèle de la charge toxique pour des expositions allant de 1 à 30 minutes, la valeur ***n*** était de 1,40 et les toxicités humaines pour le sarin **(**CLt_{01}, $CLt_{05,}$ CLt_{50} et CLt_{95}) pour des humains pesant 70 kg et respirant 15 L par minute ont été estimées être respectivement de 11, 16, 36 et 83 ; 18, 25, 57 et 132 ; 24, 34, 79 et 182 $mg.min/m^3$ pour 2, 10 et 30 min d'exposition. Ces valeurs sont recommandées pour l'usage général pour la population humaine totale. Le rapport empirique employé dans les calculs peuvent ne pas être valides pour les durées d'exposition >30 min.

Executive summary

Background: Hazard assessments of a known or potential chemical warfare agents (CWA) for the military require knowledge of the corresponding human toxicity. As these cannot be determined directly, human estimates must be extrapolated from animal toxicity experiments. For inhalation challenges, toxicity is usually expressed as the LCt_{50}, the exposure (the product of the concentration (***C***) of an agent and the exposure time (***T***)) required to kill 50% of a population. However, for the development of protective measures and the estimation of casualty rates, LCt_{05} (or better, LCt_{01}) values are more useful. While the military literature repeatedly states that the LCt_{50} varies with the ***CT*** product, for many agents, the toxicity and exposure time are related by the "toxic load model" or C^nT, where ***n*** is determined empirically. Use of the ***CT*** product can lead to serious errors in predicting hazards and potential casualties.

The techniques for extrapolation from animal data to human estimates have been very controversial. While there are a variety of procedures for the estimation of human LCt_{50} values, no reproducible and reliable methods have been available for the estimation of human LCt_{01} and LCt_{05} values.

Results: To address these deficiencies, a novel procedure has been devised using the toxic load model to, first, obtain more reliable values for toxicity in animal species and, second, to create a toxic load model for humans, from which a range of toxicity estimates can be readily obtained. The methodology is simple, rational, and consistent. No values are inferred, no assumptions are made based upon other, separate data, and subjective assessments to justify including or rejecting data have been minimized. However, as the toxic load models for both animals and humans are empirical in nature, toxicity estimates can only be made within the range of the data used for the derivations.

The procedure has been applied to the data from 38 historical animal studies of sarin (GB) toxicity involving 7 species, 762 animal groups and 6621 animals. Toxic load models were developed for each species, a pseudo-human data set was derived and a human toxic load model prepared. The amount of data used in the calculations is sufficiently large that the effects of small numbers of aberrant data points upon the calculations are minimized by weight of numbers. Similarly, the potential effect of additional information on the LCt_{50} would be minimal, but the LCt_{05} and LCt_{95} values might be slightly affected. Based on the toxic load model for 1 - 30 min exposures, the ***n*** value was 1.40 and the human GB toxicities (LCt_{01}, LCt_{05} LCt_{50} and LCt_{95}) for 70 kg humans breathing 15 L/min were estimated to be 11, 16, 36 and 83; 18, 25, 57 and 132; and 24, 34, 79 and 182 mg.min/m^3 for 2, 10 and 30 min exposures, respectively. These values are recommended for general use for the total human population for exposures between 1 and 30 min. Values for other exposure times may be calculated from the toxic load model. However, because of the empirical relationships employed, calculations for longer exposures may not be valid.

Prior to this work, the best assessment (LCt_{50} value) of human toxicity for GB, provided to US forces at the time of the 1990-91 Gulf War, was 35 mg.min/m^3 for a 2 min exposure of a 70 kg human breathing 15 L/min. Previous estimates of human toxicity had been in the range 50-100 mg.min/m^3 and did not indicate the critical exposure times and respiration rates involved. The LCt_{50} value from this study of 36 mg.min/m^3 for a 2 min exposure is in good agreement with the US Gulf War estimate, while the predicted values for longer exposures (e.g. 57 mg.min/m^3 for a 10 min exposure of a 70 kg human breathing 15 L/min) are similar to earlier estimates. This work also reports the first LCt_{01}, LCt_{05} and LCt_{95} values derived directly from experimental animal toxicity data.

Significance: A robust methodology has been developed for the estimation, not only of human LCt_{50} values, but also the exposure required to produce any level of lethality in a given population. The results of this study for GB, and analogous work for other CWA, used in conjunction with atmospheric dispersion models, would provide much more reliable estimates of casualties to be expected from chemical attacks.

Future Plans: Animal toxicity data for other CWA will be evaluated using the method developed.

Bide, R.W., Armour, S.J. and Yee, E. (2004). Estimation of human toxicity from animal inhalation toxicity data: 2. (Abridged). GB Toxicity reassessed using newer techniques for estimation of human toxicity. (DRDC Suffield TR 2004 - 167). Defence R&D Canada – Suffield. UNCLASSIFIED.

Sommaire

Contexte : L'évaluation des risques, pour l'armée, d'un agent de guerre chimique connu ou potentiel (agents chimiques de guerre) requiert d'en connaître la toxicité correspondante sur les humains. Comme il n'est pas possible de déterminer cette dernière directement, les estimations de l'effet sur les humains doivent être extrapolées à partir des expériences de toxicité sur les animaux. Pour les tests de provocation d'inhalation, la toxicité est généralement exprimée par $CLt_{50,}$ l'exposition étant le produit de la concentration (***C***) d'un agent et la durée d'exposition (***T***) requise pour tuer 50% de la population. Pour la mise au point de mesures de protection et l'estimation des taux de pertes, CLt_{05}, (ou mieux CLt_{01}) sont cependant des valeurs plus utiles. Alors que la documentation militaire répète que CLt_{50} varie avec le produit ***CT***, pour beaucoup d'agents, la toxicité et la durée d'exposition sont liées au « modèle de la charge toxique » ou C^nT quand ***n*** est déterminé empiriquement. L'utilisation du produit ***CT*** peut entraîner de graves erreurs dans la prédiction des risques et des victimes potentielles.

Les techniques d'extrapolation à partir des données animales vers les estimations humaines ont beaucoup prêté à la controverse. Alors qu'il existe une variété de procédures pour estimer les valeurs humaines CLt_{50}, il n'y a pas de méthode reproductible ni fiable pour calculer le CLt_{01} humain et les valeurs CLt_{05}.

Résultats : Pour remédier à ces faiblesses, une nouvelle procédure utilisant le modèle de la charge toxique, a été créée, d'abord pour obtenir des valeurs fiables de toxicité chez différentes espèces d'animaux puis pour créer un modèle de charge toxique chez les humains, à partir duquel un éventail d'estimation de toxicité peut être facilement obtenu. La méthodologie est simple, rationnelle et constante. Aucune valeur n'est inférée, aucune hypothèse n'est basée sur d'autres hypothèses ; les données séparées et les évaluations subjectives justifiant l'inclusion ou le rejet des données ont été minimisées. Cependant, comme les modèles de charge toxique chez les animaux et les humains sont empiriques en nature, les estimations de toxicité ne peuvent qu'être faites dans les limites des données utilisées pour les dérivations.

La procédure a été appliquée aux données tirées de 38 études historiques sur la toxicité du sarin (GB) chez les animaux comprenant 7 espèces, 762 groupes d'animaux et 6621 animaux. Les modèles de la charge toxique ont été mis au point pour chaque espèce, un ensemble de données pseudo humaines a été dérivé et un modèle de charge toxique a été préparé. Le montant de données utilisées dans les calculs est suffisamment important pour que l'effet du petit nombre de données simples aberrantes provenant des calculs soit minimisé par la quantité des données. De manière similaire, l'effet potentiel de l'information additionnelle sur CLt_{50} serait minime, mais les valeurs CLt_{05} et CLt_{95} seraient légèrement affectées. En se basant sur le modèle de la charge toxique pour une exposition allant de 1 à 30 minutes, la valeur ***n*** était de 1,40 et les toxicités GB chez les humains (CLt_{01}, CLt_{05} CLt_{50} et CLt_{95}) pour des humains de 70 kg, aspirant 15L par minute ont été estimées être respectivement de 11, 16, 36 et 83 ; 18, 25, 57 et 132 ; et 24, 34, 79 et 182 $mg.min/m^3$ pour des expositions de 2, 10 et 30 minutes. Il est possible de calculer les valeurs pour d'autres durées d'exposition à partir du modèle de la charge toxique. Cependant, les relations employées étant empiriques, les calculs pour les durées plus longues d'exposition risquent de ne pas être valides.

Antérieurement à ces travaux, la meilleure évaluation (valeur de CLt_{50}) de la toxicité GB chez les humains fournie aux Forces américaines durant la Guerre du Golfe en 1990- 1991, était de 35 mg.min/m^3 pour une exposition de 2 minutes chez un humain de 70 kg, aspirant 15 L/min. Les estimations précédentes de la toxicité humaine étaient de 50 à 100 mg.min/m^3 et n'indiquaient pas les durées critiques d'exposition et les taux de respiration n'étaient pas considérés. La valeur CLt_{50} de l'étude de 36 mg.min/m^3 pour une exposition de 2 minutes correspond à l'estimation américaine de la Guerre du Golfe, alors que les valeurs prédites pour des expositions d'une durée plus longue (p.ex. 57 mg.min/m^3 pour une durée d'exposition de 10 min chez un humain de 70 kg, aspirant 15L/min) sont similaires aux estimations antérieures. Ces travaux documentent aussi les premières valeurs CLt_{01}, CLt_{05}, et CLt_{95} directement dérivées des données expérimentales de la toxicité chez les animaux.

La portée des résultats : Une méthodologie robuste a été mise au point, non seulement pour les valeurs CLt_{50} humaines mais aussi pour les durées d'exposition requises visant à produire n'importe quel niveau de létalité chez une population donnée. Les résultats de cette étude du GB ainsi que ceux des travaux analogues sur les agent chimiques de guerre, fourniraient des estimations plus fiables sur le nombre de victimes auquel on pourrait s'attendre en cas d'attaque chimique si elles étaient utilisées en conjonction avec les modèles de dispersion atmosphérique.

Plans futurs : Les données de toxicité chez les animaux pour d'autres CWA seront évaluées en utilisant la méthode mise au point.

