Low-Level Toxicology and the Human Toxicity Estimates

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LOW-LEVEL TOXICOLOGY AND THE HUMAN TOXICITY ESTIMATES

During the course of Operation Desert Storm it became obvious that there was a need to re-evaluate the human toxicity estimates for the classical chemical warfare agents (CWA): G, V, and H. Under the auspices of the US Army Surgeon General, a comprehensive report entitled "Review of Existing Toxicity Data and Human Estimates for Selected Chemical Agents and Recommended Human Toxicity Estimates Appropriate for Defending the Soldier" was published in 1994 [1]. The document, which has since become known as the "Reutter-Wade" report was quite controversial for two primary reasons: 1) it pointed out that, contrary to popular belief, 50 years of research on CWA indicated that "Haber's Law" did not adequately describe the concentration-time profile for most agents and 2) it recommended reducing many of the existing human toxicity estimates because they appeared to have been based upon calculations for munitions expenditures (overkill)—rather than objective estimates of human toxicity. Perhaps the single most important finding of the report was the fact that the existing CWA toxicological database had been largely acquired in the process of developing chemical weapons—dose-response curves were incomplete and dealt with the high ends of the curves, and concentration-time profiles did not exist. There were virtually no data points for inhalation exposures longer than 10 minutes, and most of the data were for 2-minute exposures. After considerable controversy over the revised human toxicity estimates proposed by Reutter and Wade, it was agreed that the existing toxicological data were not designed to address the questions that were being asked, and a multi-year, multi-million dollar study [Low Level Toxicology (LLT)] was funded to begin filling the data gaps. The funding for that study ends in 2007, and there are still many unknowns. However, a wealth of new toxicity data has been obtained, and coupled with meta-analysis of the historical toxicity database, there is considerably more confidence in many of the currently recommended human toxicity estimates. The manuscript details the findings and conclusions of the LLT program and puts those data into the perspective of the recommendations originally made by Reutter and Wade.

1.0 PERSPECTIVES ON THE ORIGINAL HUMAN TOXICITY ESTIMATES

1.1 History

Chemical warfare agents (CWA) were primarily designed as offensive weapons. For decades, this fact was the primary determinant of how knowledge about CWA was acquired and used. Potential CWA were tested for toxicity and rejected if they did not meet minimum potency requirements in screening studies. Agents were evaluated in much the same way as conventional weapons; efficiency and efficacy were paramount.

Most of the original human toxicity estimates were derived in the 1940s and 1950s. Operationally, they were framed for the purpose of munitions expenditures. Effective doses were estimated from the

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perspective of overkill—an LD_{50} or LCt_{50} was intended to kill a minimum of 50% of the LEAST sensitive individuals [2].

1.2 Data

Intended offensive use of CWA governed the approach to the type of toxicological data that were collected. Complete dose-response curves were not determined because the interest was in the upper part of the dose-response curve—doses killing > 50% of the group. Sometimes, such data were extrapolated DOWN to the LCt_{50} or LD_{50}. In general, there was little interest in the bottom half of the dose-response curve or the probit slope. For vapour exposures there was little interest in the concentration-time profile.

Most of the data for inhalation (IH) studies were limited to exposures of 2 or 10 minutes. (Occasional studies focused on exposures of a few seconds duration.) The experimental exposure durations were based on expected cloud durations and limitations of the vapour generating systems. [It is difficult to maintain stable, low concentrations for longer exposures, AND it is difficult to have the vapour concentration in equilibrium when exposures are very short.]

1.3 Dogma

One of the determinants regarding the types of IH studies that were done during this period was the prevailing belief that Haber's Rule ($Ct = k$) described the concentration-time profile. What is curious is that the limited concentration-time profile data for several agents did not support this theory, but people were not interested in estimates for longer exposures, and the assumption of Haber's Rule simplified modelling.

2.0 RETHINKING THE HUMAN TOXICITY ESTIMATES

2.1 Pre-Gulf War

For decades, it was the perception of the military community that the existing human toxicity estimates were "gold standards". Somehow, the caveats on the estimates had been lost, and users had lost sight of the fact that risk assessment is a dynamic process—as more data and/or better modelling methods become available, toxicity estimates should be reviewed and revised, as necessary.

In the late 1980s, ECBC did some allometric modelling of recent inhalation (IH) studies on G-type agents. The analysis indicated that the existing human estimates seemed too high—the potency of the agents was underestimated. Wondering about the discrepancy with the existing estimates, ECBC began delving into how the original estimates were made.

