



**ERCBH<sub>2</sub>S**

**A Model for Calculating Emergency  
Response and Planning Zones for  
Sour Gas Facilities**

**Volume 2: Emergency Response Planning Endpoints**



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**ENERGY RESOURCES CONSERVATION BOARD**  
**ERCBH2S: A Model for Calculating Emergency Response and Planning Zones for Sour Gas**  
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## SUMMARY

The Alberta Energy Resources Conservation Board (ERCB) requires industry to pre-plan incident responses within the emergency planning zone (EPZ). Emergency response actions are taken to prevent significant exposure to hydrogen sulphide (H<sub>2</sub>S) near the release site. Further from the release site the concentration decreases until at the outer limit of the EPZ, as defined by the ERCB EPZ toxic load endpoint, the exposure should not result in unconsciousness. The ERCB EPZ Endpoint requires the specification of an exposure concentration and time pair and an exponent  $n$  to define the toxic load.

Volume 2 Emergency Response Planning Endpoints provides a summary of the work done to-date to define the endpoint required for the computer model ERCBH<sub>2</sub>S. The extensive stakeholder engagement process undertaken to assist the ERCB in its EPZ Endpoint selection is documented. An overview of hydrogen sulphide lethality data and exposure criteria that was developed for the November 2004 multi-stakeholder meeting is provided. It is a summary of emergency response and planning criteria used by other jurisdictions and the animal lethality data they referenced. Following the meeting, the ERCB had a review and assessment of the technical quality of lethality data proposed for use in “toxic load” calculations in support of hydrogen sulphide exposure endpoints for emergency planning purposes. The study rated the quality of lethality data identified in the overview and is attached.

This report then focused on the 22 animal lethality studies that received a moderate rating in order to determine the ERCB EPZ Endpoint. Results from 175 tests on 2291 mice and rats are summarized. About half of the studies were done in Alberta. A statistical analysis of the data was done using the probit method. On an individual study basis there was a good comparison to what the study researchers presented. When all of the data from the different species and studies were combined the goodness of fit to the toxic load model was poor, but acceptable. No data was eliminated from the combined analysis. Based on the data analysis, an exponent  $n$  of 3.5 was selected.

The probit analysis also provides the median lethal load (L50) and the variability of the response in the population of test animals. The highest confidence is in the L50. The ERCB L50 Endpoint objective is to prevent lethality so the no deaths data was reviewed in more detail. A study that used unconsciousness in mice as the endpoint was also available to define a load that prevents unconsciousness.

The toxic load that causes an effect in an animal is adjusted to a human by dividing by uncertainty factors. A review of the mathematics and of the various types of uncertainty factors applied by other agencies revealed considerable confusion when they are applied to toxic loads. The data analysis clearly shows the load (the product of time and concentration raised to a power  $n$ ) causes the effect (lethality). The confusion arises when traditional approaches for the dose of a hazardous substance ( $n=1$ ) are mistakenly applied to the load.

The uncertainty factor for adjusting the rat/mouse L50 load to the human L50 load is 20. This is based on multiplying and rounding upwards factors of three (3) for interspecies variability, three (3) for intraspecies variability and two (2) for the increased inhalation rate during an emergency.

The human ERCB L50 represents a toxic load for 50% lethality, including the susceptible population and is defined by:

$$ERCBL50 = C^{3.5}t = 2.279 \cdot 10^{10} \text{ ppm}^{3.5} \text{ minutes} = \frac{4.557 \cdot 10^{11}}{20}$$

$$\text{Probit} = -29.415 + 1.443 \cdot \ln(C^{3.5}t)$$

The endpoint scaling factor from rat/mouse L50 data to no deaths in animals is five (5). The endpoint scaling factor from rat/mouse L50 data to no unconsciousness in animals is fifteen (15), based on multiplying factors of three (3) for 50% unconsciousness from the L50 and five (5) for no unconsciousness from the 50% unconsciousness load.

To extrapolate from the rat/mouse L50 data to an endpoint that is *protective of death* in humans, an uncertainty factor of 100 (endpoint scaling factor of 5 multiplied by uncertainty factor of 20) is appropriate. To extrapolate from the rat/mouse L50 data to an endpoint that is *protective of unconsciousness* in humans, an uncertainty factor of 300 (endpoint scaling factor of 15 multiplied by uncertainty factor 20) is appropriate.

A **three hundred-fold** uncertainty factor is recommended for the ERCB non-unconsciousness endpoint to provide an adequate margin of safety. This endpoint has been set at 130 ppm for 60 minutes with an exponent *n* of 3.5. By definition this endpoint will also be protective of lethality as it is set to a lower toxic load.

The ERCB EPZ endpoint has been set at 100 ppm for 60 minutes with an exponent *n* of 3.5 to provide a more conservative margin of safety. The following table compares H<sub>2</sub>S exposure endpoints:

H2S Exposure Endpoints			
Load Equation L= tC <sup>n</sup> with exponent <i>n</i> = 3.5			
Exposure Time (t minutes)	H <sub>2</sub> S Concentration (C ppm)		
	ERCB EPZ UF=759	No Unconsciousness UF=300	50% Lethality UF=20
3	235	307	665
15	149	194	420
30	122	159	345
60	100	130	283
120	82	107	232
180	73	95	207

The uncertainty factors required to produce the ERCB EPZ Endpoint is 759, about two and one half times the value of 300 supported by the unconsciousness data analysis. With this extra safety factor, exposure to H<sub>2</sub>S at the ERCB EPZ Endpoint will not result in unconsciousness that would impair escape.

The H<sub>2</sub>S exposure endpoints were also compared to two human exposure studies with high concentration exposures. The comparison showed that the proposed ERCB L50 probit

parameters are based on reasonable uncertainty factors and that the ERCB EPZ Endpoint should not result in unconsciousness.

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# 1 INTRODUCTION

In December 2000, the *Provincial Advisory Committee on Public Safety and Sour Gas* published recommendations pertaining to emergency planning, preparedness and response. Some of the Advisory Committee recommendations called for a review of the calculation method of emergency planning zones (EPZ) for sour operations. To address these recommendations the ERCB has revised its *Directive 071, Emergency Preparedness and Response Requirements for the Petroleum Industry* for sour wells, sour pipelines, and sour production facilities. A significant change is the requirement to use the ERCBH2S computer software. ERCBH2S is a complex tool that calculates site-specific EPZs using thermodynamics, fluid dynamics, atmospheric dispersion modelling, and toxicology. The development of ERCBH2S has been a considerable undertaking with much input from many stakeholders across a range of backgrounds, disciplines and expertise.

Documents pertaining to ERCBH2S are:

Directive 71, Emergency Preparedness and Response Requirements for the Petroleum Industry (ERCB Directive 071, 2010)	This directive provides the requirements for the industrial operator. It covers not only sour operations but any activity where a hazard exists with the potential to cause a risk to the public.
Preface	Written for industrial operators and public with a particular interest in ERCBH2S. It provides an overview of the ERCB hazard management process and presents a higher level summary of the key components of the ERCBH2S software.
Volume 1 Technical Reference Document	Written for the technical specialist and to document the complex science within ERCBH2S. It provides the science required to calculate the EPZ and the basis for selecting the components used to make the calculations within ERCBH2S.
Volume 2 Emergency Responses Planning Endpoints (This document)	Written for the technical specialist with a particular interest in toxicology. It presents the data available to choose an EPZ endpoint, toxicological calculations and the EPZ endpoint values.
Volume 3 User Guide	Written for the ERCBH2S user, it provides a description on how to install and operate the computer software application with tutorial notes.

This document, Volume 2, provides a summary of the work done to-date to define the endpoint required for the computer model ERCBH2S. The selection of the endpoint has been a long process. Appendix 1 documents the extensive stakeholder engagement process undertaken to assist the ERCB in its EPZ endpoint selection. Appendix 2 provides an Overview of Hydrogen Sulphide Lethality Data and Exposure Criteria. Appendix 3 is a Review and Assessment of the Technical Quality of Lethality Data Proposed for Use in “Toxic Load” Calculations in Support of Hydrogen Sulphide Exposure Endpoints for Emergency Planning Purposes.

The ERCB requires industry to pre-plan its priority response within the EPZ. Actions are taken immediately to prevent exposure to high concentrations of H<sub>2</sub>S which could result in unconsciousness. The ERCB EPZ endpoint reflects this objective and is for emergency planning only. It is not an exposure level that will be monitored before action is taken.

As defined by the ERCB EPZ toxic load endpoint, the exposure to the ERCB EPZ endpoint should not result in unconsciousness. The equation for toxic load is:

$$Load = Time * Concentration^n$$

Toxic load depends on both the concentration and time with the concentration weighted by the power  $n$ , a number greater than 1. The ERCB EPZ endpoint requires the specification of the exponent  $n$  and a concentration-time pair to define the toxic load.

This report starts by summarizing the animal lethality test data that received a moderate rating in Appendix 3. In these tests, rats and mice are exposed to controlled H<sub>2</sub>S concentrations for a controlled time and the number of deaths is recorded. The animals either die during the exposure or shortly afterwards (within a day). The data is statistically analyzed to determine the LC50 for an exposure time which is the concentration that 50 percent of the animals would die if exposed for the time duration. A simplified analysis of the LC50-time pairs is then done to estimate the exponent  $n$ . All of the data must be considered to define the toxic load when no unconsciousness is expected to meet the ERCB EPZ objective. This requires a more complex statistical approach known as probit analysis. Tabular and graphical results of the probit analysis are presented. This is followed by sections on the toxic loads that correspond to no deaths and no unconsciousness in test animals.

The toxic load that causes an effect in an animal is adjusted to a human by dividing by the uncertainty factor. The next section discusses uncertainty factors; it starts with the mathematics to make sure they are understood and correctly applied, and then the various types used by other agencies are summarized. Incorrect applications are pointed out. The section concludes with the suggested ERCB uncertainty factors.

With the animal LC50 and no death toxic load from the probit analysis and the uncertainty factors to adjust animal loads to humans, the ERCB endpoints can now be defined. In the process of defining the ERCB EPZ, the ERCB L50 probit parameters are defined. These are important for risk analysis of the chance of lethality. The proposed ERCB probit parameters are compared to other published values. As a check, the proposed ERCB endpoints are compared to the limited human exposure data available.

The ERCB EPZ toxic load endpoint used in ERCBH2S is 100 ppm for 60 minutes with an exponent  $n$  of 3.5. The uncertainty factors required to produce these endpoints are provided.

## 2 ANIMAL LETHALITY DATA

Table 1 provides the H<sub>2</sub>S animal lethality data that received a moderate or higher grade in a recent review<sup>1</sup> (see Appendix 3 by CANTOX 2005). This signifies that the authors' findings and conclusion are reasonably technically robust, and that the data add to the knowledge about concentration-time response characteristics of H<sub>2</sub>S lethality. The table provides the author, study code, species (mouse or rat) and sex, exposure time (minutes), exposure concentration (ppm), the numbers of animals tested, the number of animals that died and the percent response. Entries are listed alphabetically by author and species, then in increasing time, percent killed and concentration.

In the 8 studies there were 175 lethality tests on 2291 mice and rats with 780 deaths during the exposure (p=34%). There were 97 tests on 1556 mice with 489 deaths during the exposure (p=31%) and 78 tests on 735 rats with 291 deaths during the exposure (p=40%). Exposure concentrations ranged from 217 to 1655 ppm and exposure times ranged from 1 to 360 minutes.

The Clanachan, Lopez and Prior studies were funded by Alberta Environment. Of the moderately rated lethality tests available, about ½ were done in Alberta on about ¾ of the animals.

The Clanachan study has not been referenced by other regulatory jurisdictions (see documentation of the emergency response criteria Appendix 2). It was referenced in the GASCON2 ERCB 90-B reports by Rogers but not used extensively. Besides testing 1256 mice for lethality, 1140 mice were tested for the righting reflex (equivalent to unconsciousness), which will be discussed later in this report.

The test data for study NC035 by Prior was generated from the probit equations and other information provided in the report. Note that the figure in the Prior report does not match the probit equation but was used to determine the percent response for each test.

The three Lopez studies provide 0% or 100% lethality data points only and cannot be used to determine an LC50.

In the following sections this lethality data will be used to determine the exponent in the toxic load equation and the load that is lethal to 50% of the rats and mice. Uncertainty factors will then be applied to adjust the animal data to humans for L50 and EPZ endpoints.

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<sup>1</sup> Appendix 3 presents the results of work commissioned by the EUB to grade the quality of the H<sub>2</sub>S toxicity studies used by others jurisdictions, and the basis for the EUB H<sub>2</sub>S endpoint, against published benchmarks. No studies achieved a 'high grade' because the guidelines were strictly and consistently applied and all studies suffered from some deficiency. However, some of the deficiencies were minor and studies of moderate quality were considered reasonable to use in toxic load calculations.

**Table 1 Mouse and Rat Lethality Data (time – concentration - %response) with Moderate Grading**

Entry	Authors	Study Code	Species (male, female)	Exposure Time (t, minutes)	H2S Concentration (C, ppm)	Number Tested	Number Killed	% Killed
1	Clanachan (1979)	NC002	mouse m,f	1	1000	20	0	0%
2	Clanachan (1979)	NC002	mouse m,f	1	1100	20	0	0%
3	Clanachan (1979)	NC002	mouse m,f	1	1200	20	0	0%
4	Clanachan (1979)	NC002	mouse m,f	1	1300	20	0	0%
5	Clanachan (1979)	NC002	mouse m,f	2.5	800	20	0	0%
6	Clanachan (1979)	NC002	mouse m,f	2.5	900	20	0	0%
7	Clanachan (1979)	NC002	mouse m,f	2.5	1000	20	0	0%
8	Clanachan (1979)	NC002	mouse m,f	2.5	1100	20	1	5%
9	Clanachan (1979)	NC002	mouse m,f	2.5	1200	20	2	10%
10	Clanachan (1979)	NC002	mouse m,f	2.5	1300	20	3	15%
11	Clanachan (1979)	NC002	mouse m,f	5	800	20	0	0%
12	Clanachan (1979)	NC002	mouse m,f	5	900	20	0	0%
13	Clanachan (1979)	NC002	mouse m,f	5	1000	20	0	0%
14	Clanachan (1979)	NC002	mouse m,f	5	1100	20	4	20%
15	Clanachan (1979)	NC002	mouse m,f	5	1300	20	12	60%
16	Clanachan (1979)	NC002	mouse m,f	5	1200	20	13	65%
17	Clanachan (1979)	NC002	mouse m,f	7.5	700	20	0	0%
18	Clanachan (1979)	NC002	mouse m,f	7.5	800	20	0	0%
19	Clanachan (1979)	NC002	mouse m,f	7.5	900	20	0	0%
20	Clanachan (1979)	NC002	mouse m,f	7.5	1000	20	0	0%
21	Clanachan (1979)	NC002	mouse m,f	7.5	1100	20	8	40%
22	Clanachan (1979)	NC002	mouse m,f	7.5	1200	20	14	70%
23	Clanachan (1979)	NC002	mouse m,f	7.5	1300	20	17	85%
24	Clanachan (1979)	NC002	mouse m,f	10	700	20	0	0%
25	Clanachan (1979)	NC002	mouse m,f	10	800	46	0	0%
26	Clanachan (1979)	NC002	mouse m,f	10	900	46	0	0%
27	Clanachan (1979)	NC002	mouse m,f	10	1000	46	9	20%
28	Clanachan (1979)	NC002	mouse m,f	10	1100	46	25	54%
29	Clanachan (1979)	NC002	mouse m,f	10	1200	46	34	74%
30	Clanachan (1979)	NC002	mouse m,f	10	1300	46	44	96%
31	Clanachan (1979)	NC002	mouse m,f	12.5	600	20	0	0%
32	Clanachan (1979)	NC002	mouse m,f	12.5	700	20	0	0%
33	Clanachan (1979)	NC002	mouse m,f	12.5	800	20	0	0%
34	Clanachan (1979)	NC002	mouse m,f	12.5	900	20	0	0%
35	Clanachan (1979)	NC002	mouse m,f	12.5	1000	20	6	30%
36	Clanachan (1979)	NC002	mouse m,f	12.5	1100	20	13	65%
37	Clanachan (1979)	NC002	mouse m,f	12.5	1200	20	17	85%
38	Clanachan (1979)	NC002	mouse m,f	12.5	1300	20	20	100%
39	Clanachan (1979)	NC002	mouse m,f	15	600	20	0	0%
40	Clanachan (1979)	NC002	mouse m,f	15	700	20	0	0%
41	Clanachan (1979)	NC002	mouse m,f	15	800	20	0	0%
42	Clanachan (1979)	NC002	mouse m,f	15	900	20	2	10%
43	Clanachan (1979)	NC002	mouse m,f	15	1100	20	13	65%
44	Clanachan (1979)	NC002	mouse m,f	15	1000	20	14	70%
45	Clanachan (1979)	NC002	mouse m,f	15	1200	20	19	95%
46	Clanachan (1979)	NC002	mouse m,f	15	1300	20	20	100%
47	Clanachan (1979)	NC002	mouse m,f	30	500	20	0	0%