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Introduction

In the post WWII period, the toxicity of GB was studied extensively as the Western Nations mounted international cooperative programs to assess the military impact of the then newly appreciated nerve agents and to develop protective and therapeutic measures to counteract the observed threat. These assessments required estimates of the toxicity of the agents for humans, particularly LCt_{05} or better for protection. The most reliable source of human toxicity data is fully documented human exposures. However, such information is difficult to obtain and what little data that is available contain only marginal estimates of the dosages involved.

In the absence of human data, the toxicologist must rely upon animal toxicity data and make extrapolations to the human condition. Experimental/mathematical procedures are available for the estimation of human LCt_{50} [1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13]. However, these procedures fail when applied to LCt_{05} and LCt_{01} and estimations of these values have had to rely upon subjective comparisons and multiple assumptions that vary between experts with the result that much controversy surrounds this activity. Consequently, toxicologists have been very reticent about providing estimates of LCt_{01} and LCt_{05}.

Currently, the most common method used to define the median or 50% toxicity of a substance (LD_{50}) is to generate, experimentally, a sigmoid toxicity exposure - response curve at a fixed exposure time. The mortality-exposure data is plotted as a PROBIT relationship (linear) of per cent effect vs dosage. Some standard techniques have been developed [14, 15, 16, 17, 18] for estimating the LD_{50}. In inhalation toxicity studies, the dose, which usually cannot be measured, is substituted by the exposure concentration (***C***) - time (***T***) product or ***CT*** and the measure of toxicity is the $\boldsymbol{LCt_{50}}$ or the concentration of substance which, when inhaled for a fixed time, will produce 50% mortality in a given population. Unfortunately, with nerve agents, as exposure times change the LCt_{50} does not remain constant [4, 8, 19, 20, 21] i.e. Haber's Rule [22] of $\boldsymbol{CT = k}$ is not obeyed. In fact, in many relationships, for a fixed per cent effect the toxicity correlates to the exposure concentration and time by the equation

(1) $$\boldsymbol{k = C^n T}$$

where **k** is a numerical constant, ***C*** is the exposure concentration of toxic material (mg/m^3 in this paper), ***T*** is the exposure time (min in this paper) and ***n*** is the "toxic load exponent". The ***n*** value can range from 0.85 to >3.5 [23]. It cannot be emphasized enough, that this $\boldsymbol{C^n T}$ model is an empirical relationship that must be derived from experimental values and is therefore limited by the experimental data available. The values derived are only as good as the fit of the model to the data. Further, the validity of the estimates will vary with the dispersion and reliability of the data used in the calculations i.e. the better the fit to the model, the better the data set and the better the resulting toxicity estimates. There are known instances in which the model does not apply [11, 24].

The common method employed [6, 10, 11, 13] for evaluating the constant ***n*** (Eq. 1) is to do a series of experiments to obtain LCt_{50} values at different fixed exposure times (i.e. a PROBIT plot with one independent and one dependent variable (1V) as Eq. 2) and then to plot (Fig. 1) the resulting $\log_{10}(LCt_{50})$ values obtained against $\log_{10}(\boldsymbol{T})$. The slope of the resulting line is (1-1/***n***). These processes require an experimental design (progressive exposure concentrations at fixed times) which was not always followed by early workers studying the toxicity of GB. However, this does not mean that the data gathered are of no consequence.

The process described above generally provides reasonable estimates of the effect of exposure time upon the LCt_{50}. However, attempts to obtain other values (such as LCt_{05}) by the same method usually fail because of the high variability or dispersion in the slope and intercept of the PROBIT line encountered in the experimental data from successive 1V experiments. This effect is clearly seen when slopes of the PROBIT lines are plotted against exposure time (Fig. 1). In turn, the toxicity values that are removed from the central LCt_{50} also will be progressively scattered.

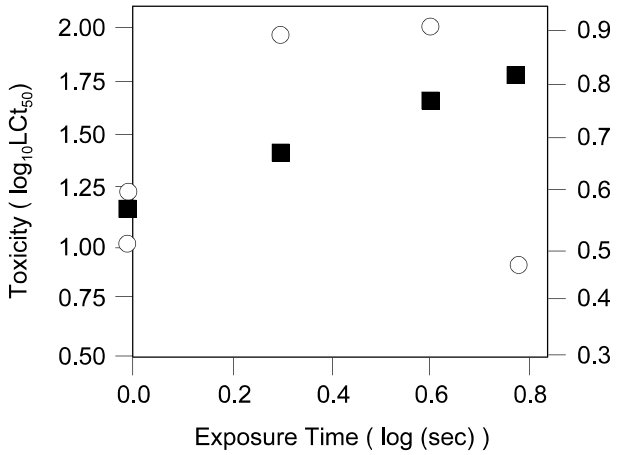

Fig. 1. LCt_{50} and PROBIT slope values are seldom both linear as exposure time changes. The toxicity (LCt_{50}) of GB to rats ( ) varies as the exposure time increases in a log-linear fashion. The slope of the PROBIT lines (○) used to generate the LCt_{50} do not. Data plotted are from McPhail, 1955 [25].

The procedure described above for estimation of the effect of exposure time, produces a liner relationship between toxicity (LCt_{50}) and exposure time but, in reality, what is being generated is the 50 per cent line of a surface, - a two variate (2V) surface plot relating toxicity (LCt_{50}), ***C*** and ***T***. The usual 1V relationship at a fixed exposure time is a slice through the 2V surface at a given value of ***T***. The less common relationship obtained when ***T*** is varied at fixed ***C*** (a procedure often used in radiation studies) is a slice through the 2V surface at 90 degrees to that generated at fixed ***T***. Using modern computer techniques, it is practical to go directly to a 2V planar surface (model) relating ***C***, ***T*** and per cent effect [23, 26, 27, 28, 29]. In the 1V method, the relationship at a fixed ***T*** is expressed by;

(2) $$\boldsymbol{Y} = a_0 + b_1 \operatorname{Log}_{10}\boldsymbol{C}.$$

This equation can be generalized to the 2V relationship, also referred to as the “toxic load model”

(3) $$\boldsymbol{Y} = b_0 + b_2 \operatorname{Log}_{10}\boldsymbol{C}^{\boldsymbol{n}}\boldsymbol{T} \quad \text{or} \quad (4) \quad \boldsymbol{Y} = b_0 + b_1 \operatorname{Log}_{10}\boldsymbol{C} + b_2 \operatorname{Log}_{10}\boldsymbol{T}$$

involving the exposure time, ***T***, explicitly. Here, ***Y*** is the PROBIT value, ***C*** is the exposure concentration, ***T*** is the exposure time, ***n*** is the “toxic load” exponent and a_0, b_0, b_1 and b_2 are empirical numeric constants. In Eq. 3, $\boldsymbol{n} = b_2/b_1$. Note that constants a_0 and b_0 are dependent upon the units of ***C*** and ***T*** whereas the other constants and ***n*** are not. With sufficient data available, the empirical constants (and,

Table 1; *Statistics of the Data Base: Numbers of studies, groups, animals and selected toxicities*

Species	Total studies	Accepted studies	Numbers in accepted studies		Range of exposure times (min)		LCt_{50} (mg.min/m^3)	
			Groups	Animals	Low	High	2min	10min
Mouse	10	9	229	3350	0.17	30	215	316
Rat	15	10	254	2460	0.17	30	114	192
G. pig	5	3	61	372	0.17	12	87	145
Rabbit	5	4	59	136	0.17	15	78	122
Cat	3	3	38	82	0.17	10	62	90
Dog	6	5	54	91	0.25	10	88	122
Monkey	5	3	67	130	0.17	10	45	67
Sheep[a]	1	0	.	.	.	.	219	227
Pig[a]	1	0	.	.	.	.	.	35
Total	51	37	762	6621	0.17	30		
Pseudo-data sets								
Dog		32	708	6530	0.17	30	90	142
Human[b]		37	762	6621	0.17	30	36	57

[a] Data not used in the calculations

[b] 70 kg human breathing 15 L/min

hence, the 2V planar surface) can be evaluated easily using a modern desktop computer and a maximum likelihood method [27, 28]. From the constants of the 2V surface, estimates are readily obtained for the values of LCt_{01}, LCt_{05}, LCt_{50} and LCt_{95} as a function of exposure time.

Because the 2V calculations encompass all ***CT*** values, a fixed ***T*** is not imposed upon the experimental design and many early experiments, carried out using variable exposure times and/or concentrations, may be used with the 2V model. Further, as the first step in the 2V calculation is to reduce all the data to dead:alive (1:0 or 0:1) type individual data, data collected by the "up and down" method [30, 31, 32], commonly used with large animals, may be incorporated and/or evaluated directly. Thus, the amount of data available for evaluation may be greatly increased.

Having achieved reasonable methods for estimation of the toxicity to animals, the second part of the problem, and the most controversial, is the estimation of human toxicity from the animal data. This process requires some form of correction for body size. In general, the inter-species relationships between body size/weight/shape, physiology and toxicology have been well defined [9, 33, 34, 35, 36, 37, 38, 39, 40, 41]. Leading from this, various relationships have been developed for estimation of human toxicity

from animal data [1, 2, 3, 6, 9, 13, 33, 42, 43, 44]. For GB, an excellent correlation is obtained [4, 8, 45, 46, 47, 48] between the minute volume to body weight ratio and the observed toxicity. This relationship, used in conjunction with the allometric relationship between body weight and respiratory volume for animals and man [11, 13, 34, 35] forms the basis for the method proposed in this study for obtaining human toxicity estimates at short exposure times.

The objective of this study was to describe a process to obtain reasonable estimates of human inhalation toxicity developed from, and fully based upon, animal toxicity data. The values obtained were to include LCt_{05}, LCt_{50} and LCt_{95} for a range of exposure times and the limits of the information were to be clearly defined. In this paper, we describe a process, using the newer computational methods now available on desktop computers, first, to obtain 2V representations of the animal toxicity data for several species and then an estimated 2V surface for the human from which specific human toxicity estimates may be derived. The wealth of animal toxicity data available for GB has been used to illustrate the process.

This work has been presented in several fora [45, 46, 47, 48] to encourage discussion and criticism. To date, the responses have been positive.

Methods

Data concerning the toxicity of GB (isopropyl methylphosphonofluoridate, Sarin, CAS # 107-44-8, MW 140.1) were gathered from the open and the military literature. Since most of the work was reported before the advent of electronic literature data bases, a manual search of Chemical Abstracts was undertaken for the years 1945 to 1965 followed by searches for references to the papers identified.