It was quickly learned that most of the usual sources for the toxicity estimates were reviews of reviews of review articles, and many were unreferenced. When the sources were referenced, it was learned that multiple authors often independently cited a single point source or the ultimate source of their citation was the same single, point source.

With this, ECBC began tracing the human toxicity estimates back to their origins in order to find the data, assumptions, and rationales upon which they were based. The minutes of the former Human Estimates Committee (1950s and 1960s) were reviewed, and data for the G and V agents were retrieved from the original technical reports and laboratory notebooks. Data for mustard and the World War I era agents were traced from to the British and American "Red Books" back to original technical reports (when possible) dating as far back as 1918.

2.2 Gulf War

For several reasons, when the Gulf War began looming in 1990, the confidence in the existing estimates was rather low: (1) The methodology used to derive them was not up to the standards of today. (2) Often the human and animal data did not obviously support the existing values. (3) There were often several vastly different estimates for the same given endpoint for the same given exposure scenario. The Gulf War really focused the latter problem; people in the field realized that they had markedly disparate information for theoretically identical exposure scenarios.

2.3 Post-Gulf War

"Lessons learned" from the Gulf War resulted in the formation of the Chemical Defense Equipment Process Action Team (CDE-PAT). Per the U.S. Army Surgeon General, who wanted to have everyone "reading from the same sheet of music", one of the tasks of the CDE-PAT was to evaluate the existing human toxicity estimates and make recommendations, as required. Two years later a SECRET report entitled "Review of Existing Toxicity Data and Human Estimates for Selected Chemical Agents and Recommended Human Toxicity Estimates Appropriate for Defending the Soldier" [also known as the "Reutter-Wade Report"] was published. The "selected chemical agents" were GA, GB, GD, GF, VX and H. The selected routes of exposure were battlefield-relevant—IH and ocular (OC) vapor exposure, percutaneous (PC) liquid exposure, and PC vapor exposure.

The report was not well received, because it advocated lowering many of the human toxicity estimates, *i.e.* the agents appeared to be more potent than had been thought for decades, and the implications were enormous. People reacted—rather than reading WHY the recommendations were made, and they failed to realize that the recommended estimates were objective estimates of the human toxicity—not overkill assessments for munitions expenditures. The proposed toxicity estimates for IH/OC exposures were the most controversial. The "discussion" was heated and far-reaching. While the debate was raging as to what the "numbers" should be, two other important points made in the report came into the cognizance of the user community: (1) there were a huge number of data gaps and (2) for vapor exposures Haber's Rule should not be assumed. Ultimately, it was recognized that more data—data designed from the perspective of estimating human toxicity, rather than creating chemical weapons, were required.

3.0 DATA GAPS

So what were the requisite data? The most efficacious operationally relevant route of is IH/OC exposure to vapour, and emphasis is put on those data.

3.1 Concentration-Time Profiles and Toxic Load Exponents

Effectively, there were no reliable concentration-time profiles for the agents for longer exposure durations. Data for 2- and 10-minute exposures for GB (Figure 1) indicated a toxic load exponent of about 1.5 for the LCt_{50}. However, there was no confidence in extrapolating beyond those exposure durations. Crude modelling of VX lethality data indicated that the toxic load exponent might be less than one. Data for the other agents were even more sparse, and there were no concentration-time profiles for endpoints other than lethality.

3.3 Less-Than-Lethal-Endpoints

3.3.1 Miosis

There only agent for which there were quasi-reliable data was GB, and no probit slope could be determined from the available data. There were absolutely no data for GF. As indicated above, the concentration-time profiles were unknown.