Entry	Authors	Study Code	Species (male, female)	Exposure Time (t, minutes)	H2S Concentration (C, ppm)	Number Tested	Number Killed	% Killed
48	Clanachan (1979)	NC002	mouse m,f	30	600	20	0	0%
49	Clanachan (1979)	NC002	mouse m,f	30	700	20	0	0%
50	Clanachan (1979)	NC002	mouse m,f	30	800	20	1	5%
51	Clanachan (1979)	NC002	mouse m,f	30	900	20	7	35%
52	Clanachan (1979)	NC002	mouse m,f	30	1000	20	12	60%
53	Clanachan (1979)	NC002	mouse m,f	30	1100	20	17	85%
54	Clanachan (1979)	NC002	mouse m,f	30	1200	20	20	100%
55	Clanachan (1979)	NC002	mouse m,f	30	1300	20	20	100%
56	Lopez et al (1987)	NC027	rat m	240	400	12	0	0%
57	Lopez et al (1989)	NC031	rat m	3	1655	5	5	100%
58	Lopez et al (1986)	NC069	rat m	360	300	12	12	100%
59	MacEwen and Vernot (1972)	NC072	mouse m	60	504	10	0	0%
60	MacEwen and Vernot (1972)	NC072	mouse m	60	400	10	2	20%
61	MacEwen and Vernot (1972)	NC072	mouse m	60	635	10	5	50%
62	MacEwen and Vernot (1972)	NC072	mouse m	60	800	10	8	80%
63	MacEwen and Vernot (1972)	NC072	rat m	60	400	10	0	0%
64	MacEwen and Vernot (1972)	NC072	rat m	60	504	10	0	0%
65	MacEwen and Vernot (1972)	NC072	rat m	60	635	10	1	10%
66	MacEwen and Vernot (1972)	NC072	rat m	60	800	10	9	90%
67	Prior et al (1988)	NC035	rat m,f	120	453	12	0	0%
68	Prior et al (1988)	NC035	rat m,f	120	537	24	1	4%
69	Prior et al (1988)	NC035	rat m,f	120	546	24	2	8%
70	Prior et al (1988)	NC035	rat m,f	120	567	24	6	25%
71	Prior et al (1988)	NC035	rat m,f	120	587	12	6	50%
72	Prior et al (1988)	NC035	rat m,f	120	604	24	17	71%
73	Prior et al (1988)	NC035	rat m,f	120	630	24	22	92%
74	Prior et al (1988)	NC035	rat m,f	120	760	12	12	100%
75	Prior et al (1988)	NC035	rat m,f	240	257	12	0	0%
76	Prior et al (1988)	NC035	rat m,f	240	398	24	1	4%
77	Prior et al (1988)	NC035	rat m,f	240	417	12	1	8%
78	Prior et al (1988)	NC035	rat m,f	240	458	24	6	25%
79	Prior et al (1988)	NC035	rat m,f	240	501	12	6	50%
80	Prior et al (1988)	NC035	rat m,f	240	548	24	18	75%
81	Prior et al (1988)	NC035	rat m,f	240	631	24	23	96%
82	Prior et al (1988)	NC035	rat m,f	240	976	12	12	100%
83	Prior et al (1988)	NC035	rat m,f	360	217	24	0	0%
84	Prior et al (1988)	NC035	rat m,f	360	297	24	2	8%
85	Prior et al (1988)	NC035	rat m,f	360	316	24	6	25%
86	Prior et al (1988)	NC035	rat m,f	360	335	36	18	50%
87	Prior et al (1988)	NC035	rat m,f	360	377	24	22	92%
88	Prior et al (1988)	NC035	rat m,f	360	515	24	24	100%
89	Tansy et al (1981)	NC047	rat m,f	240	400	10	3	30%
90	Tansy et al (1981)	NC047	rat m,f	240	440	10	3	30%
91	Tansy et al (1981)	NC047	rat m,f	240	475	10	7	70%
92	Tansy et al (1981)	NC047	rat m,f	240	500	10	8	80%
93	Tansy et al (1981)	NC047	rat m,f	240	525	10	8	80%
94	Tansy et al (1981)	NC047	rat m,f	240	554	10	9	90%
95	Tansy et al (1981)	NC047	rat m,f	240	600	10	10	100%
96	Zwart et al (1990)	NC056	mouse f	5	665	5	0	0%
97	Zwart et al (1990)	NC056	mouse f	5	854	5	0	0%
98	Zwart et al (1990)	NC056	mouse f	5	1308	5	2	40%

Entry	Authors	Study Code	Species (male, female)	Exposure Time (t, minutes)	H2S Concentration (C, ppm)	Number Tested	Number Killed	% Killed
99	Zwart et al (1990)	NC056	mouse f	10	665	5	0	0%
100	Zwart et al (1990)	NC056	mouse f	10	856	5	0	0%
101	Zwart et al (1990)	NC056	mouse f	10	1301	5	5	100%
102	Zwart et al (1990)	NC056	mouse f	30	321	5	0	0%
103	Zwart et al (1990)	NC056	mouse f	30	504	5	0	0%
104	Zwart et al (1990)	NC056	mouse f	30	581	5	0	0%
105	Zwart et al (1990)	NC056	mouse f	30	737	5	0	0%
106	Zwart et al (1990)	NC056	mouse f	30	629	5	1	20%
107	Zwart et al (1990)	NC056	mouse f	30	668	5	1	20%
108	Zwart et al (1990)	NC056	mouse f	30	694	5	2	40%
109	Zwart et al (1990)	NC056	mouse f	60	320	5	0	0%
110	Zwart et al (1990)	NC056	mouse f	60	576	5	1	20%
111	Zwart et al (1990)	NC056	mouse f	60	553	5	2	40%
112	Zwart et al (1990)	NC056	mouse f	60	694	5	2	40%
113	Zwart et al (1990)	NC056	mouse f	60	502	5	3	60%
114	Zwart et al (1990)	NC056	mouse f	60	671	5	4	80%
115	Zwart et al (1990)	NC056	mouse m	5	665	5	0	0%
116	Zwart et al (1990)	NC056	mouse m	5	854	5	0	0%
117	Zwart et al (1990)	NC056	mouse m	5	1308	5	1	20%
118	Zwart et al (1990)	NC056	mouse m	10	665	5	0	0%
119	Zwart et al (1990)	NC056	mouse m	10	856	5	0	0%
120	Zwart et al (1990)	NC056	mouse m	10	1301	5	4	80%
121	Zwart et al (1990)	NC056	mouse m	30	321	5	0	0%
122	Zwart et al (1990)	NC056	mouse m	30	504	5	0	0%
123	Zwart et al (1990)	NC056	mouse m	30	581	5	0	0%
124	Zwart et al (1990)	NC056	mouse m	30	668	5	0	0%
125	Zwart et al (1990)	NC056	mouse m	30	737	5	0	0%
126	Zwart et al (1990)	NC056	mouse m	30	629	5	1	20%
127	Zwart et al (1990)	NC056	mouse m	30	694	5	1	20%
128	Zwart et al (1990)	NC056	mouse m	60	320	5	0	0%
129	Zwart et al (1990)	NC056	mouse m	60	502	5	0	0%
130	Zwart et al (1990)	NC056	mouse m	60	553	5	0	0%
131	Zwart et al (1990)	NC056	mouse m	60	576	5	2	40%
132	Zwart et al (1990)	NC056	mouse m	60	671	5	3	60%
133	Zwart et al (1990)	NC056	mouse m	60	694	5	4	80%
134	Zwart et al (1990)	NC056	rat f	5	665	5	0	0%
135	Zwart et al (1990)	NC056	rat f	5	854	5	0	0%
136	Zwart et al (1990)	NC056	rat f	5	1308	5	5	100%
137	Zwart et al (1990)	NC056	rat f	10	665	5	0	0%
138	Zwart et al (1990)	NC056	rat f	10	856	5	5	100%
139	Zwart et al (1990)	NC056	rat f	10	1301	5	5	100%
140	Zwart et al (1990)	NC056	rat f	30	321	5	0	0%
141	Zwart et al (1990)	NC056	rat f	30	504	5	0	0%
142	Zwart et al (1990)	NC056	rat f	30	581	5	0	0%
143	Zwart et al (1990)	NC056	rat f	30	595	5	0	0%
144	Zwart et al (1990)	NC056	rat f	30	694	5	0	0%
145	Zwart et al (1990)	NC056	rat f	30	668	5	1	20%
146	Zwart et al (1990)	NC056	rat f	30	737	5	1	20%
147	Zwart et al (1990)	NC056	rat f	30	629	5	5	100%
148	Zwart et al (1990)	NC056	rat f	60	320	5	0	0%
149	Zwart et al (1990)	NC056	rat f	60	502	5	0	0%

Entry	Authors	Study Code	Species (male, female)	Exposure Time (t, minutes)	H2S Concentration (C, ppm)	Number Tested	Number Killed	% Killed
150	Zwart et al (1990)	NC056	rat f	60	553	5	0	0%
151	Zwart et al (1990)	NC056	rat f	60	576	5	0	0%
152	Zwart et al (1990)	NC056	rat f	60	590	5	0	0%
153	Zwart et al (1990)	NC056	rat f	60	671	5	4	80%
154	Zwart et al (1990)	NC056	rat f	60	694	5	4	80%
155	Zwart et al (1990)	NC056	rat m	5	665	5	0	0%
156	Zwart et al (1990)	NC056	rat m	5	854	5	2	40%
157	Zwart et al (1990)	NC056	rat m	5	1308	5	5	100%
158	Zwart et al (1990)	NC056	rat m	10	665	5	0	0%
159	Zwart et al (1990)	NC056	rat m	10	856	5	3	60%
160	Zwart et al (1990)	NC056	rat m	10	1301	5	5	100%
161	Zwart et al (1990)	NC056	rat m	30	321	5	0	0%
162	Zwart et al (1990)	NC056	rat m	30	504	5	0	0%
163	Zwart et al (1990)	NC056	rat m	30	581	5	0	0%
164	Zwart et al (1990)	NC056	rat m	30	595	5	0	0%
165	Zwart et al (1990)	NC056	rat m	30	668	5	0	0%
166	Zwart et al (1990)	NC056	rat m	30	694	5	2	40%
167	Zwart et al (1990)	NC056	rat m	30	737	5	2	40%
168	Zwart et al (1990)	NC056	rat m	30	629	5	4	80%
169	Zwart et al (1990)	NC056	rat m	60	320	5	0	0%
170	Zwart et al (1990)	NC056	rat m	60	502	5	0	0%
171	Zwart et al (1990)	NC056	rat m	60	553	5	0	0%
172	Zwart et al (1990)	NC056	rat m	60	576	5	0	0%
173	Zwart et al (1990)	NC056	rat m	60	590	5	0	0%
174	Zwart et al (1990)	NC056	rat m	60	671	5	3	60%
175	Zwart et al (1990)	NC056	rat m	60	694	5	3	60%
	Total Mouse and Rat		175			2291	780	
	Total Mouse		97			1556	489	
	Total Rat		78			735	291	

Note: Data entries have been carefully checked, some entries may appear to be in error compared to others for the same time but reflect natural variability in animals.

### 3 REPORTED LC50-TIME PAIRS

The term LC50 defines the 50<sup>th</sup> percentile Lethal Concentration for an exposure time. The LC50 is derived from the statistical analysis of the % response-concentration-time exposure data given in the previous section.

Table 2 provides a summary of the *reported* LC50 values in the moderately rated studies. Note the reported LC50 value does not always agree with the calculated value as will be discussed later. An exponent *n* of 3.5 (=7/2) has been used in the load and exposure calculation in Table 2 and will be justified in subsequent sections.

**Table 2 Reported LC50 and Time Pairs with Moderate Grading with L50 for *n* of 3.5**

Authors	Study Code	Species	Number Tested	Exposure Time (minutes)	H2S LC50 (ppm)	L50 = t*LC50 <sup>(7/2)</sup> (minutes*ppm <sup>7/2</sup> )
Zwart et al (1990)	NC056	rat	30	10	829	1.64E+11
Prior et al (1988)	NC035	rat	156	360	335	2.48E+11
Zwart et al (1990)	NC056	rat	80	30	721	3.02E+11
Clanachan (1979)	NC002	mouse	120	5	1207	3.05E+11
Clanachan (1979)	NC002	mouse	140	7.5	1132	3.66E+11
MacEwen and Vernot (1972)	NC072	mouse	40	60	634	3.85E+11
Zwart et al (1990)	NC056	mouse	60	50	671	3.91E+11
Zwart et al (1990)	NC056	rat	70	50	679	4.08E+11
Zwart et al (1990)	NC056	mouse	70	30	793	4.21E+11
Clanachan (1979)	NC002	mouse	296	10	1097	<b>4.37E+11</b>
Tansy et al (1981)	NC047	mouse	70	240	444	4.43E+11
Clanachan (1979)	NC002	mouse	160	15	1003	4.79E+11
Clanachan (1979)	NC002	mouse	160	12.5	1059	4.83E+11
Zwart et al (1990)	NC056	mouse	30	10	1150	5.16E+11
Clanachan (1979)	NC002	mouse	120	2.5	1734	5.43E+11
MacEwen and Vernot (1972)	NC072	rat	40	60	712	5.78E+11
Prior et al (1988)	NC035	rat	156	120	587	5.88E+11
Prior et al (1988)	NC035	rat	144	240	501	6.76E+11
Clanachan (1979)	NC002	mouse	180	30	961	8.25E+11
	Tests	12 mouse 7 rat 19 both	2122		Average mouse Average rat Average both	4.66E+11 4.23E+11 4.50E+11

Note: listed smallest to largest load, median in bold

The 19 values are listed from smallest to largest L50. The average mice and rats L50 of 4.50 10<sup>11</sup> is near the median of 4.37 10<sup>11</sup> minutes\*ppm<sup>7/2</sup>. The average mouse L50 is about 10% higher than the average rat L50. Zwart tested at 60 minutes but reported an LC50 for 50 minutes in the summary based on a multi-variable analysis. LC50s were not provided for all studies listed in Table 1 so the total number of animals tested is not the same (2122 vs. 2291).