From the individual animal response data, the LCt_{50}, PROBIT slope and intercept were calculated by one or both of the classic methods [15, 16, 17, 18, 49]. In some instances, when the classic methods could not be employed because of insufficient data in the critical 16-84% toxicity range, a 1V version (a special case of the 2V version) of the DRDC Suffield program was also used. The 2V surface calculations were carried out using programs (Annex A) prepared in at DRDC Suffield [27, 28] in S-Plus®, Version 3.4 for UNIX (Mathsoft, Inc., Seattle, Washington) running on a Hewlett-Packard model 715/50 workstation under HP-UX (Hewlett-Packard, 3000 Hanover St., Palo Alto, California). All the computations were preformed in double precision to minimize the effects of truncation in the statistical calculations.

A step-by-step procedure to calculate the human toxicity estimates is provided. Briefly stated, the process assembles and analyses the data for each species, calculates the toxicity, concentration, exposure time surface for each species, establishes an interspecies relationship, creates a pseudo-human data set from the animal data and uses the pseudo-human data to calculate a 2V surface for the human. Specific toxicological estimates may then be read from the surface.

The literature values for the toxicity of GB to humans, both published estimates and reports of human exposures, were reviewed and the values compared to the estimates obtained from the animal studies by the proposed methods.

Evaluation of the available animal toxicity data

Literature values were collected from as many sources as possible to provide the basic data for this project. For a study to be included in the basic data set, which considered only GB toxicity to mammals, the "raw" experimental data - i.e. the exposure concentration, the exposure time and the per cent response - had to be available for re-evaluation. In all, studies involving 9 species were identified. Following the procedures outlined below, some of the data were eliminated from the final data base. A summary of the animal numbers, groups, studies, high and low exposure times and some representative toxicity values are provided in Table 1.

The object, for each species, was to create a summary data set that was as large as possible while being cohesive and representative. To this end, the data from each paper was recalculated to provide $\boldsymbol{C}$ in mg/m^3 and $\boldsymbol{T}$ in min, the data were re-analysed and compared to the authors' stated result and, when possible, a 2V surface calculation was made. All of the data for that species were then combined, a 2V surface calculated and, at one $\boldsymbol{T}$, the ratio of LCt_{95}/LCt_{05} (Ratio) was calculated. This Ratio was used as a simple, but effective, measure of the dispersion of the data. It is directly related to the common 1V PROBIT slope obtained at fixed $\boldsymbol{T}$ (Eq. 2). Also, because the 2V surface (toxic load model) is a planar surface, the Ratio is constant for all $\boldsymbol{T}$. For each experiment, the $\boldsymbol{CT}$ values for each group of animals were plotted on a graph of $\log_{10}\boldsymbol{CT}$ *vs.* $\log_{10}\boldsymbol{T}$ and lines representing the LCt_{05}, LCt_{50} and LCt_{95} from the combined 2V surface were added to each graph. A similar graph of the combined data was also prepared. Examination of these graphs should indicate whether the data from one (or more) experiment(s) were clearly separated from the remainder (Fig. 2). If there were experiments with data separated from the rest, those data were removed, experiment by experiment, and, each time, the 2V toxicity surface for the species was recalculated and re-plotted. If the result, in each case, was a marked reduction in the Ratio and a marked increase in the slope constants, b_1 and b_2, the data from that experiment was considered for exclusion from the final data set. This process was repeated until a data set was obtained for which most of the remaining data were within the LCt_{05} and LCt_{95} lines of the 2V surface. The data were re-examined and a final decision was made concerning the content of the data set for that species. Representative toxicity values for each species were extracted and tabulated (Table 1).

Exclusion of data for <10 sec exposures

As a final step in the process, the animal data were arbitrarily truncated to eliminate all exposures of less than 10 sec. In mice, rats, guinea pigs and monkeys, the toxicity resulting from exposures <10 sec appeared to follow a different pattern from that of longer exposures. There were no clear indications of a cause for this phenomenon although the extreme technical difficulty of obtaining a reliable exposure of <10 sec may be indicated. In several of the species used (cat, dog, monkey), the time for a single breath is more than 1 sec [52, 53, 54, 55]. It becomes almost impossible to ensure that the animal was inhaling

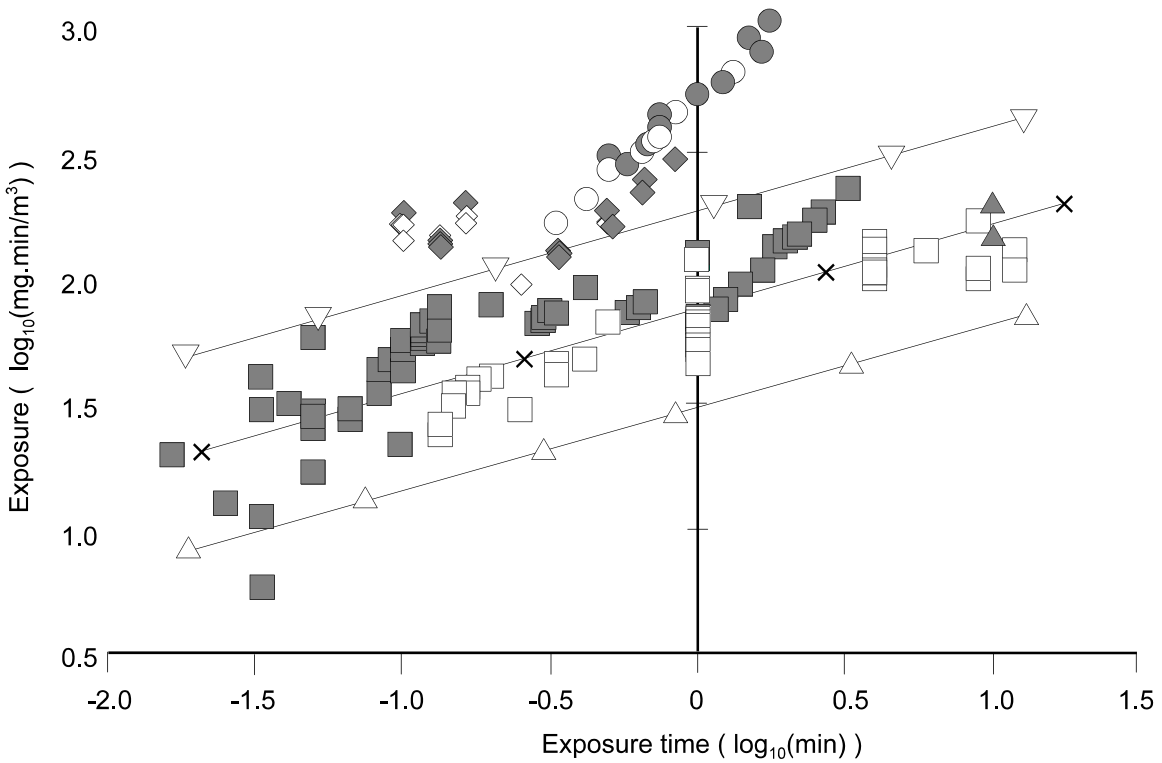

Fig. 2 The total guinea pig data with the LCt_{05}, LCt_{50} and LCt_{95} lines superimposed. Each point represents the position (***CT***) of a group in that data set. Two of the 5 studies depicted were discarded from the final data set. As only single animals were exposed, the results were alive (○,) or dead (,) animals. Each group of animals in the other three studies are represented at the exposure ***CT*** without any attempt to represent toxic response. The lines (**X**, **Î , Ï**) represent the LCt_{50} LCt_{05} and LCt_{95}, respectively, of the 2V surface calculated from the data of the 3 accepted studies.

Table 2; *Surface constants for guinea pig data*

		Calculated surface constants				
Item	Treatment	b_0	b_1	b_2	***n***	Ratio
(1)	All data	-2.0492	1.1185	1.0496	1.0656	873
(2)	(1) less [50]	-4.5300	2.0120	1.6738	1.2021	43
(3)	(2) less [51]	-7.4042	2.8936	2.3728	1.2195	13.7
(4)	**(3) $10 sec**	**-7.8797**	**4.2902**	**2.8510**	**1.5048**	**5.84**

Bold print indicates the accepted data surface

Table 3; *Surface constants for mouse data*

Item	Treatment	Calculated surface constants b_0	b_1	b_2	***n***	Ratio
(1)	All data	-6.2710	2.6383	2.2517	1.1717	17.7
(2)	(1) less high [57][a]	-6.3579	2.7730	2.1584	1.2847	15.4
(3)	**(2) $10 sec**	**-8.3461**	**3.6907**	**2.7947**	**1.3206**	**7.79**

[a] Ref. 57 contains two studies with different results. The author questioned the data set with higher values. Only the low data set was used. Bold print indicates the accepted data surface.

during the exposure and ***T*** becomes meaningless. For rabbits, there were no data for exposures <1 min. For cats and dogs, the exclusion of data for exposures <10 sec made no difference to the resulting toxicity calculations because, in the groups exposed for <10 sec, all animals survived and the recorded ***CT*** values were well separated from values which were predicted to be lethal. For exposures >10 sec, the data for all 7 species appeared to follow a similar pattern of toxicity change under a power law relationship as the exposure time increased i.e. the 2V surface or toxic load model (Eq. 3).

Toxicity in guinea pigs

The guinea pig data is presented first to illustrate the process of evaluating the animal data.

The literature search provided 5 papers [20, 21, 50, 51, 56] with animal response data for guinea pigs exposed to GB. Using all of the guinea pig data, the calculated surface was very shallow as indicated by the low values of b_1 and b_2 and the very large value of the Ratio (Table 2). When the data were plotted (Fig. 2), the data from one study [50] was clearly aberrant and the data from a second study [51] also appeared to be higher than the rest. When data from the first study [50] was removed and the surface re-calculated, significant improvements were achieved in the Ratio, b_1 and b_2 (Table 2). Removal of the second study [51] improved the values again and when the <10 sec data were removed, there was another marked change in the Ratio and in the constants of the surface. Removing other experimental data also produced changes in the surface constants but these were much smaller and not considered great enough to warrant the further reduction in the numbers of animals in the data set.