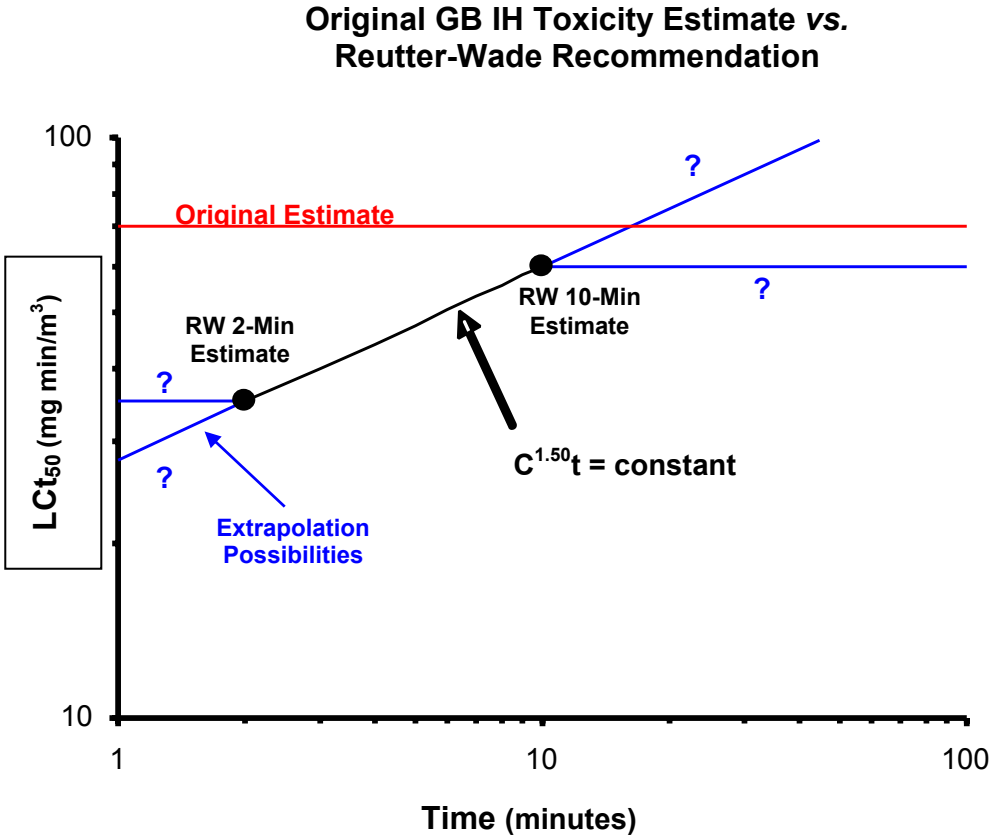

Figure 1: Concentration-Time Profile for GB at the Time of the Gulf War

3.3.2 Severe Effects

Data for severe effects (prostration, collapse convulsions) were not available for most of the agents, and the degree of confidence in the revised estimates was low. Analysis by Reutter and Wade of the existing data had indicated that the probit slope was more shallow than that for lethality, but this did not pass the "common sense" test because the physiological/toxicological mechanisms underlying severe effects were part of the continuum to lethality.

3.3.3 Sub-clinical Effects

The only existing data for sub-clinical effects consisted of cholinesterase (ChE) inhibition. The correlation between degree of inhibition and clinical effects was known to be poor—particularly with long exposures to low concentrations. The reliability of ChE inhibition as a marker for higher doses was equivocal.

4.0 THE LOW-LEVEL TOXICOLOGY PROGRAM

The Low-Level Toxicology Program (LLT) was conceived to generate some of these requisite data [3,4,5,6,7,8,9,10,11]. LLT began with some "seed money" in 1998 and subsequently became a multi-million dollar project extending through FY 07. It was designed to fill selected data gaps GB, GD (limited data), GF, and VX. The Program was NOT intended to address Gulf War Illness. This discussion will focus on the IH/OC studies done under LLT.

It was recognized that the necessary data not only needed to fill the specific data gaps outlined by Reutter and Wade [1], but also needed to provide a framework for future data collection and fulfil the requirements for modelling and casualty estimation. Toward this end, emphasis was placed upon obtaining: (a) concentration-time profiles (and their underlying toxic load exponents) for exposure durations ranging from minutes to hours, (b) probit slopes, and (c) non-lethal endpoints.

The species selected for study were the rat and the pig. The rat is a standard species for IH toxicology. The pig was selected because Reutter and Wade had determined that GB IH toxicity models as a function of body weight (BW) [Figure 2], and in order to best model the data for humans, a relatively large species was required. Moreover, pigs are excellent respiratory models for humans and do not have the carboxylesterase enzyme that protects many rodent species from nerve agents. However, (1) there were limits on the size of the animal that could be put into the exposure chamber and (2) preliminary studies in rats indicated a significant gender difference in sensitivity, so the Göttingen minipig was selected because it is sexually mature at a relatively young age and is small enough to fit into the chambers.