### 3.1 Exponent based on LC50 data

The equation for load and exposure are:

$$Load = Time * Concentration^n$$

$$Exposure = Concentration * Time^{1/n}$$

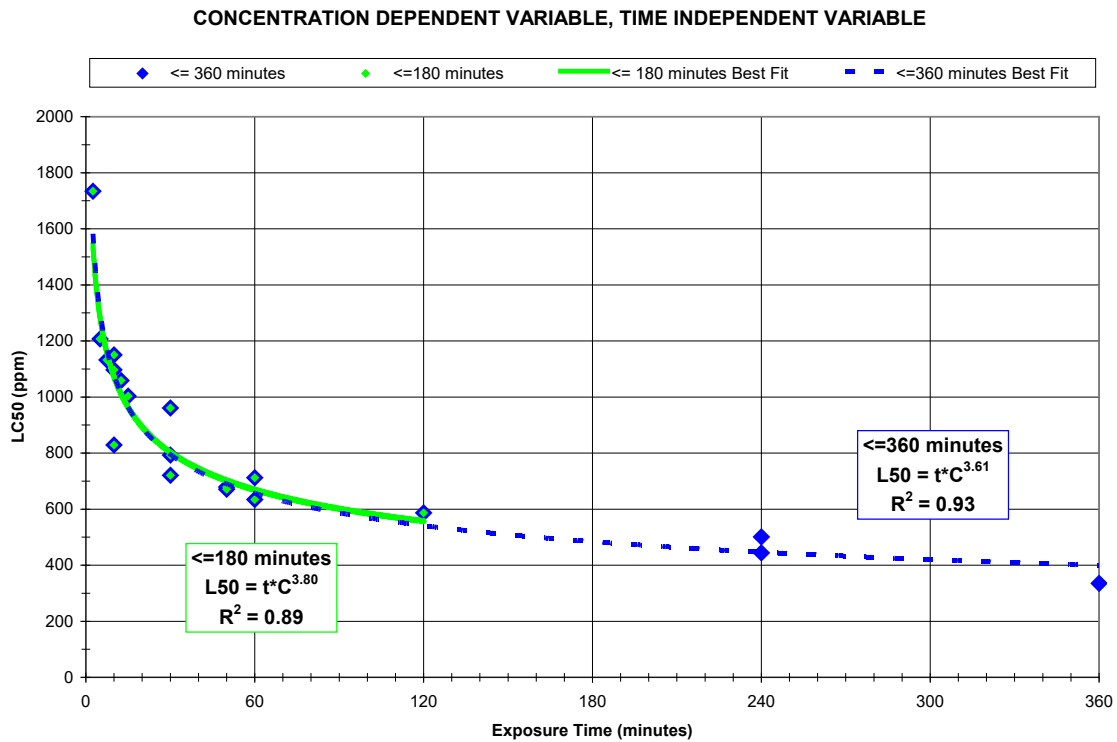
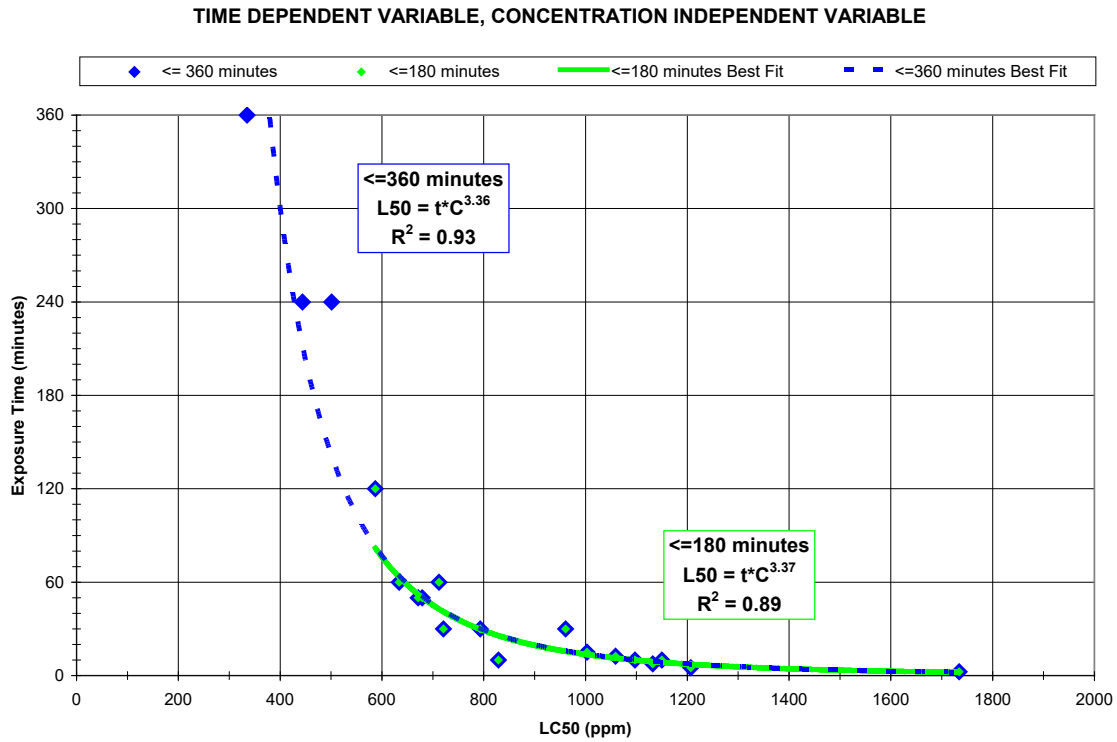
These non-linear equation are known as Haber's rule and results in higher toxic gas concentrations requiring less time to produce the same effect (for exponents  $n$  greater than 1). If the exponent  $n=1$  the equations are the linear dose relation.

Lethality data can be used to estimate the value of the exponent  $n$  in the toxic load equation in several ways. The preferred approach is to perform a multi-variable (% response – time - concentration) probit analysis, as discussed in the next section. This accounts for uncertainty in the predicted response based on the variability in the time and concentration. Alternately, the LC50 - exposure time data can be used for an initial estimate, as done below.

In laboratory animal lethality studies, for each exposure test at a specified exposure time and concentration, the number of fatalities is recorded. The time and concentration are carefully controlled with very little margin of error. The variability is in the response of the animals. The LC50 for each exposure time is derived from the statistical analysis of the % response-concentration data with the exposure time a constant. It is not possible to derive the exponent  $n$  if the time is constant.

Data from different exposure times can be analyzed to determine the exponent  $n$ . Figure 1 is a plot of the reported LC50 concentration and time pairs in Table 2. The data is presented two ways; the top plot shows time as the dependent variable and concentration as the independent variable ( $x = \text{concentration}$ ,  $y = \text{time}$ ). The bottom plot is the opposite, with concentration as the dependent variable and time as the independent variable ( $x = \text{time}$ ,  $y = \text{concentration}$ ). The equations for the best fit lines corresponding to Haber's Rule for toxic load are also provided. The top plot assumes the error is in the time variable while the bottom plot assumes the error is in the concentration variable. Notice the exponents for the equations derived both ways are not identical because the data does not perfectly fit the curves. If the goodness of fit was perfect with  $r^2=1$ , the exponents in the top and bottom plots would be the same. This can create some confusion in determining the exponent from LC50 data. Since there is uncertainty in the percent response which depends on both time and concentration, the average value should be used as summarized below:

Variables	Exposure Time less than 3 hours	Exposure Time less than 6 hours
Time Dependent (error), Concentration Independent	$n = 3.36$	$n = 3.37$
Concentration Dependent (error), Time Independent	$n = 3.80$	$n = 3.61$
Average	$n = 3.58$	$n = 3.49$

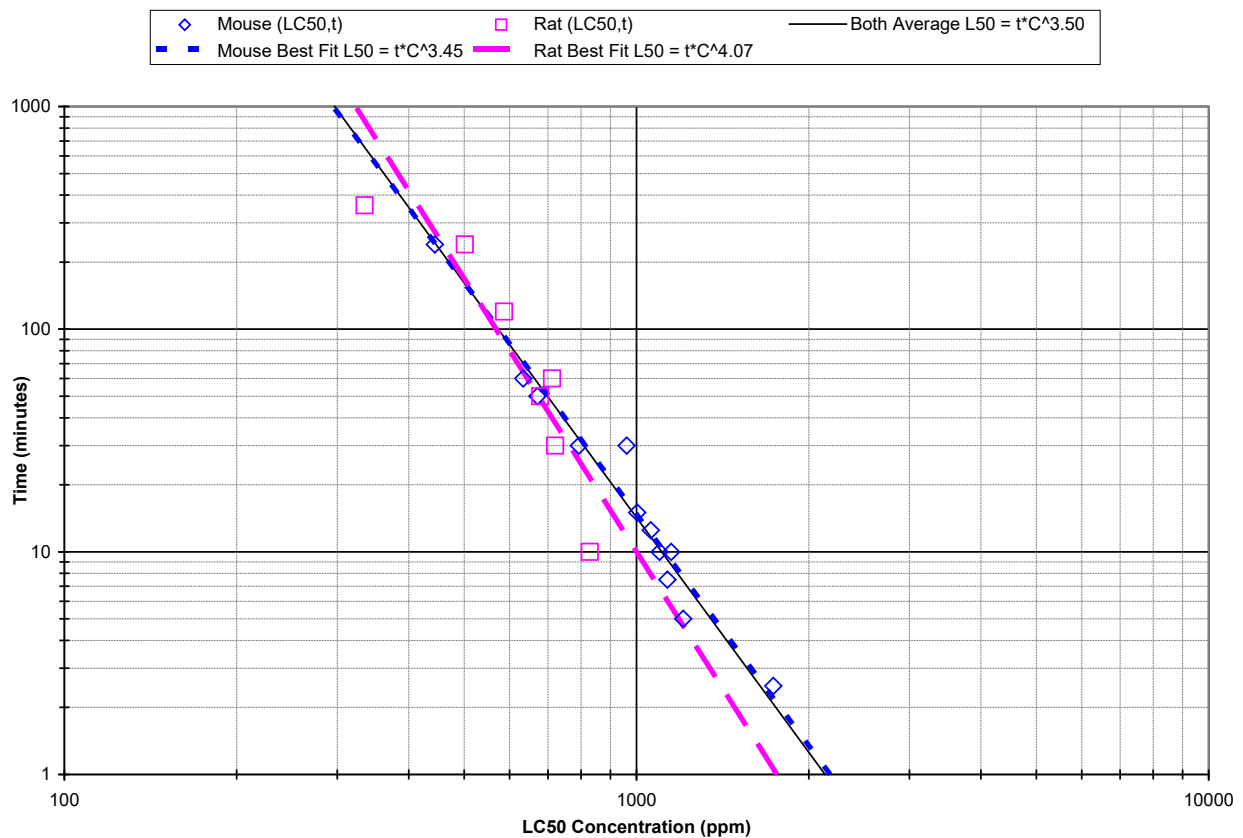


**Figure 1** LC50 and Time Pairs with Moderate Grading Presented Two Ways to Determine Exponent (the average  $n$  of top and bottom plot should be used)

In the EPZ requirements, a maximum exposure duration of 3 hours has been set. Results for exposure times less than 3 hours ( $\leq 180$  minutes) are compared to times under 6 hours ( $\leq 360$  minutes) in Figure 1. The exponent  $n$  increases to 3.58 from 3.49 with shorter exposure times, but the goodness of fit decreases. Based on this simplified analysis of the data, an  $n$  of 3.5 is recommended

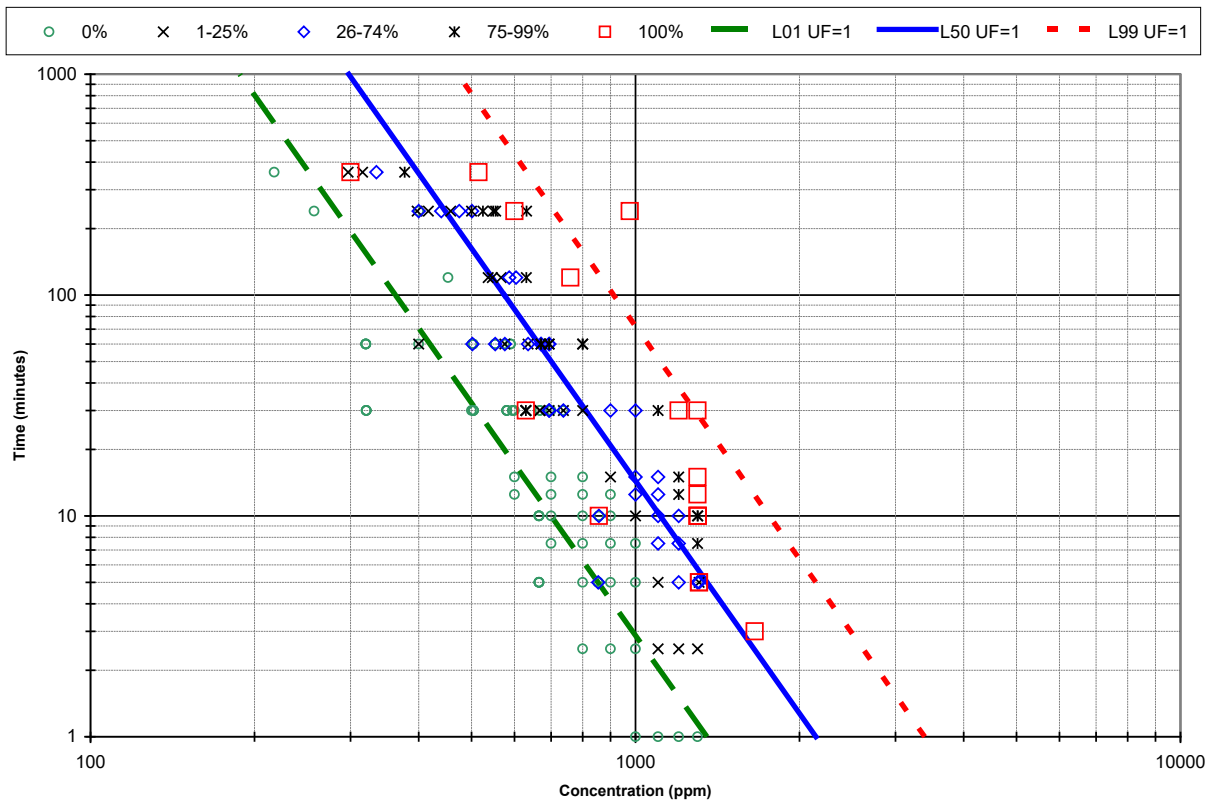
The goodness of fit indicates that 93% of the change in L50 is due to the change in the exposure time or concentration. These results verify that Haber’s rule adequately describes the load relationship between the lethal concentration and exposure time for animals exposed to  $H_2S$ .

Figure 2 is a log-log plot of the LC50 data from Table 2 with the data plotted by species. The curved lines of equal toxic load on the upper plot of Figure 1 are a straight line on a log-log plot of Figure 2. Concentration and time points below and to the left of the L50 line represent a lower load and will have a lower chance of lethality. The solid black line represents the “eye-ball” fit to the average L50 of  $4.50 \cdot 10^{11}$  minutes\*ppm<sup>7/2</sup> and has a slope of -3.5, which is an exponent  $n$  of 3.5.