Toxicity in mice

The literature search provided 10 studies in 9 reports [20, 21, 56, 57, 58, 60, 61, 62, 63] with animal response data for mice exposed to GB. In addition, some recent 20 min exposure data from DRDC Suffield were included [64]. Of the 10 studies, the data from two [57, 63] appeared to be separated from the body of the rest. When the data from the second report [63] were removed (Table 3), the Ratio increased and the slope constants b_1 and b_2 decreased so this data was retained. The first report [57] contained two studies with mice which used different exposure apparatus with different results. Based on the author's comments, only the lower values were included in the data base. When the data for exposure <10 sec were removed, marked improvements in the Ratio, slope constants and ***n*** were achieved.

Toxicity in rats

The literature search provided 12 reports [20, 21, 51, 56, 57, 58, 59, 61, 65, 25, 66, 67] with animal response data for rats exposed to GB. In addition, recent data for exposures 20 and 30 min [68, 69[1]] and some data from the DRDC Suffield archives [McPhail, unpublished data, 1950, 1951] were included. When the data for the two studies furthest removed from the remainder [51, 57] were progressively taken out of the calculation (Table 4), improvements in both the Ratio and the slope constants b_1 and b_2 were achieved. The removal of two more studies [58, 59] again improved the situation. When the data for exposures <10 sec were removed, a further a marked improvement was achieved. At this point in the data analysis, one study [25] was lost because all exposures were <10 sec. Removing more data did not have useful effects.

Table 4; *Surface constants for combinations of rat data*

		Calculated surface constants				
Item	Treatment	b_0	b_1	b_2	***n***	Ratio
(1)]	All rats	-5.1513	1.5108	1.1266	1.3411	150
(2)	(1) less [57]	-6.4289	2.0198	1.3475	1.4989	42.5
(3)	(2) less [51]	-7.5920	2.4069	1.5897	1.5140	23.2
(4)	(3) less [58]	-7.8613	2.4971	1.6450	1.5180	20.7
(5)	(4) less [59]	-8.8637	2.8208	1.8703	1.5082	14.7
(6)	**(5) less [25] $10 sec**	**-6.8900**	**3.5048**	**2.3603**	**1.4849**	**8.68**

Ref. 25 was eliminated for item 6 because all exposures were < 10 sec.

Bold print indicates the accepted data surface.

Toxicity in rabbits

The literature search provided 5 studies [56, 61, 70, 71, 72] with animal response data for rabbits exposed to GB. In one paper [70], exposure data were reported for six rabbits of which four received exposures 2 - 20 times higher than lethal. In addition, the exposure times were not clearly established so this study was not used. The other rabbit data were accepted. There were no data for <10 sec exposures. Using the data from 4 studies, the calculated constants for the exposure-response surface were; b_0 = -14.1908, b_1 = 7.8466, b_2 = 5.6377, ***n*** = 1.3918 and Ratio = 2.63.

Toxicity in cats

The literature provided three studies [21, 56, 73] with usable mortality data. Two of these [56, 73] contained 10 min exposures only. In the third study [21], cats were exposed to GB for times between 1.2 and 240 sec. Because the toxicity data were collected with both variable ***C*** and ***T*** values, the data was analysed by the 2V procedure. The 10 min LCt_{50} values were judged to be comparable within experimental error. Scrutiny of the data for the shortest exposure times showed that no lethal response was elicited and these data had no effect upon the resulting 2V surface calculations.

[1] This data was obtained originally in 1998 as a personal communication. Data for shorter and longer exposure times, included in the published papers, were not included.

Table 5; *Surface constants for dog data*

Item	Treatment	Calculated surface constants b_0	b_1	b_2	***n***	Ratio
(1)	All dog data	-3.0102	1.6523	1.5655	1.0554	96
(2)	(1) less [74]	-9.7796	5.2592	3.6910	1.4249	4.22
(3)	**(2) $10 sec**	**-13.0059**	**6.7965**	**6.1468**	**1.1057**	**3.05**

Bold type indicates the accepted data surface.

All of the data from the three papers were deemed acceptable. For consistency with the data from other species, the exposures <10 sec were removed. A 2V surface, calculated from this data set had the constants; b_0 = -18.5512, b_1 = 10.7994, b_2 = 8.1973, ***n*** = 1.3174 and the Ratio = 2.02.

Toxicity in dogs

The literature provided 6 studies [21, 56, 74, 75, 76, 77] with animal response data for dogs exposed to GB. A calculation using all of the dog data produced a shallow, broad, 2V surface with low slope constants and a large Ratio (Table 5). In a one study [74], the sizes of the dogs were very disparate, much of the data was above the range of the remaining dog data and the variability of the data was very large. Removing the data from this study from the surface calculations markedly reduced the Ratio and increased the slope constants b_1 and b_2. When exposures <10 sec were removed from this data set, there was a further decrease in the Ratio and increases in the b_1 and b_2 constants. The marked reduction in the ***n*** value as the <10 sec data were removed is the result of the combination of small numbers of animals involved (91) and the majority of the animals (69) being exposed at either one or two min, values central to the majority of the animal data. Thus, the ***n*** value of 1.1057 did affect (lower) the average ***n*** for the animal species but did not affect the subsequent calculation of pseudo-human values (*vide infra*).

Toxicity in monkeys

The literature provided 5 studies [21, 51, 56, 60, 78] with animal response data for monkeys exposed to GB. Using all of the monkey data, the calculated constants indicated an exposure-response surface that was very broad and shallow (Table 6). The data from two papers [51, 78] contained much higher toxicity values and were progressively removed from the data base with concomitant improvements in b_1, b_2 and Ratio. The ***n*** value first increased as the above papers were removed and then was lowered when the exposures <10 sec were removed. However, the b_1, b_2 and Ratio values improved further with the removal of the exposures <10 sec.

Toxicity in sheep

The literature search provided one study [51] with animal response data for sheep exposed for 0.17 and 2 min to GB. The two LCt_{50} values of 227 and 219 mg.min/m^3, respectively, were not consistent with the

Table 6; *Surface constants for monkey data*

Item	Treatment	Calculated surface constants b_0	b_1	b_2	***n***	Ratio
(1)	All data	-1.9281	1.0912	0.8547	1.2768	1034
(2)	(1) less [51]	-2.1803	1.2917	09436	1.3690	352
(3)	(2) less [78]	-2.9350	1.8908	1.3075	1.4461	54
(4)	**(3) $10 sec**	**-4.9943**	**3.2018**	**2.4851**	**1.2884**	**10.7**

. Bold print indicates the accepted data surface.

data for the remaining animal species in that the higher LCt_{50} value was recorded at the shorter exposure time. The sheep data were not included in the human estimate calculations.

Toxicity in pigs

One study [79] was found with animal response data for pigs exposed for 10 min to GB. The LCt_{50} was 34. The pigs used were very young and the authors of the paper judged the data to be non-representative of adults. The pig data were not used in the human estimate calculations.

Table 7; *Constants of predicted 2V surfaces for all species*

Species	b_0	b_1	b_2	***n***	Ratio
Mouse	-8.3461	3.6907	2.7947	1.3206	7.79
Rat	-6.8900	3.5048	2.3603	1.4849	8.68
Guinea pig	-7.8797	4.2902	2.8510	1.5048	5.84
Rabbit	-14.1907	7.8466	5.6377	1.3918	2.63
Cat	-18.5512	10.7994	8.1973	1.3174	2.02
Dog	-13.0059	6.7965	6.1468	1.1057	3.05
Monkey	-4.9943	3.2018	2.4851	1.2884	10.7
	Average ***n*** value; i.e. toxic load exponent				1.3448 ± 0.1457
Calculated data sets[a]					
Dog	-6.7881	3.6345	2.5988	1.3985	8.04
Human (by range of exposure times; min)					
0.17-30	-5.3097	3.6297	2.6158	1.3876	8.06
0.33-30	-6.3087	4.3214	3.1060	1.3913	5.77
0.5-30	-6.2387	4.2998	3.0457	1.4117	5.82
1-30	**-6.5748**	**4.5242**	**3.2208**	**1.4005**	**5.34**
2-30	-6.9698	4.7807	3.4296	1.3939	4.88

[a] Values for the pseudo-dog and pseudo-human data sets generated for this study.
Bold type indicates recommended surface for general use.

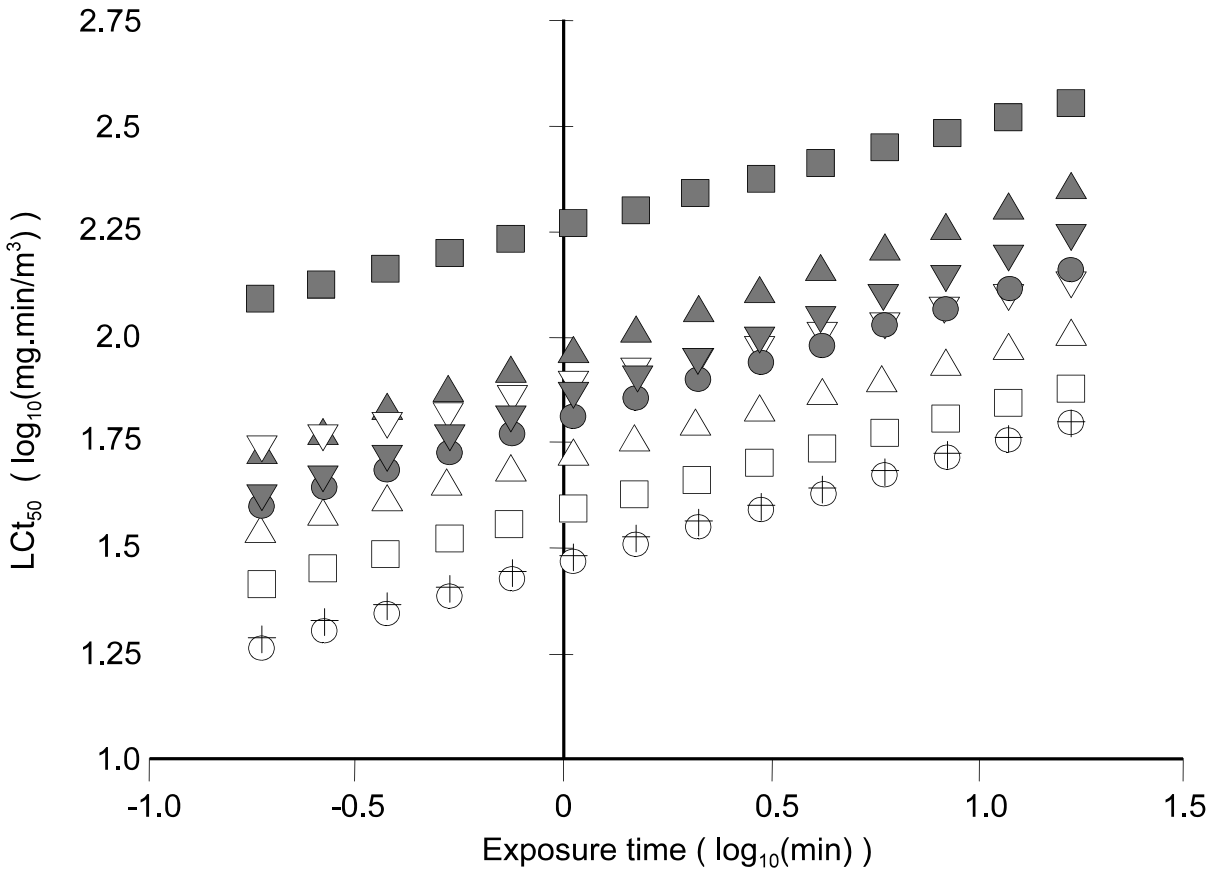

Fig. 3 Plots of LCt_{50} *vs* ***T*** generated from the 2V surface, calculated for each species, are parallel within experimental error. The plots are; mice ( ), rats ( ), guinea pigs ( ), cats ( ), dogs (L), rabbits ( ), monkeys (□) and, by two calculations, humans (○, +).