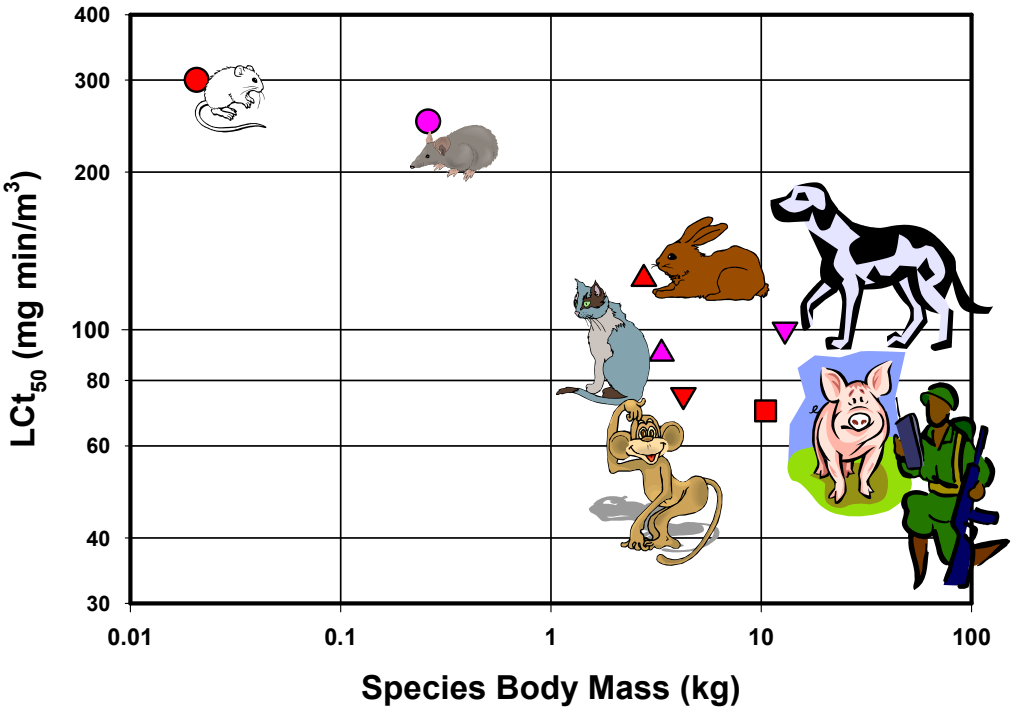

Figure 2: 10-Minute LCt_{50} *vs.* Body Mass for GB

The first agent investigated was GB. There are more historical data for GB than for the other agents, and it was desirable to use GB as a "benchmark" because it was improbable that there would ever be as much data for the other agents as there are for GB. It was also essential to ensure that the new data were

comparable (within experimental error) to the older data, and this was best accomplished with the largest data set. Finally, GB is the most volatile of these agents, so it was relatively simple to generate stable, low concentrations of GB vapour. Hence, toxicity studies with GB could be ongoing while the agent generation systems and chemical analysis were worked out for the lower volatility agents.

5.0 WHAT HAS BEEN LEARNED FROM LLT

Studies on GB, GF, and VX have been completed (the data for the VX IH studies in Göttingen minipigs are still undergoing analysis with regard to the human toxicity estimates). To maximize battlefield relevancy, all IH studies were whole-body exposures on unanesthetised animals. The data include concentration-time profiles and probit slopes for exposures as long as six hours and lethal and sub-lethal endpoints. Several revisions for the human toxicity estimates have been recommended, and these changes have been incorporated into FM 3-11.9. The findings for the individual agents are summarized below.

5.1 GB

5.1.1 Rat

Exposures ranging from 5 minutes to six hours were done in male and female Sprague-Dawley rats. ECt_{50}s, for endpoints ranging from lethality to miosis, and their associated probit slopes and toxic load exponents, were determined. [See Figure 2.] The significant findings are as follows:

- The LCt50 for 10-minute exposures closely parallels the historical data.
- There is a significant gender difference with the females being more sensitive at all time-points and endpoints. However, for lethality the gender difference decreases as the exposure duration increases.
- Haber's Rule (Ct = k) does not describe the concentration-time profiles for lethality/severe effects and miosis. Both are better approximated by the toxic load model ($C^n t = k$; n = 1.66 for lethality and 1.96 for miosis), but it is curvilinear (on a log-log scale).
- The rat is surprisingly good model for miosis.
- Clinically, miosis was the first noticeable effect (FNE), and there was no inhibition of ChE inhibition at the ECt_{50} for miosis at dosages ranging from 2.5 to 7 times the ECt_{50} for miosis (depending on the duration studied).

5.1.2 Göttingen Minipig

The studies in Göttingen minipigs encompassed exposure durations ranging from 10 minutes to three hours. Lethal and sub-lethal endpoints were investigated. ECt_{50}s, probit slopes, and concentration-time profiles were determined.