**Figure 2 LC50 and Time Pairs with Moderate Grading showing  $n \approx 3.5$**

Figure 3 is a log-log plot of the concentration-time-percent response data from Table 1. The percent response data is given in ranges of 0%, 1-25%, 26-74%, 75-99% and 100%. Lines of constant toxic load or exposure with an exponent  $n$  of 3.5 are provided for comparison. L01, L50 and L99 toxic loads (or exposure) for mice and rats based on the probit analysis of all of the data are provided. Note that uncertainty factors have not been applied. The L50 and E50 line defines the LC50 and LT50 that meet the load or exposure equation. For example, moving horizontally across on the 100 minute line, 1% of the animals would die at about 375 ppm, 50% of the animals would die at about 575 ppm and 99% of the animals would die at about 900 ppm. Moving vertically up on the 1000 ppm line, 1% of the animals would die at about 3 minutes, 50% of the animals would die at 15 minutes and 99% of the animals would die at about 75 minutes.



**Figure 3** % Response, Concentration and Time Triplets with Moderate Grading showing Probit Analysis Results for L01, L50 and L99 with  $n$  of 3.5

The L50 line falls in the middle of the data, as it should. The L01 line runs through the 0% points and the L99 line borders the 100% points. When data from many sources is compared, there may be a few inconsistencies, such as L100 points to the left of the calculated L50 or L99. No data was disregarded as outliers in the data analysis. The probit method to determine the response curves and the exponent  $n$  when response-concentration-time data are considered independently will be discussed in the next section.

## 4 PROBIT ANALYSIS

The word probit is a contraction of the term ‘probability unit’. Probits are a convenient mathematical device for transforming the probability of response for a normal distribution to a linear scale. Probit equations, maximum likelihood estimation, goodness of fit and the results will be discussed. Probit statistical methods have an important role in the design of animal experiments, in the interpretation of toxic load response data and in estimating the parameters of correlation. The number of animals used in gas toxicity experiments is low and the statistical interpretation of the results is therefore crucial.

### 4.1 Probit Equations

The probit equation can be derived from exposure data that provides the concentration, time and percentage of response. Population response to toxic gas follows a lognormal distribution with concentration and time which is expressed as:

$$Y = a + b_1 \ln C + b_2 \ln t \quad (4.1)$$

where:  $Y$  is the probit, a measure related to percentage of an exposed population that suffers a given level of damage ranging from irritation to fatalities  
 $a$ ,  $b_1$ , and  $b_2$  are regression coefficients,  
 $\ln$  is the natural logarithm function (base  $e \sim 2.72$ ),  
 $C$  is the exposure concentration (ppm), and  
 $t$  is the exposure duration (minutes).

This is a linear equation with regression coefficient  $a$  being a constant,  $b_1$  is the slope giving the change in probits for each increase in  $C$  by a factor of  $e$  (base  $e \sim 2.72$ ), and  $b_2$  is the slope giving the change in probits for each increase in  $t$  by a factor of  $e$ .

In most animal exposure studies to determine LC50 for a specified time, the concentration is varied from test to test (using different animals) and the number of animals that die at the end of the specified exposure time is recorded. The fraction of animals that would die at a different time can not be determined from these studies. The time is constant so  $b_2 \ln t$  is constant and included in the constant  $a$  and the equation becomes:

$$Y = a_c + b_c \ln C \quad (4.2)$$

In a few animal exposure studies to determine LT50 for a given, the time is varied from test to test (using different animals) and the number of animals that die at the end of the specified exposure concentration is recorded. The fraction of animals that would die at a different concentration can not be determined from these studies. The concentration is constant so  $b_1 \ln C$  is constant and included in the constant  $a$  and the equation becomes:

$$Y = a_t + b_t \ln t \quad (4.3)$$

In some studies, both concentration and time are varied and the data is fitted to Equation (4.1) to determine the relationship between the LC50 and LT50. With some algebraic manipulation of (4.1), the form of the equation used in hazard analysis can be derived:

$$\begin{aligned}
 Y &= a + b_2 \ln C^n t, \text{ or} \\
 Y &= a + b_1 \ln C t^{1/n} \text{ with} \\
 n &= \frac{b_1}{b_2}
 \end{aligned}
 \tag{4.4}$$

These equations give the same probit for the same  $C$  and  $t$  pair. Note that:

$$\begin{aligned}
 L &= \int_{\text{time}} C^n dt = C^n t, \text{ or} \\
 E &= \int_{\text{time}} \frac{C}{n} t^{\left(\frac{1}{n}-1\right)} dt = C t^{1/n}
 \end{aligned}
 \tag{4.5}$$

for a constant concentration over time

so,

$$\begin{aligned}
 Y &= a + b_2 \ln L, \text{ or} \\
 Y &= a + b_1 \ln E
 \end{aligned}
 \tag{4.6}$$

Note that  $L$  (minutes·ppm<sup>n</sup>) and  $E$  (ppm·minutes<sup>1/n</sup>) have different units, and are related through:

$$\begin{aligned}
 L &= E^n, \text{ or} \\
 E &= L^{1/n}
 \end{aligned}
 \tag{4.7}$$

Uncertainty factors can be applied to  $C$  or  $t$ , as shown below:

$$Y = a_{UF} + b_1 \ln \frac{C}{UF_C} + b_2 \ln \frac{t}{UF_t}
 \tag{4.8}$$

$UF_C$  and  $UF_t$  do not have to be the same. Upon fitting the  $C$  and  $t$  data with uncertainty factors applied, the intercept  $a$  changes while the slopes  $b_1$  and  $b_2$ , and thus the exponent  $n$  are unchanged. Rearranging and introducing  $n$ :

$$\begin{aligned}
 Y &= a_{UF} + b_2 \ln \frac{C^n}{UF_C^n} \frac{t}{UF_t}, \text{ or} \\
 Y &= a_{UF} + b_1 \ln \frac{C}{UF_C} \frac{t^{1/n}}{UF_t^{1/n}}
 \end{aligned}
 \tag{4.9}$$

Composite uncertainty factors can be defined:

$$\begin{aligned}UF_L &= UF_C^n UF_t, \text{ or} \\UF_E &= UF_C UF_t^{1/n}\end{aligned}\tag{4.10}$$

*This is important as the uncertainty factors on concentration or time are often interchanged without regard or knowledge of the relationship to each other and the impact they have on the load or exposure. They are related by:*

$$\begin{aligned}UF_L &= UF_E^n, \text{ or} \\UF_E &= UF_L^{1/n}\end{aligned}\tag{4.11}$$

$UF_L$  and  $UF_E$  should have different numerical values for the same  $UF_C$  and  $UF_t$ , unless  $n$  is one. For a dose,  $n$  equals 1 and the uncertainty is usually applied to the concentration so  $UF_t$  is set to one. Regrouping (4.9):

$$\begin{aligned}Y &= a_{UF} - b_2 \ln UF_L + b_2 \ln L, \text{ or} \\Y &= a_{UF} - b_1 \ln UF_E + b_1 \ln E\end{aligned}\tag{4.12}$$

The second term in the above equations that includes  $UF_L$  and  $UF_E$  are constant and can be combined with  $a_{UF}$  and thus Equations (4.12) has the same slope  $b_2$  or  $b_1$  as Equations (4.4) but with the constant being different to account for the uncertainty factors.

A probit  $Y$  of 5 corresponds to the 50<sup>th</sup> percentile, so

$$\begin{aligned}L_{50} &= \exp\left(\frac{5 - a}{b_2}\right), \text{ or} \\E_{50} &= \exp\left(\frac{5 - a}{b_1}\right)\end{aligned}\tag{4.13}$$

From the L50 or E50 the corresponding LC50 and LT50 can be calculated. Although we may be uncertain about the concentration and time it is the load or exposure that causes the effect, as demonstrated in the previous sections. The Load  $L$  or Exposure  $E$  is the causative factor which the uncertainty factor must be applied to. Problems are avoided if a consistent approach of applying the uncertainty factor to the load is followed.

## 4.2 Maximum Likelihood Estimation

The probability that  $n, (n-1), \dots, 3, 2, 1, 0$  subjects respond when all members of a batch react independently to a stimulus is described by the binomial distribution. It can be shown that in experiments with small numbers of animals that the confidence limits for 50% mortality are wide and that those for other percentage mortalities are even wider. For 50% mortality, 2 to 8 deaths in a group of 10 is the range for 95% confidence levels. For 10% mortality, 0 to 3 deaths in a group of 10 is the range for 95% confidence levels. For 90% mortality, 7 to 10 deaths in a group of 10 is the range for 95% confidence levels. Thus for a given confidence, level it is necessary to use more animals to determine a 10<sup>th</sup> Percentile Lethal Concentration (LC10) or 90<sup>th</sup> Percentile

Lethal Concentration (LC90) than a 50<sup>th</sup> Percentile Lethal Concentration (LC50). Alternatively, for a given number of animals the confidence in the LC10 and LC90 values is less than that in the LC50. The probit method accounts for the increased confidence levels as the response approaches 50% and the limited number of animals tested.

The animal lethality response data from the exposure studies are fitted with a regression line. The maximum likelihood estimation described by Finney (1971) commonly used in probit analysis is used in this study. This approach weights the data point by the number of observations and how far the predicted response is from 50%. For example, observations with a 50% predicted response are trusted more and have a weighting coefficient that is about double that of observations at 10 or 90%. If the predicted response is 0.1 or 99.9%, the weighting coefficient is about 1/58 that of predictions at 50%. The 0 and 100% observed response data are used in the analysis and will have greater affect on the predicted regression line if they result in predicted responses in the 10 to 90% range.

*An implication of this is that there is very little confidence in using 0% response observations to determine a no observed adverse effect exposure level if it does not 'fit' the other data. The predicted no death load should be based on the probit analysis for a 1% response. Care should be used in applying no response exposure observations directly to set no observable adverse effects exposure levels.*

### **4.3 Goodness of Fit**

“The probit is no more than a convenient mathematical device for solving certain equations. Probit analysis provides the dose response curves; suggestions that the statistical analysis is completed must be avoided. The method is only appropriate for data from subjects tested once each. The complete independence of the subjects tested at different loads and of the binomial distribution associated with them, is implicit in the theory of probit analysis. To test whether the predicted line is an adequate representation of the data, a chi-squared ( $\chi^2$ ) test is used. The  $\chi^2$  test for heterogeneity of discrepancies between observed and predicted numbers is valid only when the expected numbers are not ‘small’. If the 0% and 100% observations do not match the predictions for the load, the contribution to  $\chi^2$  can be large. A value of  $\chi^2$  within the limits of random variation indicates satisfactory agreement between theory (the predicted line) and the observations. A significantly large  $\chi^2$  may arise either because individual test subjects do not react independently, or because the predicted line does not adequately describe the relation between load and probit. The former increases the dispersion of the observations about the predicted line in a random manner. A heterogeneity factor can be introduced to adjust the variances. The latter and greater fear is that the underlying mathematical model is incorrect and there is a systematic deviation.” (Finney 1971)

The data was analysed many different ways; by individual study or with studies combined, and by individual exposure time or all exposure times. In the analysis, the calculated  $\chi^2$  are compared to the 1% confidence limits for the degrees of freedom. Often the goodness of fit test fails; this was especially true for combined data sets as the 0% and 100% observations do not match the predictions for the load. However the heterogeneity factor met the  $t$  distribution, indicating that the wider range of values is within the limits of experimental error. Each table of results is discussed below.

## 4.4 Individual Study Results

Table 3 provides the results for studies where the concentration was varied for a constant exposure time. Only 16 of the 19 reported LC50 exposure times could be calculated. Three of the studies used other times in a multi-variable analysis to determine LC50 when insufficient data was available. All curve fits pass the goodness of fit test, except one that fails.

**Table 3 LC50 Probit Analysis Results for *Each* Time**

Authors	Species	Exposure Time (minutes)	$Y = a_c + b_c * \ln(C)$		Goodness of Fit X <sup>2</sup> /df/ pass or fail	LC50 (ppm)
			$a_c$	$b_c$		
Prior et al (1988)	rat	360	-60.54	11.27	3.28/4/p	335
Clanachan (1979)	mouse	5	-59.05	9.02	5.64/4/p	1213
Clanachan (1979)	mouse	2.5	-34.31	5.35	0.54/4/p	1552
Clanachan (1979)	mouse	7.5	-73.20	11.09	3.10/5/p	1155
MacEwen and Vernot (1972)	mouse	60	-14.22	2.97	5.35/2/p	647
Clanachan (1979)	mouse	10	-64.57	9.93	2.98/5/p	1103
Zwart et al (1990)	rat	60	-118.03	18.93	1.43/5/p	665
Tansy et al (1981)	rat	240	-35.25	6.59	1.94/5/p	449
Clanachan (1979)	mouse	15	-59.78	9.37	7.56/6/p	1007
Clanachan (1979)	mouse	12.5	-72.86	11.17	2.09/6/p	1067
MacEwen and Vernot (1972)	rat	60	-67.95	11.11	0.00/2/p	713
Prior et al (1988)	rat	120	-117.07	19.15	78.96/6/f	587
Prior et al (1988)	rat	240	-41.43	7.47	19.16/6/p	501
Clanachan (1979)	mouse	30	-55.92	8.87	1.48/7/p	958
Zwart et al (1990)	mouse	30	-14.71	2.85	6.87/5/p	1017
Zwart et al (1990)	mouse	60	-21.23	3.87	2.09/4/p	883

Listed in order of increasing load with  $n$  of 3.5

The parameter  $b_c$  is the spread of the data: the higher  $b_c$  is the less change in concentration is required to produce a change in the lethality response curve (steeper slope as it passes through LC50). It ranges from 2.85 to 11.17 for mice and 6.59 to 19.15 for rats. This suggests less variability between rats than there is in mice.

The exponent  $n$  can not be determined when the time is constant. The equations to convert the LC50 for an assumed exponent are:

$$\begin{aligned} &\text{for } L = t C^n \\ &Y_L = a + b_2 \ln(t C^n) \text{ with} \\ &b_2 = \frac{b_C}{n} \text{ and} \\ &a = a_C - \frac{b_C}{n} \ln(t) \end{aligned} \tag{4.14}$$

$$\begin{aligned} &\text{for } E = C t^{1/n} \\ &Y_L = a + b_1 \ln(C t^{1/n}) \text{ with} \\ &b_1 = b_C \text{ and} \\ &a = a_C - \frac{b_C}{n} \ln(t) \end{aligned} \tag{4.15}$$

Table 4 provides the results for studies where the exposure time and exposure concentration was varied, allowing a multi-variable analysis.