Toxicity in birds and fruit flies

The toxicities of GB to birds and flies have been reported [20, 58]. The avian and insect studies are mentioned for the record, only.

Summary of animal data

The ***n*** values calculated from the data for the seven individual species were generally similar (Table 7). The mean ***n*** value was 1.345 ± 0.146 with a range of 1.1057 - 1.5048. On a graph, the lines are parallel within experimental error (Fig. 3). The scatter within the data sets, as reflected in the Ratio and the slope constants b_1 and b_2, is quite wide. The cat and rabbit data, which are the tightest distributions, are from small numbers of both animals and studies (Table 1) and, in the case of the rabbits, from only one laboratory. In contrast, the mouse, rat and monkey data are from many more reports by several laboratories. Some representative toxicity values are provided in Table 1.

Calculation of human estimates

Relating toxicity and animal size

In many cases, physiologic and toxicologic parameters are transposed between animal species using relationships between toxicity and body mass or volume. For inhalation studies, there is an allometric relationship between minute volume and body weight that may be used to define the interactions between species [1, 2, 3, 4, 6, 7, 8, 9, 10, 11, 12, 13, 33, 34, 35, 36, 37, 39, 40, 41, 42, 43, 44, 80]. For the nerve agent GB, there is an excellent correlation (Fig. 4; Table 9) between toxicity and the minute volume body weight ratio (***MV/BW***) that may be used to predict toxicity in one species from values in several others [4, 8, 46, 48].

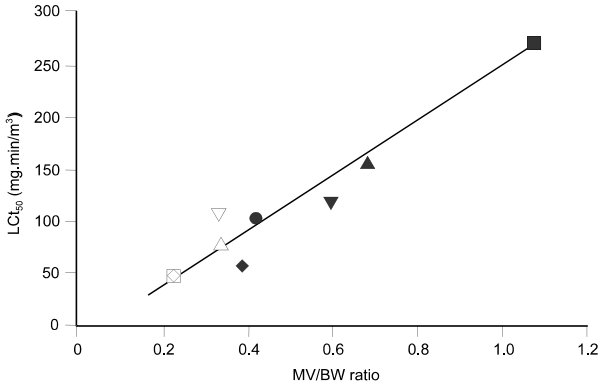

Fig. 4 An example of a regression line of MV/BW *vs* toxicity (LCt_{50}). The points are for mice ( ), rats ( ), guinea pigs ( ), cats ( ), dogs ( ), rabbits ( ), monkeys ( ) and the calculated values for humans (,).

Predicting a toxicity surface

The lines of LCt_{50} *vs* exposure time (Fig. 3) may be represented as simple linear equations of the form

$$y = a.(\boldsymbol{LCt_{50}}) + b.$$

If the lines were exactly parallel, the slopes (*a)* would be equal for all lines and the *y* intercepts (*b)* would be the unique feature of each line. To move any line to superimpose upon another would simply require that a constant be added to the appropriate *y* intercept. Further, the lines for the species are related via the ***MV/BW***. To obtain the line for another species ***H***, for which the ***MV/BW*** is known, a regression of the LCt_{50} *vs.* ***MV/BW*** is calculated at one ***T*** (Fig. 4) for the various species. The toxicity, as $\log_{10}(LCt_{50})$, for species ***H*** is calculated from the regression line using the ***MV/BW*** for ***H***. To obtain the*y* intercept for species ***H***, a correction factor (Table 8) is calculated by subtracting the $\log_{10}(LCt_{50})$ of ***H*** from the $\log_{10}(LCt_{50})$ at ***T*** of species ***J*** (for which the LCt_{50} *vs* at ***T*** from exposure time line is known) and then subtracting this correction factor from the *y* intercept of the line for ***J***. Because the lines are parallel, the LCt_{50} values for species ***H*** may be calculated.

Unfortunately, the LCt_{50} *vs* exposure time lines are not parallel (Fig. 3) and the slopes are not equal but only similar within the limits of experimental error.

Table 8; *Constants for calculating pseudo-dog and -human values*

Species	Dog constants	Human constants
Mouse	-0.3694	-0.7932
Rat	-0.1093	-0.5147
Guinea pig	0.0297	-0.3652
Rabbit	0.0685	-0.3461
Cat	-0.1745	-0.2334
Dog	0.0	-0.4069
Monkey	0.3350	-0.0889

Table 9; *Toxicity values for inter-species regression*

Species	MV BW	Exposure times (min) 0.53	1.05	2.10	5.3	10.5
Mouse	1.076	156	185	218	273	323
Rat	0.681	75	94	118	159	199
Gpig	0.594	55	70	88	119	151
Rabbit	0.416	54	65	79	103	125
Monkey	0.384	31	37	42	53	61
Cat	0.335	45	53	62	78	92
Dog	0.328	77	82	88	96	103
Human	0.223	28	31	35	42	48
*Correlation coefficient (**r**)*		0.870	0.909	0.938	0.964	0.973

Values are inhalation toxicity in mg.min/m^3

Therefore, to estimate the LCt_{50} ***vs T*** relationship for the human (or any other species), a series of regressions of LCt_{50} *vs.* ***MV/BW*** were made at 14 values of ***T***. All correlation coefficients (***r***) obtained (Table 9) for the LCt_{50} *vs* exposure time regressions were > 0.870 and those for 1 - 10 min ***T*** were >0.91. Then, using the "standard" ***MV/BW*** value of 0.223 previously established [34, 35] for a 70 kg human, a series of LCt_{50} values for the human were calculated from the 14 regression lines. These points, which form a predicted $\boldsymbol{LCt_{50}}$ vs ***T*** line for the human, were plotted on the same graph as the animal lines (Fig. 3). As noted in the paragraph above, any of the lines for other species may be moved on top of the proposed human line by calculating a correction factor to add to the y-intercept of the line to be moved. The fit obtained will depend upon how far from parallel to two lines really are. If a correction factor is calculated near the middle of the two lines, the values will be close in the middle and more separated at the ends. The average values of the constants to be added to each animal line to move it to the human position were calculated in this fashion (Table 8).

As the LCt_{50} *vs* exposure time lines may be moved, so can all of the data from which the lines were generated. The same $\boldsymbol{LCt_{50}}$ *vs.* ***T*** line for species ***H*** is obtained whether the toxicity *vs* exposure time line for ***J*** is calculated and then the line moved or the raw data for ***J*** are moved and then the $\boldsymbol{LCt_{50}}$ *vs.* ***T*** line for ***H*** is calculated. In the latter case, a 2V surface is created instead of just the LCt_{50} line. Thus, to obtain a predicted or "pseudo-" population for one species from the data of another, an adjustment constant is added to the $\log_{10}\boldsymbol{CT}$ of each data point to move the entire data distribution. A surface calculation may then be performed on the "pseudo" data set that has been created and toxicity estimates may be obtained from the surface. Because the 2V surface encompasses all ***CT*** values, estimates of LCt_{01}, LCt_{05} and LCt_{95} are readily obtained. The estimation of toxicity may be enhanced by moving all of the available animal data, creating a much larger "pseudo-species" data base for the 2V calculation.

Validation of the predictive process

As a verification of this "pseudo-species" approach, a "pseudo-dog" data set was created. The dog data were chosen to represent the worst case from among the species available. The appropriate constants (Table 8) were calculated as described above and added to each $\log_{10}(\boldsymbol{CT})$ of the other species to create a pseudo-dog data set for 0.17-30 min exposures. A 2V toxicity surface for the pseudo-dog was then calculated. The real- and pseudo- dog 2V surfaces were compared by plotting the LCt_{05}, LCt_{50} and LCt_{95}