- Allometric modelling based upon BW predicted the ECt_{50}s for severe and lethal effects with surprising accuracy and validated the Göttingen minipig as an excellent model for IH toxicity for these endpoints.
- For lethality, there is a statistically significant gender difference (p = 0.01), with the males (on average, across all three exposure durations) having LCt_{50}s 10% lower than the females. This bias is opposite that of the rat. For miosis, the difference is borderline significant (p = 0.06) with the female pigs being more sensitive.9
- The concentration-time profile followed the toxic load model with n = 1.32 for miosis and 1.38 for lethality.

- The Göttingen minipig is not a sensitive model for miosis. Further, the oblique contraction of the iris makes it difficult to compare with the rat or other species that have symmetrical pupillary constriction.

5.1.3 Recommendations for the Human Toxicity Estimates

Allometric modelling of the data (the above data and the historical data) for severe effects and lethality supported the median effective dosages recommended by Reutter and Wade. It was recommended (Table 1) that the probit slope estimate for severe effects should be made the same as that for lethality, since both endpoints result from the same poisoning mechanism. Otherwise, a probabilistic impossibility would occur (*i.e.* having a LCt_{XX} being less than a ECt_{XX} [12]). The probit slope recommended by Reutter and Wade was not statistically different from those observed in the two species. No change (other than equating the slope for severe effects to that of lethality) was recommended.

The ECt_{50} for mild effects (miosis, tight chest rhinorrhea) recommended by Reutter and Wade was 0.5 mg min/m^3 (2-minute exposure). However the official interim standard [13] was subsequently set at 1 mg min/m^3 [6]—despite the fact that the historical human data indicated the ECt_{50} was lower. Given the LLT rat data and the historical human data, the recommended miosis dosage was lowered to 0.4 mg min/m^3 (2-minute exposure). The data also provided a toxic load exponent and probit slope.

It has been understood that the recommended human toxicity estimates applied to only male soldiers, because the modelling was done based on 70 kg individuals—referenced against the historical human data which were largely done on males. Although there is a significant gender difference in the rat, the difference is minor, at best, in the Göttingen minipig, which is a more acceptable physiological model for the human, so no recommendations were made for adjusting the toxicity estimates for the female soldier.

5.2 GF

Prior to the LLT program, the data for GF were extremely limited, and there were few human toxicity estimates. This stems, in large measure, from the fact that before the Gulf War there was little interest in GF. There were so few data on GF that Reutter and Wade [1] referenced the potency of GF to that of GD when there were no GF data available. [There were more data for GD, but they, too were sparse. Given the fact that GD was considerably more potent than GB—for all routes of exposure, other than IH lethality, it was reasoned that this approach would err on the side of conservatism if it proved to be imprecise.]

GB LCt_{50} (2-minute exposure)	Estimate		
	Original	**R-W**	**LLT**
Dosage (mg min/m^3)	70	35	35
Toxic Load Exponent	none	1.5*	1.5
Probit Slope	7	12.0	12.0
GB ECt_{50} (Severe) (2-minute exposure)	**Original**	**R-W**	**LLT**
Dosage (mg min/m^3)	35	25	25
Toxic Load Exponent	none	none	1.5
Probit Slope	none	10.0	12.0
GB ECt_{50} (Mild) (2-minute exposure)	**Original**	**R-W**	**LLT**
Dosage (mg min/m^3)	2	0.5	0.4
Toxic Load Exponent	none	none	1.4
Probit Slope	none	none	10

*This value is derived from the LCt_{50} values for 2-and 10-minute exposures; it is not explicitly stated by Reutter and Wade.

Table 1: Human Toxicity Estimates for IH/OC Exposure to GB Vapour

5.2.1 Rat

Exposures ranging from 5 minutes to four hours were done in male and female Sprague-Dawley rats. ECt_{50}s, for endpoints ranging from lethality to miosis, and their associated probit slopes and toxic load exponents, were determined. [See Figure 2]. The significant findings are as follows:

- There is a significant gender difference with the females being more sensitive at all time-points and endpoints.
- Haber's Rule (Ct = k) does not describe the concentration time profile. The concentration-time profile is better approximated by the toxic load model ($C^nt = k$, with n = 1.98 for miosis and n = 1.27 for lethality). The 10-minute LCt_{50} is effectively the same as that for GB; however, the toxic load exponents for the two agents differ (1.27 for GF versus 1.66 for GB). Hence, the lethality concentration-time profile is different than that for GB.
- Again, the rat was surprisingly good model for miosis, and the ECt_{50} for miosis was comparable to that for GB (toxic load exponent values of 1.98 for GF *versus* 1.96 for GB).