**Table 4 Load ( $L=t*C^n$ ) Probit Analysis Results for All Times and Concentrations with  $n$  calculated**

Authors	Species	Exposure Time (minutes)	$Y = a + b_1 \ln(C) + b_2 \ln(t)$			$n = b_1/b_2$	Goodness of Fit $X^2/df/$ pass or fail	LC50 (ppm)
			$a$	$b_1$	$b_2$			
Clanachan (1979)	mouse	1	-66.89	9.77	1.50	6.53	36.8/52/p	1570
		2.5						1365
		5						1227
		7.5						1153
		10						1104
		12.5						1067
		15						1037
		30						933
Zwart et al (1990)	mouse	5	-38.93	5.62	1.88	2.99	31.8/35/p	1448
		10						1149
		30						795
		60						631
Prior et al (1988)	rat	120	-48.96	6.16	2.99	2.06	81.8/19/f	619
		240						442
		360						363
Zwart et al (1990)	rat	5	-54.71	8.54	1.03	8.27	79.4/39/f	904
		10						831
		30						726
		60						667

There were four studies with multiple times and concentrations. Only Zwart used a multi-variable analysis with all the times to determine the LC50 and  $n$ . The others used each time independently and did not determine  $n$ . The LC50 for each time is provided. The exponent  $n$  ranged from 2.06 to 8.27. The smallest value of  $n$  was determined from the longer exposure times (120 – 360 minutes). There is a significant difference between mouse and rat data by Zwart (2.99 for mouse versus 8.27 for rat).  $b_2$  ranged from 1.03 to 2.99 for rats and did not vary much for mice.

Table 5 provides a comparison of the reported and calculated results. Generally the reported LC50 are within 1% of the calculated values, with a few exceptions. The calculated values for each time are about the same as the calculated values for all times but are expected to be different as predictions are influenced by responses at other times. The reported LC50 were used in the simplified analysis of  $n$  in Section 3

**Table 5 Comparison of Reported to Calculated LC50**

Authors	Species	Exposure Time (minutes)	LC50 (ppm)		
			<i>Reported for each time</i>	<i>Calculated for each time</i>	<i>Calculated from all times</i>
Clanachan (1979)	mice	1	na	na	1570
Clanachan (1979)	mice	2.5	1734	1552	1365
Clanachan (1979)	mice	5	1207	1213	1227
Clanachan (1979)	mice	7.5	1132	1155	1153
Clanachan (1979)	mice	10	1097	1103	1104
Clanachan (1979)	mice	12.5	1059	1067	1067
Clanachan (1979)	mice	15	1003	1007	1037
Clanachan (1979)	mice	30	961	958	933
MacEwen and Vernot (1972)	mice	60	634	647	na
MacEwen and Vernot (1972)	rats	60	712	713	na
Prior et al (1988)	rats	120	587	587	619
Prior et al (1988)	rats	240	501	501	442
Prior et al (1988)	rats	360	335	335	363
Tansy et al (1981)	mice	240	444	449	na
Zwart et al (1990)	mice	5	na	na	1448
Zwart et al (1990)	mice	10	1150	na	1149
Zwart et al (1990)	mice	30	793	1017	795
Zwart et al (1990)	mice	50	671	na	671
Zwart et al (1990)	mice	60	na	883	631
Zwart et al (1990)	rats	5	na	na	897
Zwart et al (1990)	rats	10	829	na	825
Zwart et al (1990)	rats	30	721	na	722
Zwart et al (1990)	rats	50	679	na	679
Zwart et al (1990)	rats	60	na	665	664

## 4.5 Combined Study Results

Table 6 provides the results for combined data sets from the moderately rated studies, allowing a multi-variable analysis. Four combinations were analyzed, mouse only, rat only, combined mouse and rat and weighted mouse and rat.

**Table 6 Load ( $L=t*C^n$ ) Probit Analysis Results for All Data with various  $n$**

Data	Species	Exposure Time (minutes)	$Y = a + b_1*ln(C) + b_2*ln(t)$			$n = b_1/b_2$	Goodness of Fit X <sup>2</sup> /df/ pass or fail
			$a$	$b_1$	$b_2$		
1. $n$ calculated using maximum likelihood estimation method							
Mouse (97 tests)	mouse	1-60	-41.94	6.20	1.54	4.02	1980/94/f
Rat (78 tests)	rat	3-360	-25.26	4.01	1.05	3.80	345/75/f
Combined (175 tests)	both	1-360	-33.89	5.08	1.43	3.55	807/172/f
Human Weighted (175 tests)	mouse=0.5 rat=0.25	1-360	-25.79	4.09	1.37	2.99	1397/172/f
2. $n$ selected to minimize difference between predictions and observations							
Data	Species	Exposure Time (minutes)	Load $Y = a + b_2*ln(t*C^n)$			$n$	Goodness of Fit X <sup>2</sup> /df/ pass or fail
			$a$	$b_1$	$b_2$		
Mouse (97 tests)	mouse	1-60	-36.30	na	1.79	2.96	285/95/f
Rat (78 tests)	rat	3-360	-25.23	na	1.08	3.71	344/76/f
Combined (175 tests)	both	1-360	-30.04	na	1.44	3.11	734/173/f
Human Weighted (175 tests)	mouse=0.5 rat=0.25	1-360	-24.90	na	1.15	3.51	1137/173/f
3. $n$ set to 3.5							
Data	Species	Exposure Time (minutes)	Load $Y = a + b_2*ln(t*C^n)$			$n$	Goodness of Fit X <sup>2</sup> /df/ pass or fail
			$a$	$b_1$	$b_2$		
Mouse (97 tests)	mouse	1-60	-40.70	na	1.70	3.50	1910/95/f
Rat (78 tests)	rat	3-360	-24.85	na	1.11	3.50	348/76/f
Combined (175 tests)	both	1-360	-33.74	na	1.44	3.50	789/173/f
Human Weighted (175 tests)	mouse=0.5 rat=0.25	1-360	-24.92	na	1.15	3.50	1137/173/f

In Table 6 data was analyzed three ways.

1. Calculate  $n$  using the maximum likelihood estimation to determine  $b_1$  and  $b_2$ . This is the normal approach to use. The exponent ranges from 2.99 to 4.02. A value of 3.55 is obtained when all of the data is considered which is very close to the simplified analysis value of 3.49.
2. Select  $n$  to minimize the difference between predicted and observed values. In this case the maximum likelihood estimation method is repeated with different values of  $n$  to find the minimum value of the chi-squared. The biggest reduction was in the mice data set with  $n$  decreasing from 4.02 to 2.96 while  $\chi^2$  decreased from 1980 to 285.  $n$  ranged from 2.96 to 3.71 and decreased in all of the data sets except for the weighted case.
3. Set  $n$  to 3.5, the recommended value.

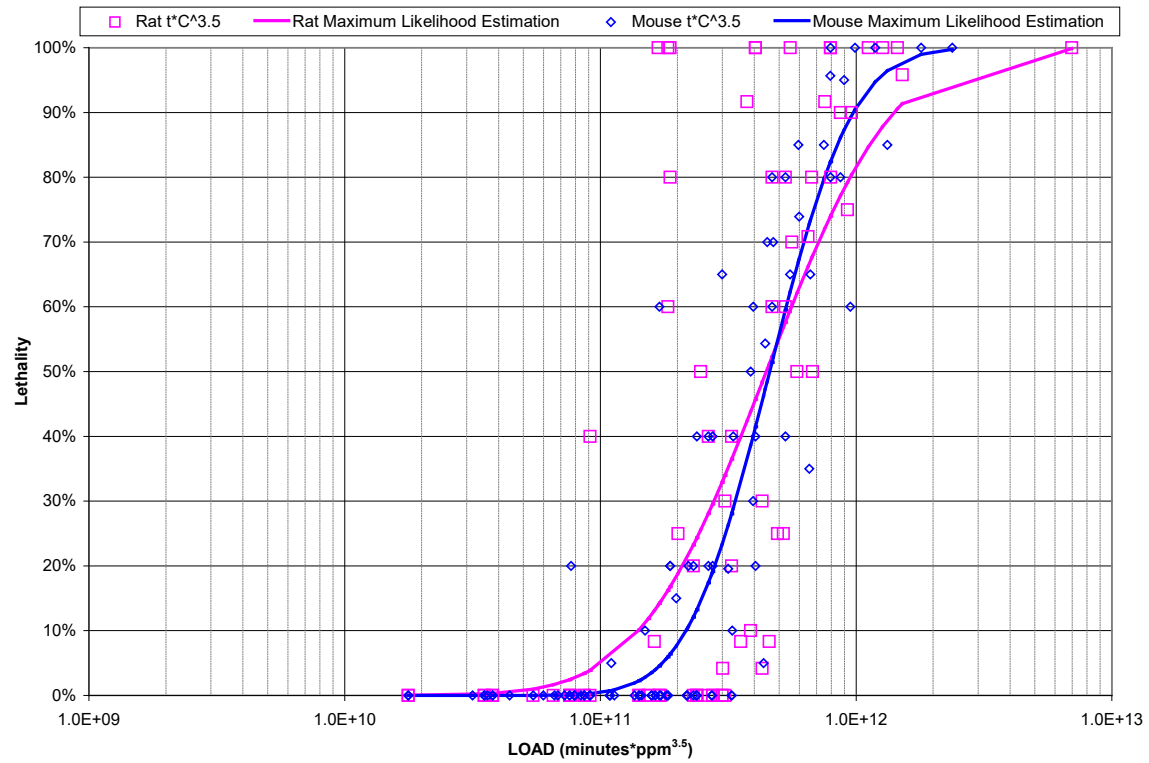
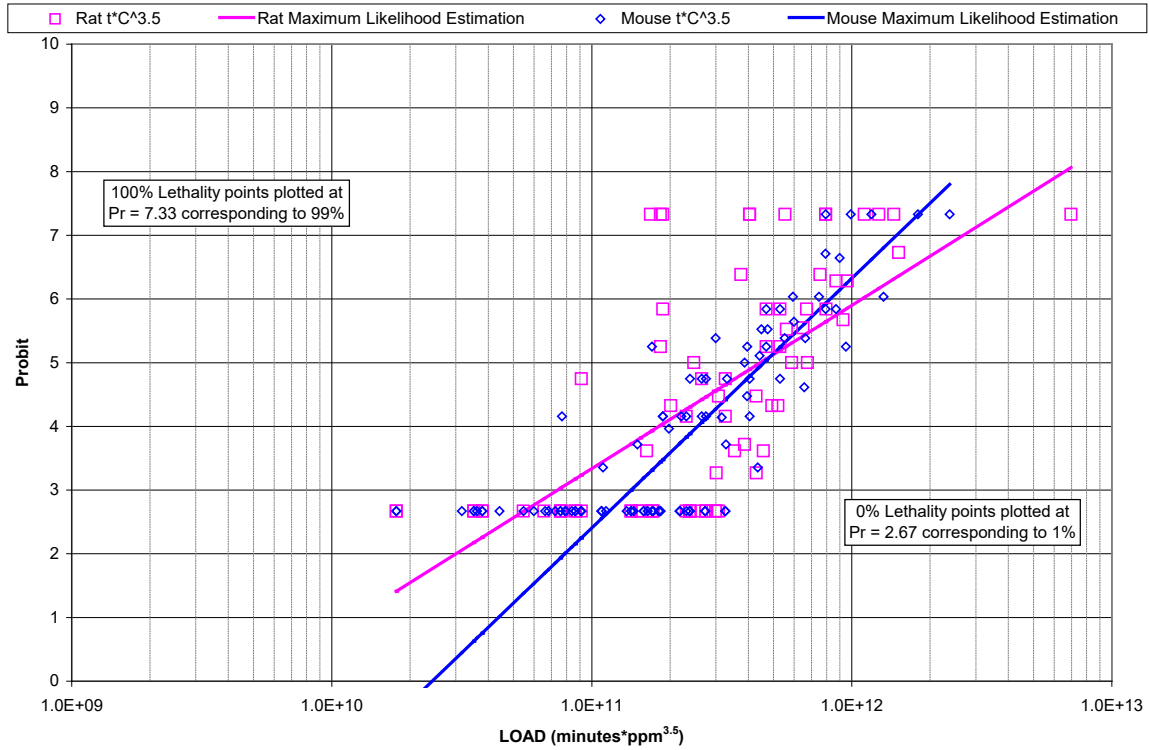
Notice that in all cases the goodness of fit test failed on  $\chi^2$ . However it passed on the homogeneity test.  $b_2$  ranges from 1.05 to 1.79 and is not sensitive to  $n$ .

The upper plot of Figure 4 to Figure 6 is the load versus the probit. An exponent of 3.5 is used to calculate the load and the probit parameters are provided in Table 6. The lower plot is the percent response. The maximum likelihood estimation calculations are done from this plot, with the resulting curve shown. The 0 and 100% response data points are plotted at a probit corresponding to 1 and 99%, respectively. However, the response corresponding to the load is used in the calculation. The lower plot is the same data and curves but with the load versus percent response. The L50 is the load for 50% lethality and is where the line crosses the 50% response which is when the probit is 5.

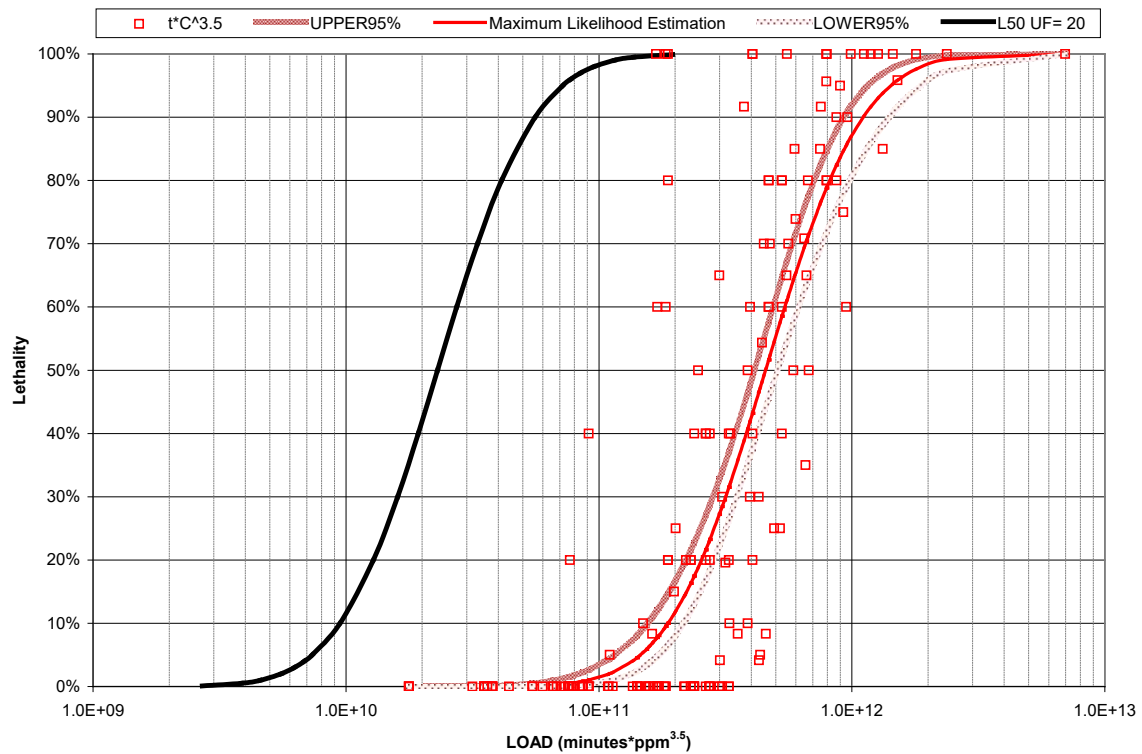
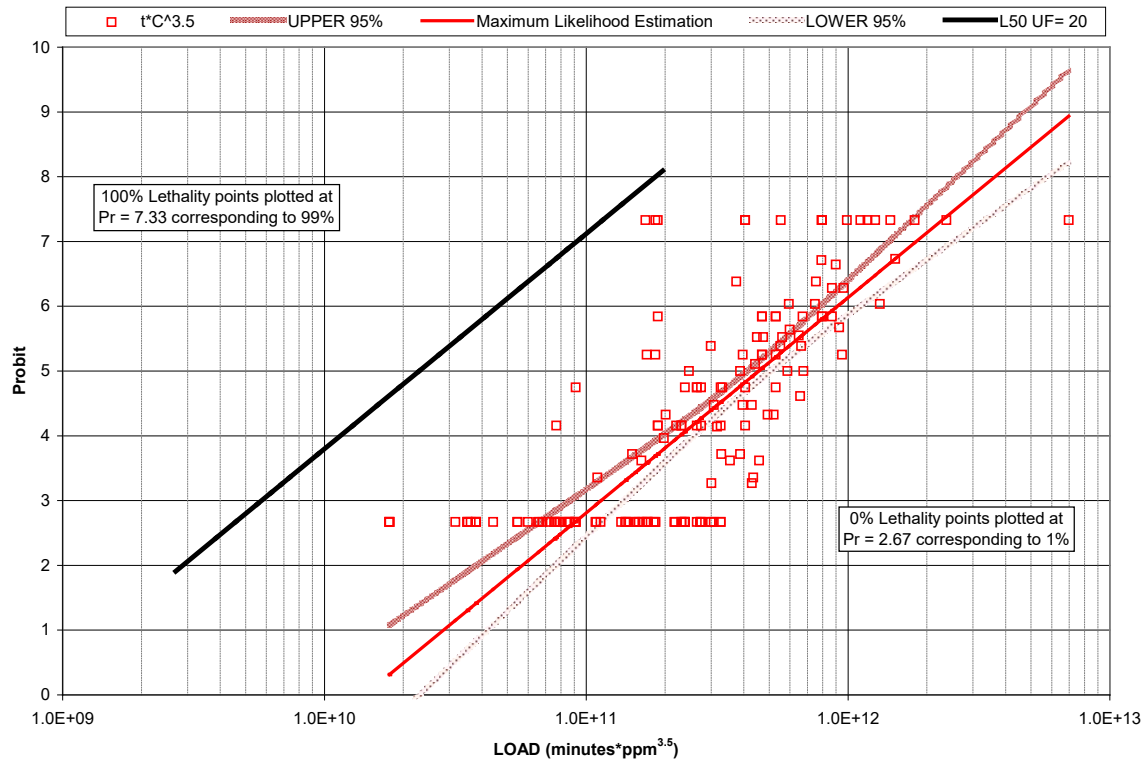
Figure 4 provides separate mice and rat data from all of the studies. The L50 for mice  $4.591 \cdot 10^{11}$  minutes  $\text{ppm}^{3.5}$  is slightly larger than for rats  $4.454 \cdot 10^{11}$  minutes  $\text{ppm}^{3.5}$ . The response curve for mice is steeper than for rats ( $b_2$  of 1.70 for mice vs. 1.11 for rats) suggesting less variability in the mice population