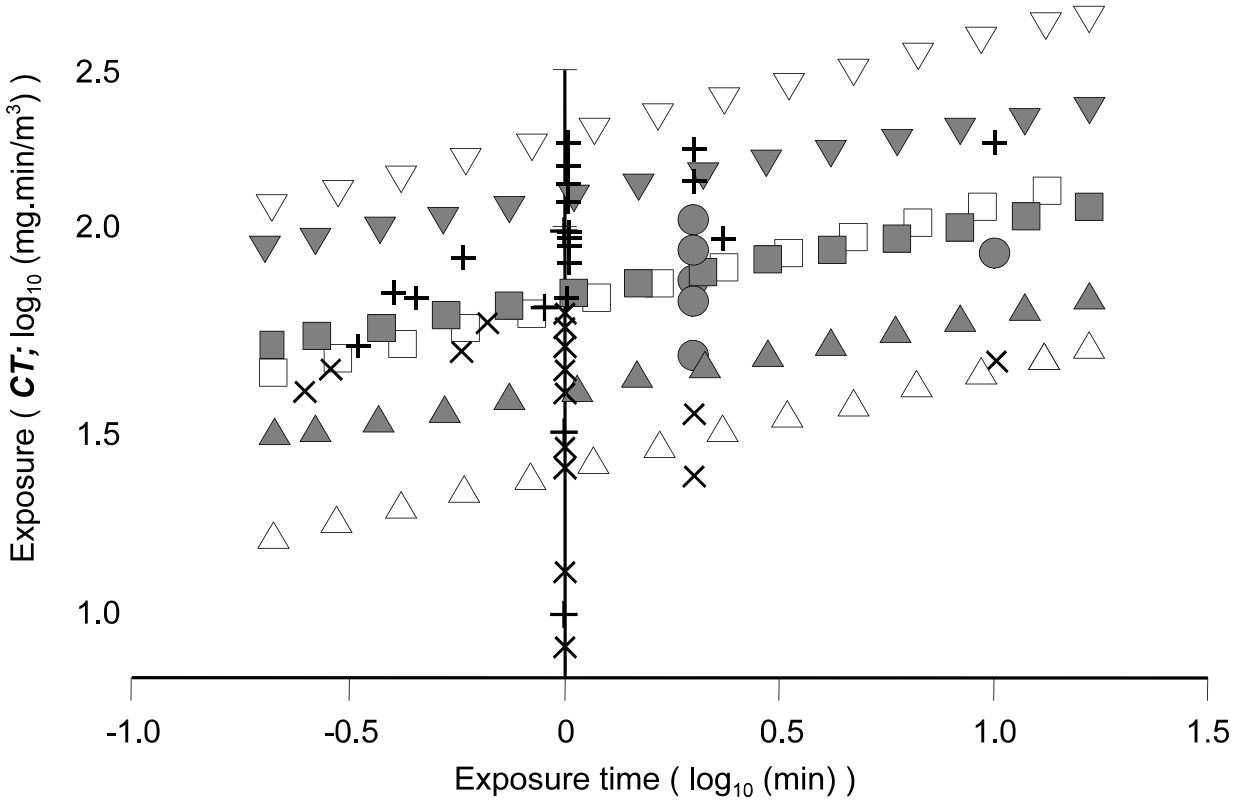

Fig. 5 Comparison of dog and pseudo-dog calculations. The accepted dog data is plotted showing 0-20% ( ), 20-80% ( ) and 80-100% (+) toxic results. Each symbol represents a group (1 to 4 animals) tested at that ***CT.*** The calculated real dog LCt_{05} ( ), LCt_{50} (■) and LCt_{95} ( ) lines and the calculated pseudo-dog LCt_{05} (ª), LCt_{50} (□) and LCt_{95} (L) lines are superimposed. Both the dog and pseudo-dog 2V surface models would adequately represent the real dog data.

lines for both surfaces upon a scatter diagram of the real dog data (Fig. 5). The surface constants for the pseudo-dog data may be compared to those for the real dog data (Table 7). As expected, the Ratio and b_1 and b_2 constants indicate a shallower, flatter surface for the pseudo-dog population. Despite this, the LCt_{05} and LCt_{95} lines (Fig. 5) of the pseudo-dog surface enclose the majority of the dog data and the LCt_{50} line of the pseudo-dog data is close to that of the real dog data. In fact, either surface may be used to describe the toxicity of GB for the "standard" dog with body weight of 10 kg [2, 34, 35]. Calculations for dogs of different size/breed must be done using an appropriate MV/BW ratio obtained from the allometric equations [34, 35] relating respiration and body weight.

Values for man estimated from the animal data

Using this process described above, a "pseudo"-human data set was created from all of the accepted animal data for 0.17-30 min exposures and a scatter diagram was prepared (Fig. 6). From this data, a 2V surface was calculated, LCt_{05}, LCt_{50} and LCt_{95} lines were generated and the lines were added to Fig. 6. The LCt_{50} line, also added to Fig. 3, was essentially superimposed upon the line obtained above by serial

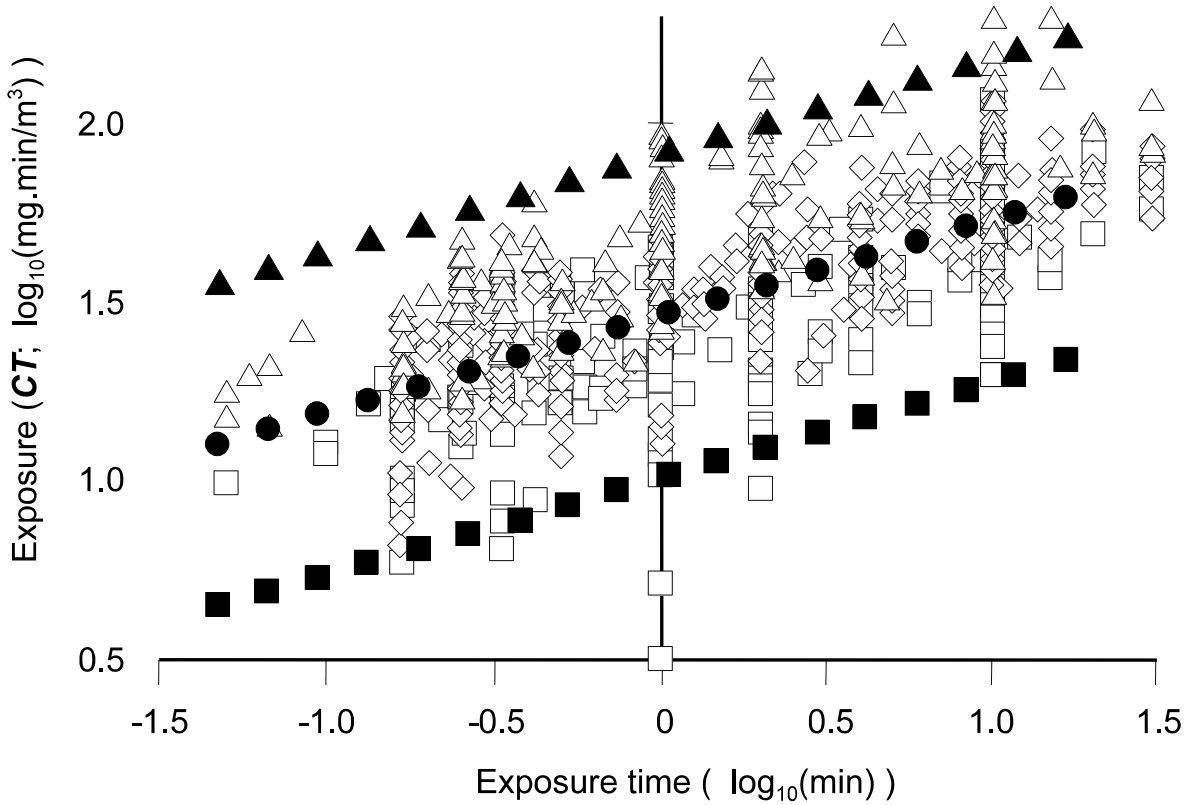

Fig. 6 The pseudo-human data distribution shown with LCt_{05}, LCt_{50} and LCt_{95} lines from the calculated surface. The pseudo-human data are plotted to show 0-20% (~), 20-80% ( ) and 80-100% ( ) toxic responses. Each symbol represents the ***CT*** value of one "experimental" group. The LCt_{05} ( ) and LCt_{95} ( ) lines include all but a few of the data points and those outside points represent either low or high responses. The LCt_{50} line (●) effectively bisects the distribution.

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regressions. The predicted LCt_{05} and LCt_{95} lines enclose the majority of the data set and the lines appear closely predictive of the pseudo-human distribution suggesting that reasonable confidence may be placed in the toxicity values indicated (Table 10).

Close inspection of the pseudo-human data set (Fig. 6) indicated that the distribution of values might be compacting as the exposure time shifted from low seconds to minutes *ie.* the distribution appeared to compress as the exposure time increased. To test this concept, several 2V surfaces were calculated from the pseudo-human data set, progressively removing the data for shorter exposure times (Table 7). As the lower exposure limit increased in four steps from 0.17 to 0.99 min, the ***n*** values of the four calculated surfaces remained essentially constant (mean ± standard deviation; 1.399 ± 0.011), the slope constants b_1 and b_2 increased, the Ratio decreased by 33% and the predicted LCt_{50} values (Table 10) remained constant. These changes indicated that the distribution was compacting without changing the central axis of the predicted 2V surface. A further change in the lower limit to 1.99 min produced changes in the Ratio and slope constants that would have been considered as improvements. However, there was a sharp increase in the predicted LCt_{50} (Table 10) of more than 3 standard deviations compared to the mean of the previous values. Therefore, the 2V surface calculated for the range 2 - 30 min was not considered further.

Effect of adding more data

To demonstrate the effects to be expected by the addition of more animal data, the human estimate calculations were redone using rat data augmented by adding one study [McPhail, 1950; Unpublished data from DRDC archives] a second time to the data base. This increased the number of animals by 260. For the 0.17 - 30 min exposure range, the ***n*** value decreased from 1.3876 to 1.3873, the Ratio decreased from 8.0597 to 8.0547 and the slope constant b_1 increased from 3.6297 to 3.6308. The indicated 2 min LCt_{50} increased from 35 to 36 mg.min/m^3 and the 10 min value increased from 55 to 56 mg.min/m^3. In summary, the changes were very slight.

Recommended toxicity values

The procedures above provide a pseudo-human data base that is derived directly from the animal data and a 2V planar model of the data from which specific toxicity estimates may be obtained. The 2V planar model fits the data distribution (Fig. 6; Tables 7, 10). The human ***LCt_{50} vs T*** estimates are generally consistent and representative as the data set is manipulated (Table 10). As demonstrated by serial calculations of the model with an increasing lower limit (Table 7), there is a much greater variability in the predicted mortality data at the shorter exposure times. The difficulty in generating exposures for the short times may be part or all of the cause of the increased variability at the low exposure times. Biologically, an exposure #10 sec to a human may be academic. Assuming breathing rates of 11 - 17/min or 5.5 - 3.5 sec/breath [52, 81, 82], a wide variation can occur in the amount of contaminated air inhaled in #10 sec according to where the subject is in the breathing cycle as the exposure is started. If the source of vapour is relatively close to the subject, the variability of concentration in the chemical cloud [29, 83], will add another large uncertainty to the delivered exposure. Therefore, despite the wide distribution of toxic effects indicated, the model for 0.17 to 30 min exposures may be the choice when short exposures are considered with the subjects close to the source.