5.2.2 Göttingen minipig

The studies in Göttingen minipigs encompassed exposure durations ranging from 10 minutes to three hours. Lethal and sub-lethal endpoints were investigated. ECt_{50}s, probit slopes, and concentration-time profiles were determined.

- Allometric modelling based upon BW predicted the ECt_{50}s for severe and lethal effects for 10-minute exposures with surprising accuracy.
- There is no statistically significant gender difference.

- The concentration-time profile followed the toxic load model for both miosis and lethality, with n = 1.60 for miosis and 1.28 for lethality. This differs from the values for GB (1.32 for miosis and 1.38 for lethality).
- The Göttingen minipig is not a sensitive model for miosis. Again, the oblique contraction of the iris makes it difficult to compare with the rat.

5.2.3 Recommendations for the Human Toxicity Estimates

The data corroborated the recommended human toxicity estimates for severe effects and lethality for 10-minute exposures. However, given the smaller toxic load exponent (relative to GB) the extrapolations to 2-minute exposures yielded higher values than had been previously recommended [1,6]. The toxicity estimates were not rounded—in order to emphasize the differences between GF and GB [Table 2].

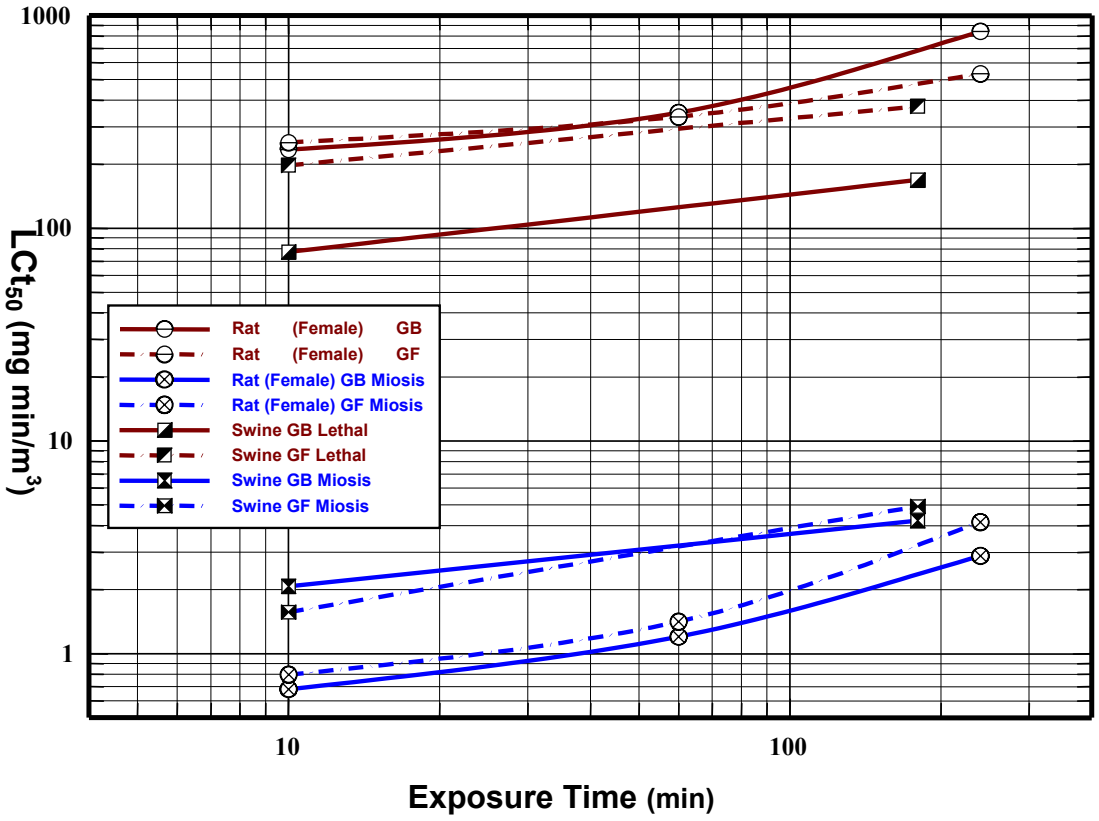

Figure 3: Concentration-Time Profiles for GB and GF in Rats and Göttingen minipigs

The miosis data for female rats were again used as the most sensitive species from which to extrapolate to humans. The ECt_{50}s for GB and GF were virtually identical. The human toxicity estimate recommended by Reutter and Wade [1] had been referenced to that for GD (there were no miosis data for GF) and was about half of the dosage recommended for GB. Given that the miosis dosage derived from the LLT data

was lower than that recommended by Reutter and Wade [1] and the interim standard [6], the body of data did not mitigate against raising the raising the recommended ECt_{50} (mild effects) for GF, and the human toxicity estimate was raised from 0.2 mg min/m^3 to 0.4 mg min/m^3 [Table 2].