Figure 5 provides combined mice and rat data from all of the studies. The species are treated as one. The L50 is  $4.557 \cdot 10^{11}$  minutes  $\text{ppm}^{3.5}$  and the  $b_2$  is 1.44, in between the values when analyzed separately. This compares well to the simplified analysis average L50 of  $4.50 \cdot 10^{11}$  minutes  $\text{ppm}^{3.5}$ . The 95 % confidence interval is also shown. The L50 corresponds to a response of  $50 \pm 6\%$ . Also shown for comparison is the response curve if an uncertainty factor of 20 is applied to the load. The combined results were used in later analysis.

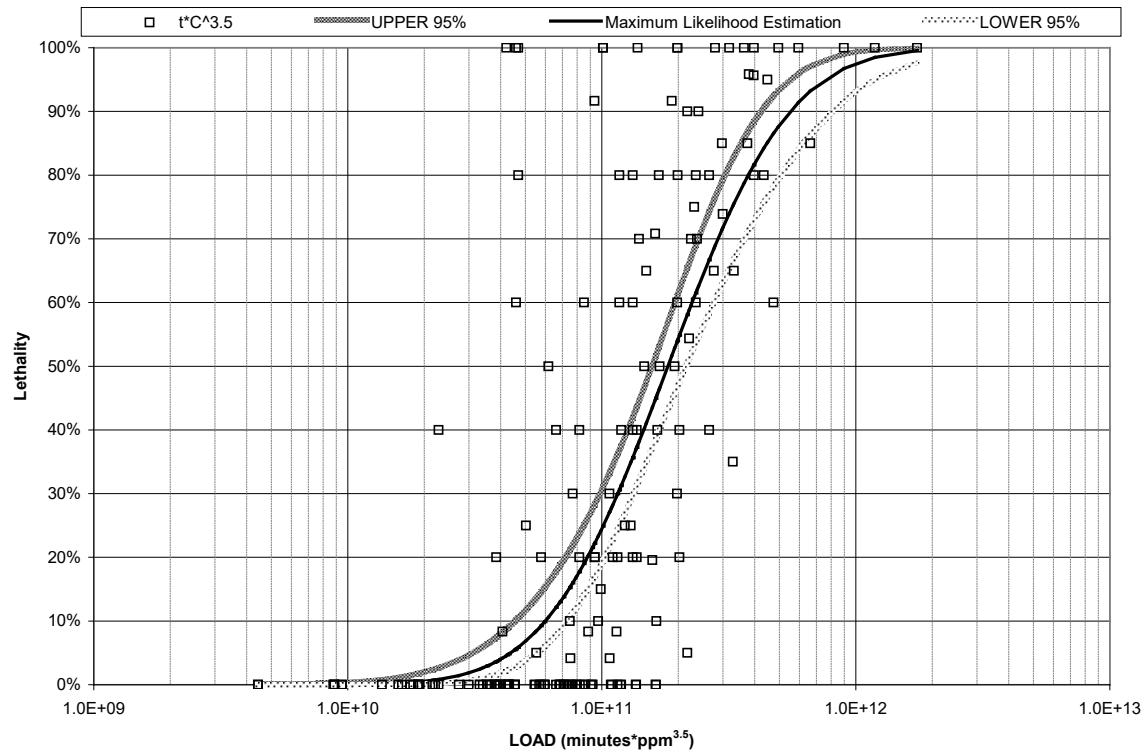
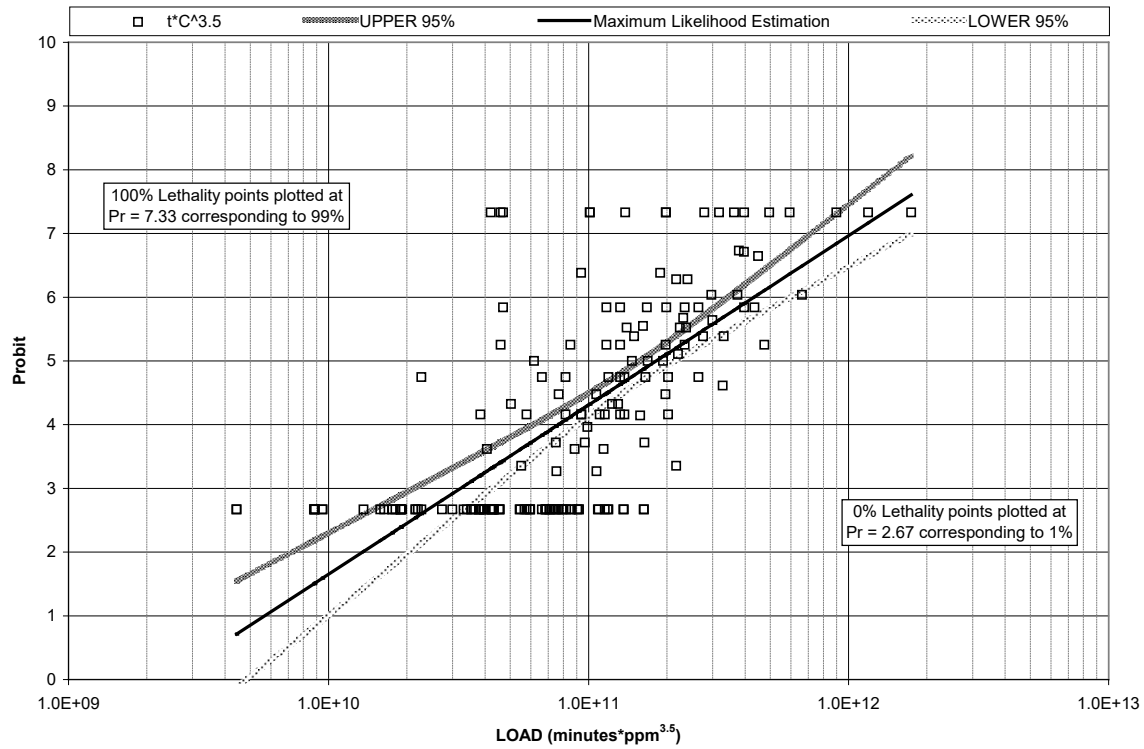
Figure 6 provides the weighted mice and rat data from all of the studies. The load for mice is multiplied by a weighting of 0.25 and for rats a weighting of 0.5 is used, as per the Dutch TNO to bring it to a load to humans. The L50 is  $1.820 \cdot 10^{11}$  minutes  $\text{ppm}^{3.5}$  and the  $b_2$  is 1.15. The 95 % confidence interval is also shown. The weighted results were not used in later analysis.



**Figure 4** Separate Mice and Rats Probit Analysis for Load with  $n$  of 3.5



**Figure 5 Combined Mice and Rats Probit Analysis for Load with  $n$  of 3.5**



**Figure 6**    **Weighted Mice and Rats Probit Analysis for Human Load with  $n$  of 3.5**

## 5 ANIMAL NO DEATH DATA

As shown in Table 1, Figure 4, Figure 5 and Figure 6 there are several exposures where no lethality was observed. The load for no observed adverse effects level LNOAEL can be defined from these animal exposures. As discussed previously, care should be used in applying no response exposure observations directly to set no observable adverse effects exposure levels.

For example, referring to Table 1, in the Clanachan study, a group of 20 mice were exposed to 1000 ppm H<sub>2</sub>S for 1 minute and none died. The concentration was increased to 1100 ppm H<sub>2</sub>S and another, different group were exposed for 1 minute and none died. This was repeated at 1200 and then 1300 ppm for 1 minute and none died. An exposure was not done at 1400 ppm so the LNOAEL for this 1 minute exposure is cautiously set at 1300 ppm, the maximum concentration for no observed deaths. This exposure data can not be used to determine an LC50 for 1 minute as no animals died. However, this data can be used with the other exposure time data to determine the sensitivity of response to time and concentration (the exponent *n*).

Clanachan then increased the exposure time to 2.5 minutes and reduced the H<sub>2</sub>S exposure concentrations to 800 ppm and none died. New groups of 20 mice were tested at 900 and 1000 ppm and none died. At 1100 ppm, 1 of the 20 mice died (5%); at 1200 ppm, 2 of the 20 mice died (10%); and at 1300 ppm, 3 of the 20 mice died (15%). This exposure data can be used to determine an LC50 for 2.5 minute as there are at least two data points that are not 0% or 100% response.

The maximum concentration for no observed deaths from each study can be used as an indicator of no lethality. The minimum, median and maximum of the maximum concentration for no observed adverse effects level LNOAEL are  $5.54 \cdot 10^{10}$ ,  $2.01 \cdot 10^{11}$  and  $3.26 \cdot 10^{11}$ , minutes\*ppm<sup>3.5</sup> respectively for the 22 studies. The L50 is  $4.56 \cdot 10^{11}$  minutes\*ppm<sup>3.5</sup> for a median L50/LNOAEL ratio of **2.27**.

Referring to Figure 5, one notes that the curves approach 0% lethality but do not cross it. The probit analysis does not readily define 0% lethality as mathematically it approaches a load of negative infinity (likewise the load for 100% lethality approaches positive infinity). 0% lethality can be defined as 1% (1/100 chance of lethality) with a probit of 2.67. Based on the probit equation for the combined data of Figure 5 the  $L1=9.07 \cdot 10^{10}$  minutes\*ppm<sup>3.5</sup> (this is where best fit line crosses Pr=2.67) for a L50/L1 ratio of **5.02** based on the probit analysis and should be used as it matches the data.

## 6 ANIMAL UNCONSCIOUSNESS DATA

Clanachan also tested mice for the loss of the righting reflex. The righting reflex is equivalent to unconsciousness and the load will be designated as RR. Table 7 provides the probit analysis results. Note that the calculated exponents  $n$  are greater than the value of 3.5 used for the entire data set. The Clanachan study was for mice exposed for 1 to 30 minutes whereas the entire data set is for rats and mice exposed for 1 to 360 minutes.

**Table 7 Unconsciousness Probit Analysis Results**

Authors	Species	Endpoint	$Y = a + b1*\ln(C) + b2*\ln(t)$ $Y = a + b2*\ln(t*C^n)$			$n$ $=b1/b2$	Goodness of Fit $X^2/df$ / pass or fail	L50 or RR50
			$a$	$b1$	$b2$			
Clanachan (1979)	mice	lethality	-44.853	na	1.855	3.5	163/53/f	L50 minutes*ppm <sup>3.5</sup> 4.662 10 <sup>11</sup>
Clanachan (1979)	mice	lethality	-66.894	9.769	1.496	6.53	37/52/p	na
Clanachan (1979)	mice	righting reflex	-32.331	na	1.440	3.5	238/55/f	RR50 minutes*ppm <sup>3.5</sup> 1.820 10 <sup>11</sup>
Clanachan (1979)	mice	righting reflex	-47.198	7.259	1.281	5.67	86/54/f	na

The Clanachan load for lethality in 50% of the mice population is 4.63 10<sup>11</sup> minutes\*ppm<sup>3.5</sup>. This compares well to the L50 for all of the data of 4.56 10<sup>11</sup> minutes\*ppm<sup>3.5</sup>. The 50<sup>th</sup> percentile righting reflex load RR50 is 1.82 10<sup>11</sup> minutes\*ppm<sup>3.5</sup>. The L50/RR50 ratio of **2.56** is less than the L50/L1 ratio of 5.01. In other words, when 50% of the population is unconscious, about 5% of the population may be dead (see Figure 7).

The median of the maximum concentration for no observed adverse effects level RRNOAEL was 7.02 10<sup>10</sup> for the 8 exposure times. The median RR50/RRNOAEL ratio is **2.59**. Based on the probit equation for the unconsciousness data of Figure 7, RR1 is 3.61 10<sup>10</sup> (this is where best fit line crosses Pr=2.67) for a RR50/RR1 ratio of **5.04** based on the probit analysis and should be used as it matches the data. As a check the L1/RR1 ratio is **3.68**.