Table 10; *Toxicity estimates for 70 kg humans breathing 15.6 L/min*

Item	Range (min)	Toxicity (mg.min/m^3) LCt_{01}	LCt_{05}	LCt_{50}	LCt_{95}
2 min exposure					
(1)	0.17 - 30	8.1	12.4	35.2	100
(2)	0.33 - 30	10.1	14.6	35.0	84
(3)	0.5 - 30	9.9	14.3	34.6	83
(4)	1 - 30	10.6	15.0	34.7	80
(5)	2 - 30	11.4	15.8	39.7	77
10 min exposure					
(6)	0.17 - 30	12.6	19.4	55.2	157
(7)	0.33 - 30	16.0	22.9	55.1	132
(8)	0.5 - 30	15.9	22.9	55.3	133
(9)	1 - 30	16.9	23.9	55.1	127
(10)	2 - 30	17.9	24.9	62.6	122
30 min exposure					
(11)	0.17 - 30	17.2	26.4	75.1	213
(12)	0.33 - 30	21.7	38.0	75.0	180
(13)	0.5 - 30	21.9	31.6	76.2	184
(14)	1 - 30	23.2	32.8	75.6	175
(15)	2 - 30	24.5	34.0	85.4	166

Mean ± St. dev.	Items (1-4)	34.9 ± 0.27
	Items (6-9)	55.2 ± 0.10
	Items (11-14)	75.5 ± 0.45

However, under more general conditions where the exposure times are longer so the breathing cycle has less effect and under conditions where the source is more distant so that the exposure concentrations are less variable, the steeper planar model for the 1 - 30 min exposure times would appear more suitable. As the former conditions are apt to be a special case, the recommended toxicity estimates for general use would be those generated from the model calculated for the 1 - 30 min exposures.

Table 11; *Recommended toxicity estimates for a 70 kg human.*

Minute volume (L/min)	Exposure time (min)	Toxicity (mg.min/m^3)			
		LCt_{01}	LCt_{05}	**LCt_{50}**	LCt_{95}
6	2	27	40	**65**	207
	10	45	62	**142**	330
	30	60	85	**197**	455
15	2	11	16	**36**	83
	10	18	25	**57**	132
	30	24	34	**79**	182
30	2	5.5	8.0	**18**	42
	10	9.0	12	**28**	66
	30	12	17	**40**	91

The recommended toxicity estimates for a 70 kg human breathing 6, 15 and 30 L/min are given in Table 11. Direct calculations from the 2V surface equation for 1 - 30 min exposures provides values for a minute volume of 15.6 L/min (Table 10). The 15.6 L/min minute volume for the human is the value obtained for a 70 kg subject from the allometric relationship of minute volume to body weight for many species [34, 35]. The values for 6, 15 and 30 L/min were obtained by simple ratios of the minute volumes involved. Values for other minute volumes may be obtained in a similar fashion. However, a simple ratio calculation should not be used with other human body weights. A calculation from the allometric the relationship between minute volume and body weight should be used instead.

Comparisons with literature values for man

Several previous studies are available which describe attempts to estimate human toxicity of the G agents [49, 84, 85, 86, 87, 88]. The most recent estimation of human toxicity, as an LCt_{50}, for GB was provided [4, 8] for the US Army as part of the preparations for the Gulf War (Operation Desert Storm). The estimated human inhalation toxicity provided in that review was 35 mg.min/m^3 for a 2 min exposure to a 70 kg human breathing 15 L/min (This breathing rate, considered as a “standard” for military purposes, represents a fit man doing light work [81, 82]). The 2 min value from the present study is 36 mg.min/m^3 (Table 11), so the two assessments are in essential agreement. Other human toxicity estimates from the literature, [49, 84, 85, 86, 87 ,88, 89, 90, 91, 92, 93] indicate LCt_{50} values of 50-100 mg.min/m^3 (several of these are based upon or refer back to the same original papers for the numbers), values closer to the 10 min toxicity value of 57 mg.min/m^3 calculated by the procedures described in this report. All of these early LCt_{50} estimates are in essential agreement given the variations in methods, exposures, breathing rates and other available information. None of the previous attempts to estimate human toxicity provided either a continuous surface describing the exposure time-concentration-toxicity response or LCt_{05} and LCt_{01} values generated directly from the animal data.

Discussion

A procedure is presented that provides an estimation of human toxicity that is totally dependent upon direct calculations from the animal data. There are no inferences of values, no assumptions based upon other data and no subjective assessments of studies to include or reject the data. The key to the process is to achieve the situation described in Fig. 3. The relationship between toxicity and exposure time may be different from the one presented in this paper but the process presented will still work effectively as long as the toxicity-exposure time relationships produce near parallel lines for each species as shown in Fig. 3. Once this point has been achieved, the mathematics and procedures required to produce the pseudo-human data set, the predicted human LCt_{xx} lines and the human estimates are trivial.

The procedure described fits a mathematical planar surface model to the experimental results. This 2V surface model predicts that the toxicity parameters, notably b_1 (the 1V PROBIT slope at $\boldsymbol{T}$) and b_2, are constant as the exposure time changes. A surface calculated using all of the pseudo-human data created from the accepted animal data, appears to represent the total data set for GB toxicity very well as almost all of the critical 20 - 80 percent mortality data fall between the LCt_{05} and LCt_{95} lines (Fig. 6). Further, the numbers generated from the surface are in good agreement with previous literature values for the LCt_{50} of GB. However, when data for the exposure times #0.99 min were progressively removed from the surface calculation, the predicted LCt_{50} values were very similar (Table 7) but b_1, b_2 and the Ratio clearly indicated that the predicted surface was becoming steeper. In turn, this indicates that the variability seen in the short exposure times was greater. As noted, this greater variability may result from technical considerations in generating the exposures. However, the result may be fortuitous in view of the large concentration differences seen in a chemical cloud [26, 29] and the resulting variability of the exposure to individuals separated by only short distances. Because the toxicity is not directly related to exposure, the values used for a given project/purpose should be chosen to best fit the scenario in question. The recommended values for general use (Table 11) are those calculated from the 1 - 30 min exposure range (Table 7). When very high transient exposures are involved, as when the subjects are close to the source of a cloud, the values for the 0.17 to 30 min toxic load model may be more appropriate.

A cautionary caveat must be clearly understood. The analysis and estimates presented are based upon empirical relationships. As such, the range of exposures for the extrapolation of toxicity from animal to human is limited by the range of the suitable animal toxicity data available. Mathematical extrapolation beyond the boundaries of the original animal data is possible (the calculated lines are mathematically limitless) but is not a recommended practice and may well lead to serious error. When this project began, there were only a few acute toxicity data available in the literature for exposure times longer than 15 min and these were only for the rat [56]. From current work, which extends the rat [68, 69] and mouse [64] data to longer times, it is clear that the empirical $\boldsymbol{C^nT}$ or toxic load relationship described in this study may be invalid for exposure times of more than 30 min. Similarly, toxicity at lower exposure times may be estimated but in this case, the surface calculated for 0.17 to 30 min, which predicts the same LCt_{50}, may provide better toxicity estimates for LCt_{05}, etc.

The concepts and the analytical methods presented here cannot be validated by comparisons to human toxicity data. Therefore, to demonstrate the functionality of the procedures proposed, a "pseudo-dog" data set was prepared in the same method as proposed for the human (Table 5, 7). The $\boldsymbol{C^nT}$ lines for the actual dog and the calculated "pseudo-dog" data are close and cross. However, from Fig. 5 it is clear that either empirical line can effectively represent the dog data.

In all, the data from 11 studies were discarded. In one [25], all of the data were for exposure times <10 sec. In another [50], the procedures described strongly suggest that the high values obtained may have been caused by rebreathing of the test atmosphere [94, 95]. With the exception of the one study noted [74], there was nothing in the reports to indicate a reason/cause for the discarded studies to digress from the remainder.

Having said the above, as in any calculation of this type, the toxicity estimates are only as good as the original data available. To minimize the effect of inter-laboratory differences, the procedure described includes a critical comparison of the data from each study to the total data for each species. In the example used, GB, there are many studies from different laboratories so that systematic errors in any one location should be either eliminated because the data would be different from the remaining studies on that species or, again, swamped by weight of numbers. Nothing will protect against a systematic or procedural error across all studies. However, there is still a preponderance of data from mice and rats and, unfortunately, it is the data from these species that, initially, have the greatest variability. This is reflected in the dispersion of the final human estimates; the relatively shallow slope (. 4.5: values between 7 and 12 have been suggested; [4, 8, 56, 86] of the toxicity surface and the 5.4 Ratio. Despite this, the animal studies and the predicted human values appear to be consistent within themselves and, in the authors' opinion, represent the best currently available estimates of the inhalation toxicity of GB for a large human population and the only values for LCt_{01}, LCt_{05} and LCt_{95} that have been derived directly from experimental numbers.

Comparisons of physiologic function between the species may be done by several allometric methods relating function to body size, weight, surface area, etc. [11]. For respiration, the historical comparisons have been minute volume compared to body weight [2, 3, 6, 11, 13, 41, 82] usually as the minute volume/body weight ratio. However, in the listed papers, there was no separation between anaesthetized and non-anaesthetized animals. In previous papers from this laboratory [34, 35], an allometric relationship between minute volume and body weight was developed for young adult, non-anaesthetized mammals. Using this relationship the equivalent minute volume for a 70 kg human and the equivalent MV/BW were determined to be 15.6 L/min and 0.223, respectively. These values were used as the human respiratory parameters in the present study.

This report describes a part of an ongoing study. The human inhalation toxicity estimates given in this report are constantly being refined and updated as more information becomes available. In particular, the data for the rat and mouse are under close scrutiny at this time in an attempt to reduce the dispersion of this data. However, the amount of data already available for calculating the human estimates is such that the addition of more data within 10 sec to 30 min exposure times should have only minor effects upon the outcome of the calculations. To illustrate this point, the rat data were augmented by 260 animals by

including one study twice and then recalculating the human estimates. The result was minimal changes in the toxicity values. Similarly, the effects on the statistical calculations of small numbers of aberrant data points are reduced by the weight of numbers.