GF LCt_{50} (2-minute exposure)	**Estimate**		
	Original	**R-W**	**LLT**
Dosage (mg min/m^3)	none	35	43
Toxic Load Exponent	none	none	1.25
Probit Slope	none	12.0	12.0
GF ECt_{50} (Severe) (2-minute exposure)	**Original**	**R-W**	**LLT**
Dosage (mg min/m^3)	none	25	31
Toxic Load Exponent	none	none	1.25
Probit Slope	none	10.0	12.0
GF ECt_{50} (Mild) (2-minute exposure)	**Original**	**R-W**	**LLT**
Dosage (mg min/m^3)	none	0.2	0.4
Toxic Load Exponent	none	none	1.5
Probit Slope	none	none	10

Table 2: Human Toxicity Estimates for IH/OC Exposure to GF Vapour

5.3 VX

Historical IH toxicity data for VX are limited. VX has a low vapour pressure, and it is not only difficult to volatilise it, but it is also difficult to maintain a steady vapour concentration—particularly for long exposure durations.

Preliminary modelling had indicated that VX does not follow the BW function that GB and GF do. The historical data also indicated that the concentration-time profile was considerably different. [Given that airborne VX is virtually equi-potent *via* IH and PC exposure—contrary to GB which has relatively low PC toxicity, the differences in the BW function and concentration-time profile could reflect the enhanced PC potency, compared to GB.]

5.3.1 Rat

Exposures ranging from 10 minutes to four hours were done in male and female Sprague-Dawley rats. ECt_{50}s, for endpoints ranging from lethality to miosis, and their associated probit slopes and toxic load exponents, were determined. The significant findings are as follows:

- For lethality, there is a marginal gender difference with the females being slightly more sensitive for 10-minute exposures if the animals were decontaminated post-exposure. However, if the rats were not decontaminated, the males were more sensitive for 10-minute exposures. For the other exposure durations (60 and 240 minutes), there was no statistically significant gender difference.

- Clinically, miosis was the first noticeable effect (FNE). The rat is surprisingly good model for miosis. For miosis, there is a significant gender difference, with the females being more sensitive at all exposure durations
- For threshold AChE depression, there is a marginal (at best) gender difference (p-value of 0.049), with females being more sensitive for all three exposure durations investigated.
- The toxic load model describes the concentration-time profiles for severe effects/lethality and miosis. However, the toxic load exponent for the former is <1 (n = 0.92), which means that the LCt_{50} and ECt_{50} (severe effects) decrease with longer exposure durations. The toxic load exponent for miosis is 1.65, which means the ECt_{50}s increase with longer exposure durations.
- The concentration-time profile for threshold AChE depression from whole-body vapour exposures has also been determined. The toxic load exponent equals 1.57. The ECt_{50} for depression is about a factor of 1.75 higher than the ECt_{50} for miosis (roughly constant potency ratio with respect to exposure duration).
- There is a statistically significant gender difference, with the females being more sensitive at all time-points and endpoints.

5.3.2 Göttingen Minipig

The responses of the Göttingen minipig to VX were unexpected .

- Dosages producing miosis were not significantly different from those producing death.
- Contrary to what was observed in the rat, the toxic load exponent for lethality/server effects was >1.