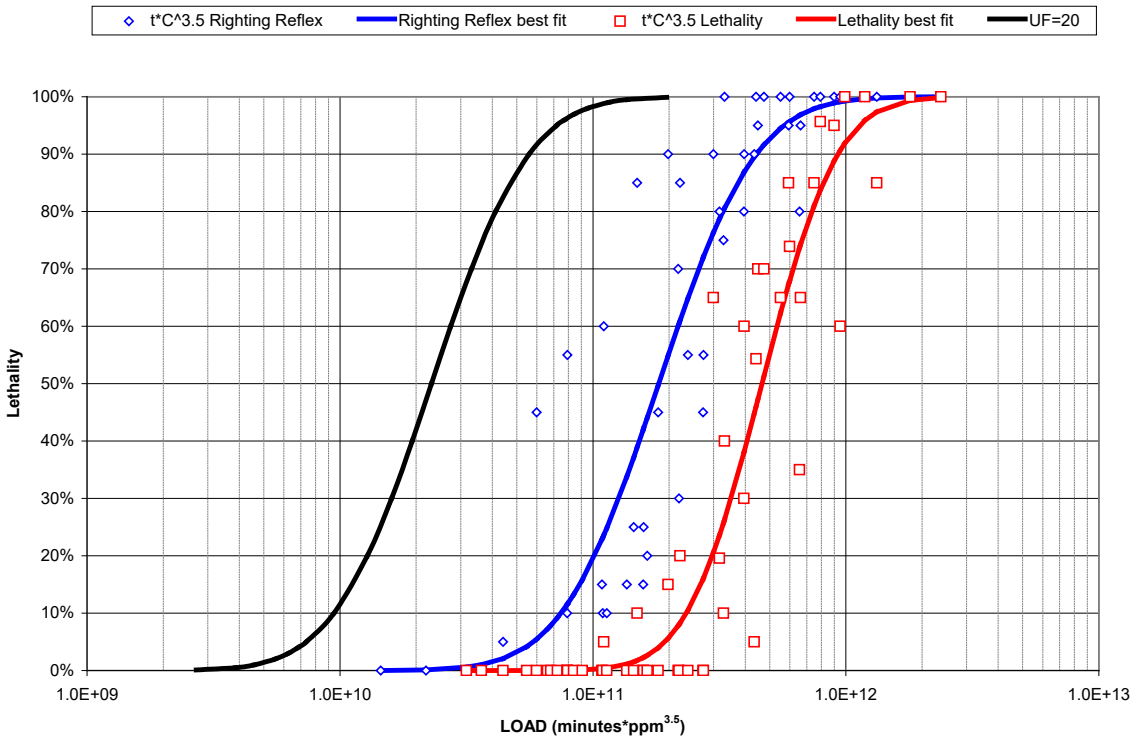
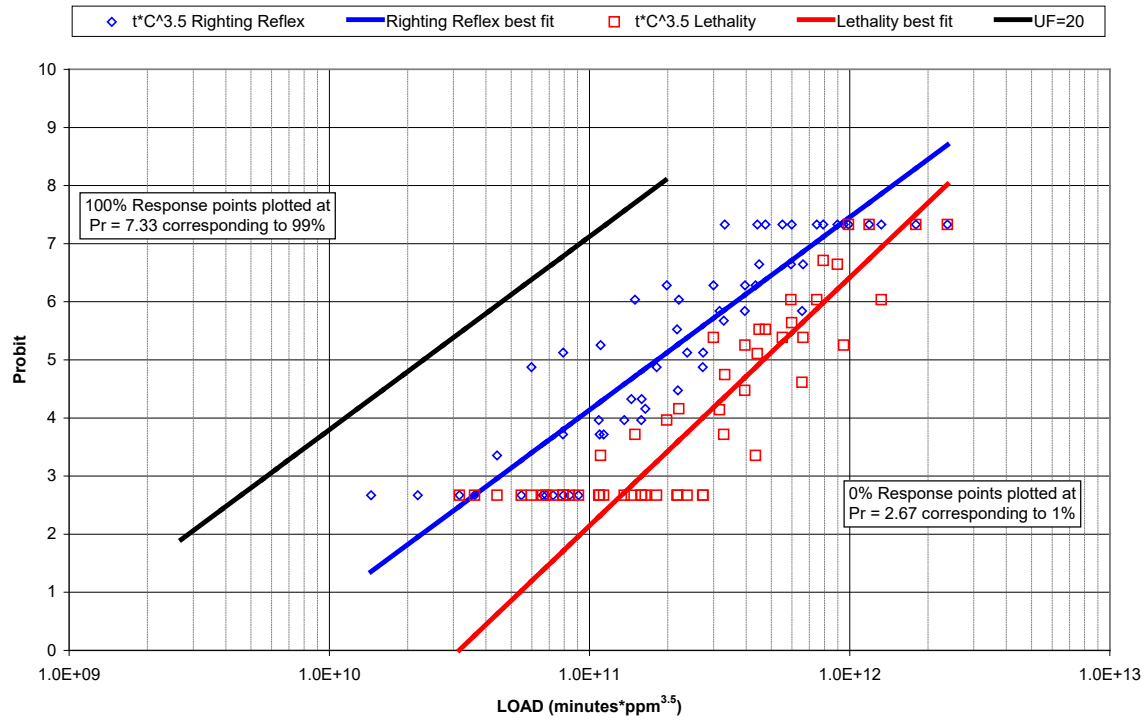
**Table 8** provides the concentration-time-response data for unconsciousness in order of increasing load. A few entries may appear to be in error since for an exposure time the % response generally increases with the concentration. But there are a few exceptions, for example: at 7.5 minutes from 1100 to 1300 ppm, at 15 minutes from 1000 to 1200 ppm and at 30 minutes from 700 to 900 ppm. These are not errors but rather examples of the variability of the mouse population response that the probit analysis accounts for. Figure 7 presents the unconsciousness data analysis and compares it to the Clanachan lethality data for an exponent  $n$  of 3.5.

**Table 8 Mouse Unconsciousness Exposure Data with Moderate Grading**

Entry	Authors	Study Code	Species (male, female)	Exposure Time (t, minutes)	H2S Concentration (C, ppm)	Number Tested (n)	Number RR Observed (r)	% RR <sup>1</sup> (p)
1	Clanachan (1979)	NC002	mouse m,f	1	800	20	0	0%
2	Clanachan (1979)	NC002	mouse m,f	1	900	20	0	0%
3	Clanachan (1979)	NC002	mouse m,f	1	1000	20	0	0%
4	Clanachan (1979)	NC002	mouse m,f	1	1100	20	1	5%
5	Clanachan (1979)	NC002	mouse m,f	1	1200	20	9	45%
6	Clanachan (1979)	NC002	mouse m,f	1	1300	20	11	55%
7	Clanachan (1979)	NC002	mouse m,f	2.5	800	20	0	0%
8	Clanachan (1979)	NC002	mouse m,f	2.5	900	20	0	0%
9	Clanachan (1979)	NC002	mouse m,f	2.5	1000	20	2	10%
10	Clanachan (1979)	NC002	mouse m,f	2.5	1100	20	12	60%
11	Clanachan (1979)	NC002	mouse m,f	2.5	1200	20	17	85%
12	Clanachan (1979)	NC002	mouse m,f	2.5	1300	20	18	90%
13	Clanachan (1979)	NC002	mouse m,f	5	800	20	0	0%
14	Clanachan (1979)	NC002	mouse m,f	5	900	20	2	10%
15	Clanachan (1979)	NC002	mouse m,f	5	1000	20	3	15%
16	Clanachan (1979)	NC002	mouse m,f	5	1100	20	17	85%
17	Clanachan (1979)	NC002	mouse m,f	5	1200	20	18	90%
18	Clanachan (1979)	NC002	mouse m,f	5	1300	20	18	90%
19	Clanachan (1979)	NC002	mouse m,f	7.5	700	20	0	0%
20	Clanachan (1979)	NC002	mouse m,f	7.5	800	20	3	15%
21	Clanachan (1979)	NC002	mouse m,f	7.5	900	20	4	20%
22	Clanachan (1979)	NC002	mouse m,f	7.5	1000	20	11	55%
23	Clanachan (1979)	NC002	mouse m,f	7.5	1100	20	20	100%
24	Clanachan (1979)	NC002	mouse m,f	7.5	1200	20	19	95%
25	Clanachan (1979)	NC002	mouse m,f	7.5	1300	20	19	95%
26	Clanachan (1979)	NC002	mouse m,f	10	700	20	0	0%
27	Clanachan (1979)	NC002	mouse m,f	10	800	20	5	25%
28	Clanachan (1979)	NC002	mouse m,f	10	900	20	6	30%
29	Clanachan (1979)	NC002	mouse m,f	10	1000	20	16	80%
30	Clanachan (1979)	NC002	mouse m,f	10	1100	20	20	100%
31	Clanachan (1979)	NC002	mouse m,f	10	1200	20	20	100%
32	Clanachan (1979)	NC002	mouse m,f	10	1300	20	20	100%
33	Clanachan (1979)	NC002	mouse m,f	12.5	600	20	0	0%
34	Clanachan (1979)	NC002	mouse m,f	12.5	700	20	2	10%
35	Clanachan (1979)	NC002	mouse m,f	12.5	800	20	9	45%
36	Clanachan (1979)	NC002	mouse m,f	12.5	900	20	11	55%
37	Clanachan (1979)	NC002	mouse m,f	12.5	1000	20	16	80%
38	Clanachan (1979)	NC002	mouse m,f	12.5	1100	20	20	100%
39	Clanachan (1979)	NC002	mouse m,f	12.5	1200	20	20	100%
40	Clanachan (1979)	NC002	mouse m,f	12.5	1300	20	20	100%
41	Clanachan (1979)	NC002	mouse m,f	15	600	20	0	0%
42	Clanachan (1979)	NC002	mouse m,f	15	700	20	3	15%
43	Clanachan (1979)	NC002	mouse m,f	15	800	20	14	70%
44	Clanachan (1979)	NC002	mouse m,f	15	900	20	15	75%
45	Clanachan (1979)	NC002	mouse m,f	15	1000	20	20	100%
46	Clanachan (1979)	NC002	mouse m,f	15	1100	20	19	95%
47	Clanachan (1979)	NC002	mouse m,f	15	1200	20	20	100%
48	Clanachan (1979)	NC002	mouse m,f	15	1300	20	20	100%

Entry	Authors	Study Code	Species (male, female)	Exposure Time (t, minutes)	H2S Concentration (C, ppm)	Number Tested (n)	Number RR Observed (r)	% RR <sup>1</sup> (p)
49	Clanachan (1979)	NC002	mouse m,f	30	500	20	0	0%
50	Clanachan (1979)	NC002	mouse m,f	30	600	20	5	25%
51	Clanachan (1979)	NC002	mouse m,f	30	700	20	9	45%
52	Clanachan (1979)	NC002	mouse m,f	30	800	20	18	90%
53	Clanachan (1979)	NC002	mouse m,f	30	900	20	16	80%
54	Clanachan (1979)	NC002	mouse m,f	30	1000	20	20	100%
55	Clanachan (1979)	NC002	mouse m,f	30	1100	20	20	100%
56	Clanachan (1979)	NC002	mouse m,f	30	1200	20	20	100%
57	Clanachan (1979)	NC002	mouse m,f	30	1300	20	20	100%

Note: Unconsciousness is based on observed Righting Reflex (RR )



**Figure 7 Unconsciousness and Lethality Data Probit Analysis for Load with n of 3.5**

## 7 UNCERTAINTY FACTORS

Uncertainty factors are often applied by regulators when setting exposure guidelines to account for uncertainties such as extrapolating from animals to humans and individual susceptibility to a toxic substance within a population. In the past the use of default uncertainty factors was common but more recently regulators have begun using data-derived uncertainty factors to avoid being overly cautious. Conversely where the effects from a particular substance are not known, sometimes greater uncertainty than the defaults are applied. Choosing an appropriate uncertainty factors is very important especially when the endpoint is to be applied in a complex computer model where an unrepresentative EPZ (large or small) can be counter to good emergency response planning. The uncertainty factor is a mix of science and policy. There is no uniquely 'right' answer when setting emergency planning requirements but there should be a reasonable margin of safety to the EPZ endpoint criterion.

### 7.1 Inhalation and Uptake of Toxic Gases

At low concentrations, H<sub>2</sub>S is a locally acting substance, exerting its effect on the organ in which it penetrates, for example the eyes, nose, throat, and lungs. At higher concentrations, H<sub>2</sub>S is a systemically acting substance that is absorbed by the lungs and transported by the blood. The breathed-in dose is:

$$D = \frac{V \cdot C \cdot t}{k_1}, \text{ with}$$

$D$  = breathed-in dose (mg)  
 $V$  = breathing minute volume (Litres/min) (7.1)  
 $C$  = concentration (ppm)  
 $t$  = exposure duration (minutes)  
 $k_1$  = unit constant

If  $V$ ,  $C$  or  $t$  is doubled the breathed-in dose is doubled. The toxic affect is defined by the load or exposure:

$$L = t \cdot C^n, \text{ or}$$
$$E = C \cdot t^{(1/n)}, \text{ with}$$

$L = E^n$  (7.2)  
 $L$  = load (minutes · ppm <sup>$n$</sup> )  
 $E$  = exposure (ppm · minutes <sup>$1/n$</sup> )  
 $n$  = exponent

The exposure  $E$  has been defined to avoid confusion with the load  $L$ . The toxic affect can be expressed either way as long as it is consistently used. The exponent  $n$  is defined by statistical analysis of exposure data where both  $C$  and  $t$  are varied and the response is observed. The load  $L$

is used in hazard analysis as it lends itself to easier integrations in time. The exposure  $E$  is used by toxicologists as it provides the correct uptake with time.

Figure 8 illustrates how the load  $L$  and exposure  $E$  change with time during an exposure to  $C_{endpoint}$  for  $t_{endpoint}$ . The numerical endpoint values are different,  $L_{endpoint} = t_{endpoint} C^n$  versus  $E_{endpoint} = C_{endpoint} t^{1/n}$  but the effect is the same (lethality). Three exponents are compared,  $n$  of 1 for a dose and  $n$  of 2 and 4 for loads/exposures.

The body absorbs the toxic gas according to the exposure equation given in the upper plot. At  $C_{endpoint}$  for  $0.5 * t_{endpoint}$  the exposure is 0.5, 0.71 and 0.84 of  $E_{endpoint}$ , respectively for  $n$  of 1, 2 and 4. The time to achieve  $0.5 * E_{endpoint}$  is 1/2, 1/4 and 1/16 of  $t_{endpoint}$ , respectively for  $n$  of 1, 2 and 4. If the concentration is doubled the exposure doubles and  $E_{endpoint}$  is achieved sooner in time.

The lower plot for load shows that at  $C_{endpoint}$  for  $0.5 * t_{endpoint}$  the load is 0.5 of  $L_{endpoint}$ , for  $n$  of 1, 2 and 4. The time to achieve  $0.5 * L_{endpoint}$  is 1/2 of  $t_{endpoint}$  for  $n$  of 1, 2 and 4. If the concentration is doubled the load increases by a factor of 2, 4 and 16, respectively for  $n$  of 1, 2 and 4 and  $L_{endpoint}$  is achieved sooner in time.

If the concentration is doubled the time to achieve  $E_{endpoint}$  or  $L_{endpoint}$  is the same at 1/2, 1/4 and 1/16 of  $t_{endpoint}$ , respectively for  $n$  of 1, 2 and 4.