Affecting all of the current attempts to obtain human estimates from animal data is the difference between the genetically similar populations of the test animals, reared in isolation, deliberately selected to be the same sex, near constant weight and age, guaranteed not to have *x, y, z* respiratory diseases, fed on a constant diet and the target human population that is genetically diverse, disease carrying, nutritionally very diverse, and of mixed age, sex, weight and body style [1, 2, 9, 42, 96]. As expected, toxicity testing of the "outbred" laboratory animal strains produces tight, steep exposure response data [64, 68, 69]. When randomly selected, genetically diverse subjects such as the population of dogs and monkeys (Fig. 5; Tables 5, 6, 7) are used , the data shows greater variability. Indeed, even serial results in the same laboratory with supposedly similar animals from the same source, produces increased variability [25, 64, 68, 69]. The animal populations used for some of the quoted studies were not the restricted, coddled subjects used today. In some instances, the animals are referred to as "dogs" or "cats" of unspecified origin, breed, size or shape. Following from this, the authors think that the calculated toxicity response surfaces presented take a large step towards ameliorating these significant population differences without resorting to adding artificial safety factors and that the results for both the animal species and the general human population are closer to the "truth" than data obtained in single studies with current laboratory species.

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Annex A: Computer codes for the 2V data analysis

Data format

The data are complied as an ASCII text file in columnar form as animals alive, animals dead, concentration ($mg.m^3$) and exposure time (sec) e.g.

Dead	Alive	Conc.	Time
0	10	24	600
5	5	54	600
10	0	70	600

The times were set in sec to eliminate decimal values that would have resulted if min were used. The resulting equations were corrected to min by changing the b_0 values appropriately.

Batch file driver

run.bat (batch file to run script file)

..\..\cmd\Splus /BATCH processn tmpfile

Computer code in "S"

1. create.data1 (S function)

```
function(file)
{
# Function to create a data set suitable for analysis by
# the glm function. First, we read in a file containing
# n columns of data---1st column is the number of animals
# that died, 2nd column is the number of animals that lived,
# 3rd column is the concentration [mg/m3], 4th column is
# the exposure time [s], and other columns may follow that
# are not relevant to the present analysis.
#
dat.tmp <- read.table(file = file, header = T)
Conc <- dat.tmp[, 3]
Time <- dat.tmp[, 4]
Number <- (dat.tmp[, 1] + dat.tmp[, 2])
Deaths <- dat.tmp[, 1]
nrws <- length(Conc)
for(i in 1:nrws) {
```

```
n1 <- Number[i]
d1 <- Deaths[i]
c1 <- seq(from = Conc[i], to = Conc[i], length = n1)
t1 <- seq(from = Time[i], to = Time[i], length = n1)
s11 <- seq(from = 1, to = 1, length = d1)
s12 <- seq(from = 0, to = 0, length = (n1 - d1))
s1 <- c(s11, s12)
if(i == 1) {
ctot <- c1
stot <- s1
ttot <- t1
}
else {
ctot <- c(ctot, c1)
stot <- c(stot, s1)
ttot <- c(ttot, t1)
}}
State <- factor(stot)
Concentration <- ctot
Time <- ttot
ctab <- data.frame(State, Concentration, Time)
ctab
}
```

2. probit1.analysis (S function)

```
function(x)
{
# Function probit1.analysis performs probit analysis on binary
# (or quantal) response data.
# Calling sequence: probit1.analysis(x)
# where x is the name of the data frame (object) containing
# the data. It is assumed that x contains 3 columns of data.
# Column 1 contains either a 0 (no response) or a 1 (response)
# indicating whether the animal lived or died, respectively;
# column 2 contains the concentrationt the animal was exposed
# to (units here are assumed to be mg/m3); and,
# column 3 contains the exposure time (in s). The function
```

```
# returns the object obtained from a generalized linear model
# fitting. Use summary() function to interrogate this object.
#
result.fit <- glm(State log10(Concentration) + log10(Time),
family = binomial(probit), data = x)
result.fit
}
```

3. LC.estimate.probit (S function)

```
function(x, pp = 0.5, Te = 600)
{
# Function LC.estimate.probit determines the lethal concentration
# that will kill a fraction pp of the population. Unless changed by
# user, it is implicitly assumed that pp = 0.5, and that the
# exposure time Te is 600 s [10 min].
# Calling sequence: LC.estimate.probit(glm.fitted.obj,pp,Te)
# where
# glm.fitted.object is result obtained from glm() call
# pp---vector of mortality fractions desired
# Te---vector of exposure times desired (in s)
# Note: either pp or Te could be a vector, but not both.
# The LC values (in mg/m3) are returned from the function.
# Response probability is based on probit model.
#
b0 <- coef(x)[1]
b1 <- coef(x)[2]
b2 <- coef(x)[3]
Y <- qnorm(pp)
tmp <- (Y - b0 - b2 * log10(Te))/b1
Conc <- 10(tmp)
Conc
}
```

4. processn (script file to call S functions)

```
f1in <- "gpphos.txt"
f1out <- "gpphos.prc"
f2out <- "gb.dat"
tmp1 <- create.data1(f1in)
```

```
tmp2 <- probit1.analysis(tmp1)
b <- as.vector(coef(tmp2))
b0 <- b[1]
b1 <- b[2]
b2 <- b[3]
n <- b1/b2
dat.tmp <- read.table(file=f1in,header=T)
Number <- (dat.tmp[,1]+dat.tmp[,2])
Totalanimals <- sum(Number)
Nogroups <- length(Number)
tmp <- seq(30,1800,by=30)
Te <- tmp
pp <- 0.01
lct1 <- LC.estimate.probit(tmp2,pp=pp,Te=Te)
lct1 <- lct1*Te/60.0
pp <- 0.05
lct5 <- LC.estimate.probit(tmp2,pp=pp,Te=Te)
lct5 <- lct5*Te/60.0
pp <- 0.16
lct16 <- LC.estimate.probit(tmp2,pp=pp,Te=Te)
lct16 <- lct16*Te/60.0
pp <- 0.50
lct50 <- LC.estimate.probit(tmp2,pp=pp,Te=Te)
lct50 <- lct50*Te/60.0
pp <- 0.84
lct84 <- LC.estimate.probit(tmp2,pp=pp,Te=Te)
lct84 <- lct84*Te/60.0
pp <- 0.95
lct95 <- LC.estimate.probit(tmp2,pp=pp,Te=Te)
lct95 <- lct95*Te/60.0
pp <- 0.99
lct99 <- LC.estimate.probit(tmp2,pp=pp,Te=Te)
lct99 <- lct99*Te/60.0
cat("b0 = ",b0,"\n","b1 = ",b1,"\n","b2 = ",b2,"\n",
"n = ",n,"\n",file=f1out)
cat("Total number of animals = ",Totalanimals,file=f1out,append=TRUE)
```

```
cat(" distributed among ",Nogroups,"
groupings.",file=f1out,append=TRUE)
cat("\n","\n","\n",file=f1out,append=TRUE)
cat("Time
[s]","LCt01","LCt05","LCt16","LCt50","LCt84","LCt95","LCt99",
sep=" ",file=f1out,append=TRUE)
cat("\n","\n","\n",file=f1out,append=TRUE)
tmp <- cbind(Te,lct1,lct5,lct16,lct50,lct84,lct95,lct99)
write(format.default(t(tmp),digits=12),file=f1out,
ncol=ncol(tmp),append=TRUE)
pp <- 0.5
Te1 <- 120.0
lct50 twomin <- LC.estimate.probit(tmp2,pp=pp,Te=Te1)
lct50 twomin <- lct50 twomin*Te1/60.0
pp <- 0.5
Te2 <- 600.0
lct50 tenmin <- LC.estimate.probit(tmp2,pp=pp,Te=Te2)
lct50 tenmin <- lct50 tenmin*Te2/60.0
cat("\n","\n","\n",file=f1out,append=TRUE)
cat("LCt[50] at 2 minutes = ",lct50 twomin,"mg-min/m3",
"\n",file=f1out,append=TRUE)
cat("LCt[50] at 10 minutes = ",lct50 tenmin,"mg-min/m3",
"\n",file=f1out,append=TRUE)
Ylow <- qnorm(0.0001)
Yhigh <- qnorm(0.99999)
logTLlow <- (Ylow-b0)/b2
logTLhigh <- (Yhigh-b0)/b2
TL <- seq(logTLlow,logTLhigh,by = 0.005)
TL <- 10TL
Y <- b0 + b2*log10(TL)
P <- pnorm(Y)*100.0
tmp <- cbind(TL,P,Y)
write(format.default(t(tmp),digits=12),file=f2out,ncol=ncol(tmp))
```

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Estimated human inhalation toxicity values for GB were calculated using a new three dimensional, non-linear dose response (toxicity) model combined with re-evaluated allometric equations relating animal and human respiration. Historical animal studies of GB toxicity containing both exposure and fractional animal response data were used to test the new process. The final data set contained 6621 animals, 762 groups, 37 studies and 7 species. The toxicity of GB for each species was empirically related to exposure concentration (***C***; mg/m^3) and exposure time (***T***; min) through the surface function

$$\boldsymbol{Y} = b_0 + b_1 \text{Log}_{10}\boldsymbol{C} + b_2 \text{Log}_{10}\boldsymbol{T} \quad \text{or} \quad \boldsymbol{Y} = b_0 + b_2 \text{Log}_{10}\boldsymbol{C}^{\boldsymbol{n}}\boldsymbol{T}$$

where ***Y*** is the PROBIT, b_0, b_1 and b_2 are constants and ***n*** is the "toxic load exponent". Between exposure times 0.17 and 30 min, the average value for ***n*** in 7 species was 1.35 ± 0.15. The near parallel toxic load equations for each species and the linear relationship between minute volume/ body weight ratio and the inhalation toxicity (LCt_{50}) for GB were used to create a pseudo-human data set and then an exposure time/toxicity surface for the human. The calculated ***n*** for the human was 1.38 ± 0.01. The pseudo-human data had much more variability at low exposure times. Raising the lower exposure limit to one min did not change the LCt_{50} but did result in lower variability. Raising the lower value to 2 min was counterproductive. Based on the toxic load model for 1 - 30 min exposures, the ***n*** value was 1.40 and the human GB toxicities (LCt_{01}, LCt_{05}, LCt_{50} and LCt_{95}) for 70 kg humans breathing 15 L/min were estimated to be 11, 16, 36 and 83; 18, 25, 57 and 132; 24, 34, 79 and 182 $mg.min/m^3$ for 2, 10 and 30 min exposures, respectively. These values are recommended for general use for the total human population. The empirical relationships employed in the calculations may not be valid for exposure times >30 min.

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GB
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Inhalation
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