Figure4. Concentration-Time Profiles for VX in Rats

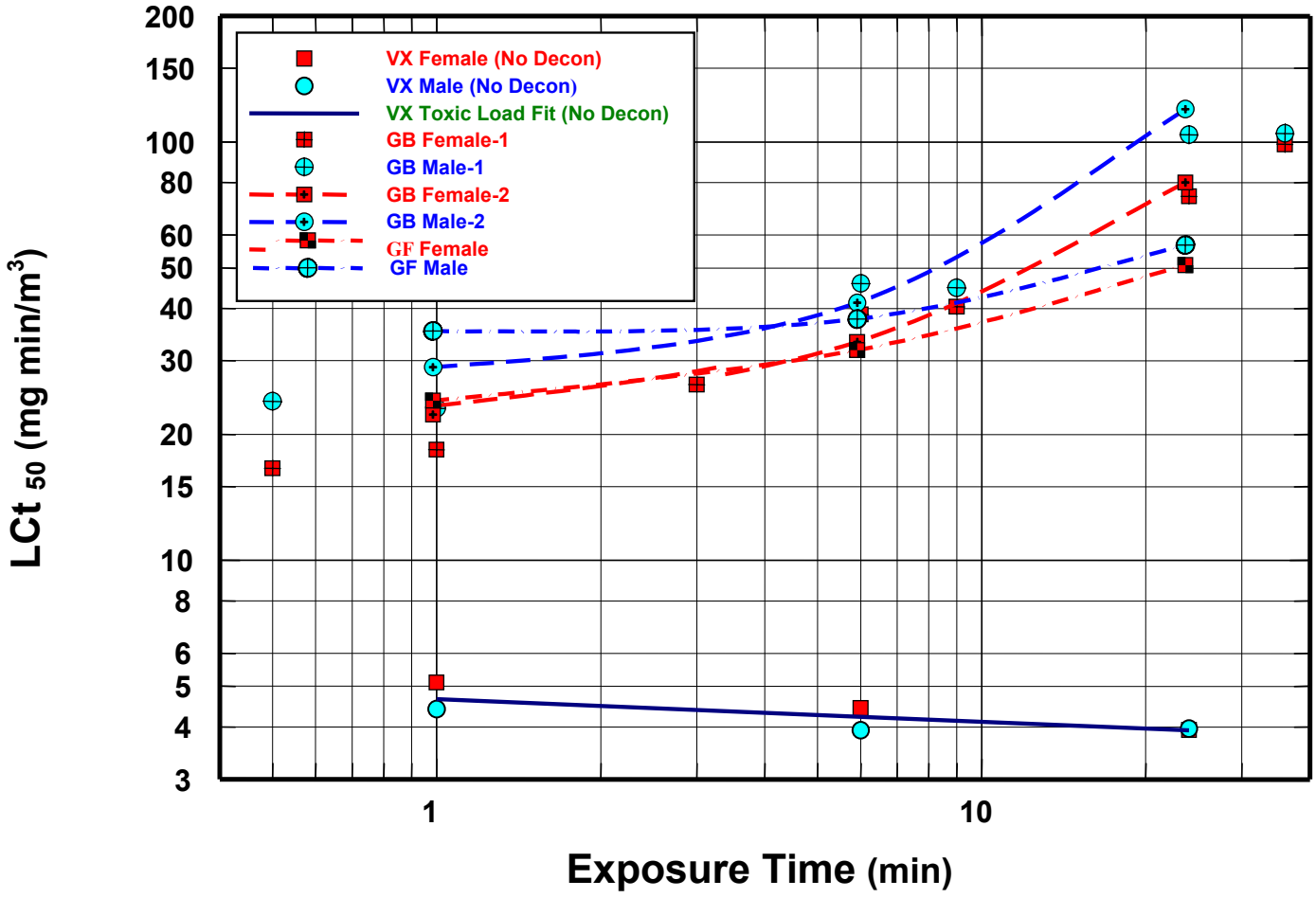

Figure4. Concentration-Time Profiles for GB and VX in Rats

5.3.3 Recommendations for the Human Toxicity Estimates

Based upon the relative potency of GB and VX in female rats and the recommendations that were made for human estimate for GB-induced miosis in humans, the recommended ECt_{50} for humans was reduced. Although the toxic load exponent for the rat was greater than one, the concentration-time profile picture for VX is so complicated, that the toxic load exponent was assumed to be one (which is somewhat more conservative for longer exposures). No recommendations for changes in the ECt_{50}s for lethality and severe effects have been made; however, the recommended probit slope was changed, based upon these data. Similar to miosis, the recommended toxic load exponent for these endpoints is currently one.

VX LCt_{50} (2-minute exposure)	Estimate		
	Original	**R-W**	**LLT**
Dosage (mg min/m^3)	30	15	15
Toxic Load Exponent	none	none	1.0
Probit Slope	none	6.3	10.0
VX ECt_{50} (Severe) (2-minute exposure)	**Original**	**R-W**	**LLT**
Dosage (mg min/m^3)	25	10	10
Toxic Load Exponent	none	none	1.0
Probit Slope	none	5.9	10.0
VX ECt_{50} (Mild) (2-minute exposure)	**Original**	**R-W**	**LLT**
Dosage (mg min/m^3)	0.09	0.09	0.04
Toxic Load Exponent	none	none	1.0
Probit Slope	none	none	4

Table 3: Human Toxicity Estimates for IH/OC Exposure to VX Vapour

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