The expressions for  $L$  and  $E$  can be combined with the breathed-in dose to define a breathed-in load or exposure:

$$D_L = \frac{V \cdot C^n \cdot t}{k_L} = \frac{V \cdot L}{k_L} = \frac{D \cdot C^{(n-1)}}{k_L}, \text{ or}$$

$$D_E = \frac{V \cdot C \cdot t^{1/n}}{k_E} = \frac{V \cdot E}{k_E} = \frac{D \cdot t^{(1/n-1)}}{k_E}, \text{ with}$$

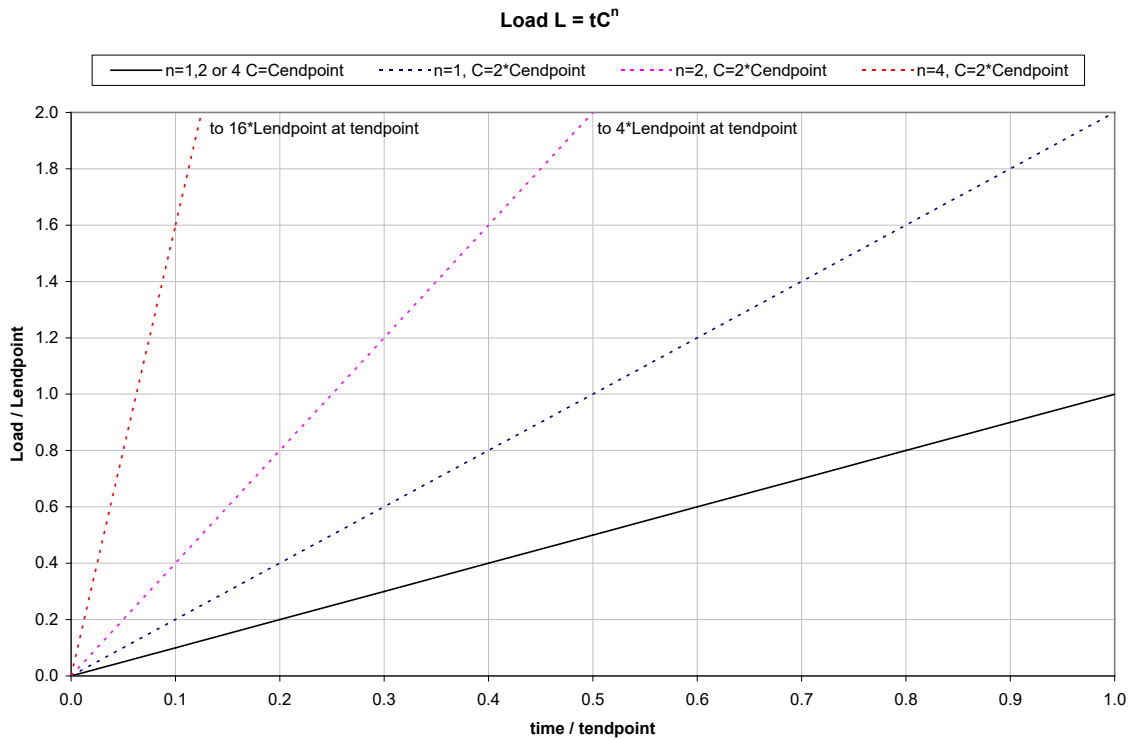
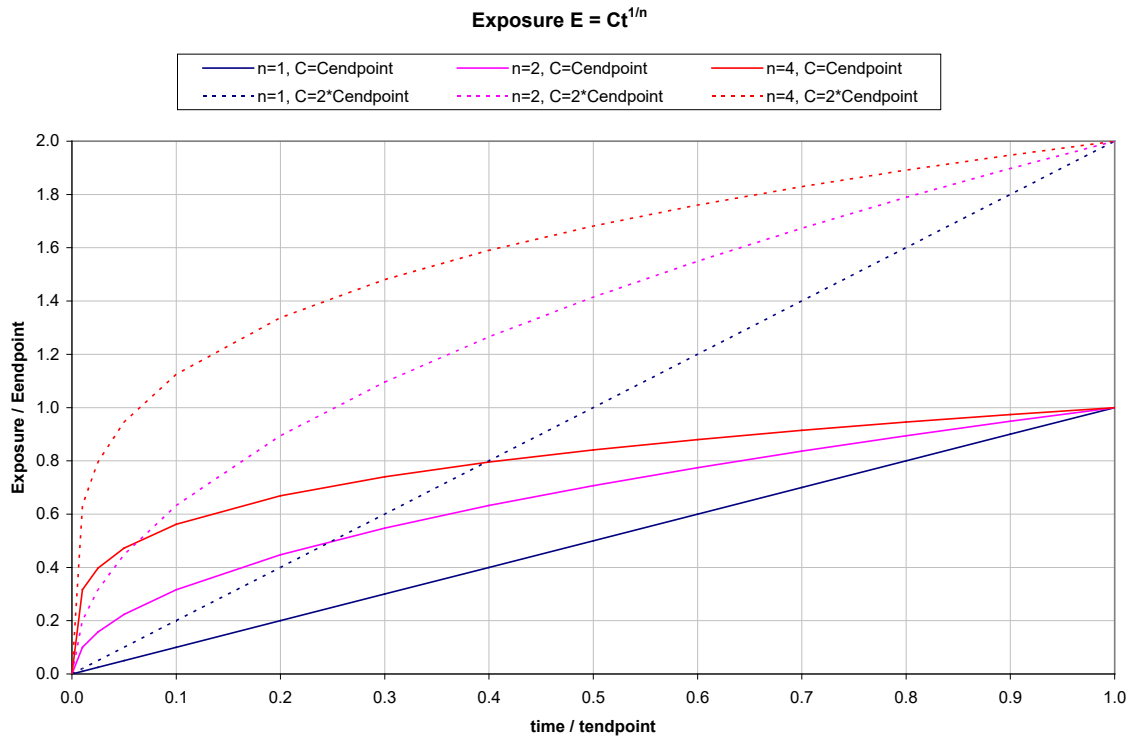
$$D_L = \text{breathed-in load (mg)} \tag{7.3}$$

$$D_E = \text{breathed-in exposure (mg)}$$

$$k_L \text{ and } k_E = \text{unit constants dependent on } k_1 \text{ and } n$$

The above terms have been defined to aid in the interpretation of the exposure data and were not referenced from toxicology textbooks. For example, for an  $n$  of 4: if  $V$  or  $t$  is doubled the breathed-in load is doubled but if  $C$  is doubled the breathed-in load increases by a factor of  $2^4 = 16$ . If  $V$  or  $C$  is doubled the breathed-in exposure is doubled but if  $t$  is doubled the breathed-in exposure increases by a factor of  $2^{(1/4)} = 1.19$ .

The exposure equation represents the uptake of the  $H_2S$ ; the fraction of the final endpoint is greater for the exposure than for the load at the same time. This is important if intermediate times are being considered, for example how much time it takes to absorb half of the endpoint. However, the final endpoint described by  $C_{endpoint}$  at  $t_{endpoint}$  is the same so the load can be used.



**Figure 8 Exposure and Load Endpoint variation with Time**

## 7.2 Extrapolation of Exposure Data from Animal to Human

When  $C$  and  $t$  exposure data is analysed for a species the  $V$  and  $k$ 's are constant and often ignored. For irritants such as  $H_2S$  at low concentrations, a measure of absorbed dose is the breathed-in load or exposure per unit surface area of the lung ( $m^2$ ). For systemically acting substances such as  $H_2S$  at lethal concentrations, a measure of absorbed dose is the breathed-in load or exposure per unit body mass (kg). The variable  $X$  with appropriate units ( $m^2$  or kg in above examples) will be used to define the appropriate pathway parameter.

$$D'_L = \frac{D_L}{X}, \text{ or } D'_E = \frac{D_E}{X}, \text{ with} \quad (7.4)$$

$$D'_L = \text{adsorbed breathed-in load per unit } X \text{ (mg/unit } X)$$

$$D'_E = \text{adsorbed breathed-in exposure per unit } X \text{ (mg/unit } X)$$

$$X = \text{parameter defining pathway with appropriate units}$$

From Equation (7.3) the breathing minute volume  $V$  and from Equation (7.4) the mass  $W$  can be used to extrapolate exposure data from animals to humans based on the same absorbed breathed-in load or exposure per unit mass. *Care must be taken when different species are compared on plots of  $C$  and  $t$  as the absorbed breathed-in load or exposure is not the same as  $V$  and  $W$  are different for each species.*

The Dosimetric Adjustment Factor  $DAF$  is introduced to adjust the absorbed breathed-in load or exposure per unit mass or area from one species to another.

$$\frac{D'_{L1}}{D'_{L2}} = DAF \frac{C_1^{n_1} \cdot t_1}{C_2^{n_2} \cdot t_2}, \text{ or} \quad (7.5)$$

$$\frac{D'_{E1}}{D'_{E2}} = DAF \frac{C_1 \cdot t_1^{1/n_1}}{C_2 \cdot t_2^{1/n_2}}, \text{ where}$$

$$DAF = \frac{V_1}{X_1} \bigg/ \frac{V_2}{X_2}$$

To achieve the same effect the same absorbed breathed-in load or exposure per unit  $X$  is required. For example, if species 1 is animal and species 2 is human, the  $DAF$  is the ratio of animal properties to human properties. The load on a human for the same effect is the  $DAF$  times the load on the animal ( $DAF = L_{human} / L_{animal}$ ).

### 7.3 Adjustment of Exposure Data for Breathing Rate

In comparing the absorbed breathed-in load or exposure for the same species ( $n_1=n_2$  and  $X_1=X_2$ ):

$$\frac{D'_{L1}}{D'_{L2}} = \frac{V_1 \cdot C_1^n \cdot t_1}{V_2 \cdot C_2^n \cdot t_2}, \text{ or}$$

$$\frac{D'_{E1}}{D'_{E2}} = \frac{V_1 \cdot C_1 \cdot t_1^{1/n}}{V_2 \cdot C_2 \cdot t_2^{1/n}} \quad (7.6)$$

A breathing minute volume corresponding to rest (case 1 at  $V_1$ ) can be adjusted to an emergency breathing minute volume that is double the rest rate (case 2,  $V_2=2V_1$ ) by:

$$\frac{L2}{L1} = \frac{V_1 \cdot D'_{L2}}{2 \cdot V_1 \cdot D'_{L1}}, \text{ or}$$

$$\frac{E2}{E1} = \frac{V_1 \cdot D'_{E2}}{2 \cdot V_1 \cdot D'_{E1}} \quad (7.7)$$

To achieve the same absorbed breathed-in load or exposure per unit mass the required load L2 (or exposure E2) under emergency breathing conditions is  $\frac{1}{2}$  of the load L1 (or exposure E1) at rest conditions. A factor of 2 increase in the breathing rate during an emergency reduces the load (or exposure) required for the same effect by a factor of 2. For an  $n$  of 4, to reduce the load by a factor of 2 the time  $t_2$  can be reduced to half with the concentrations the same, the concentration  $C_2$  can be reduced to 0.84 with the time the same, or any other combination that is defined by  $2=(t_1/t_2)(C_1/C_2)^4$ .

In summary, either load or exposure can be used in hazard analysis but care must be taken in the application of uncertainty factors. Uncertainty factors can be applied to  $E$  or  $L$  directly. Uncertainty factors can be applied to  $C$  if  $E$  is used or  $t$  if  $L$  is used, however they can not be applied to  $t$  if  $E$  is used or  $C$  if  $L$  is used as the uncertainty factor becomes raised to an exponent. ***This study will use the load with the uncertainty factor applied directly to L.*** As will be shown in the next section, uncertainty factors are not consistently applied in the selection of endpoints.

## 7.4 Types and Magnitude of UF

Table 9 summarize the types and magnitude of uncertainty factors quoted by other agencies in general and specifically for non-acute doses of H<sub>2</sub>S. The exponent  $n$  in these cases is typically one as the dose equation should apply so the load and exposure are the same. Uncertainty factors are specific to the situation, the type and the magnitude applied depends on the available data for the effect being considered.

Health Canada (HC) recommends that uncertainty factors be considered on a case-by-case basis but also provides general guidance to account for uncertainties by applying a factor of 1 to 10 to each component.

The International Programme on Chemical Safety (IPCS) is a joint venture of the United Nations Environment Programme, the International Labour Organisation, and the World Health Organization. Guidance is provided on extrapolating from a toxicity database to account for uncertainties by applying a “commonly used and appropriate factor of 100”. For interspecies extrapolation a default factor of 10 is suggested. To account for differences in the mean population and highly sensitive subjects (i.e. intraspecies extrapolation) a factor of 10 is suggested. The IPCS also provides a method for subdividing the two factors of 10 when appropriate data is available i.e. providing a ‘correction factor’.

For the inhalation Reference Concentration (RfC) for H<sub>2</sub>S, an uncertainty factor of 300 was chosen by the United States Environmental Protection Agency (US EPA) based on 3 for interspecies extrapolation, 10 for sensitive populations and 10 for sub-chronic exposure. The latter, although applicable to low level long term (i.e. chronic exposures), demonstrates the variability and subjectivity behind selecting uncertainty factors depending on the effect and data.

Alberta Health and Wellness used a 1000-fold uncertainty factor on load applied to the data from a single study to evaluate the mandatory evacuation requirement of 20 ppm H<sub>2</sub>S measured over a 3-minute average. The endpoint assessed was moderate reversible respiratory distress in rats. The toxic load model with an  $n$  of 4.36 was used but at these concentrations there is no evidence to support that it is more applicable than the dose model.

The AEGL-1 was based on persistent odors, eye and throat irritation, headache, and nausea. An UF of 3 was applied to account for intraspecies variability since minor irritation is not likely to vary greatly between individuals.

The AEGL-2 was based on focal areas of perivascular edema and an increase in protein and lactic acid dehydrogenase (LDH) in bronchioalveolar lavage fluid in rats. An UF of 3 was used to extrapolate from animals to humans since rat and mouse data suggest little interspecies variability. An UF of 3 was also applied to account for sensitive individuals since data suggest little strain variability of hydrogen sulphide toxicity among rats (total UF = 10).

**Table 9 Comparison of Uncertainty Factors Used to Extrapolate From Animal Toxicity Studies to Humans – Non-Acute Dose**

<b>Uncertainty Factor Description</b>	Health Canada (General)	International Programme on Chemical Safety (General)	United States Environmental Protection Agency (H2S RfC)	Alberta Health and Wellness (H2S Evacuation)	United States Environmental Protection Agency (H2S AEGL-1)	United States Environmental Protection Agency (H2S AEGL-2)
Observed Effect in Animals to Predicted Effect in Humans	general	general	chronic toxicity	moderate reversible respiratory distress to Irritation	Persistent odours, eye and throat irritation, headache and nausea to Mild Irritation	Disabling to Non-Disabling
<b>Interspecies Variability</b> (accounts for animals being physiologically different than people)	1-10	10	3	10	-	3
<b>Dosimetric Adjustment Factor</b> (ratio of dose in human to dose in animal to achieve same effect)	-	-	0.184 for rat	-	-	-
<b>Intraspecies Variability</b> (accounts for differences in tolerability to exposure within species average to sensitive population)	1-10	10	10	10	3	3
<b>Adequacy of Studies</b> (accounts for the inability of any single study to adequately address all possible adverse outcomes)	1-10	-	-	5	-	-
<b>Nature/Severity of Effects</b> (changes endpoint e.g. L50 to L1, or LOAEL to NOAEL, or chronic to sub-chronic)	1-10	-	10	2	-	-
<b>Uncertainty Factor</b>	1-10000 on dose	100 on dose	300 on concentration	1000 on load	3 on concentration	10 on concentration

Table 10 summarize the types and magnitude of uncertainty factors quoted by other agencies specifically for acute exposures to H<sub>2</sub>S. Note that uncertainty factors can not be compared to the each other unless the starting and final endpoints are the same.

**Table 10 Comparison of Uncertainty Factors Used to Extrapolate From Animal Lethality Studies to Humans – Acute H<sub>2</sub>S Exposures**

Uncertainty Description	United States Environmental Protection Agency (H2S AEGL-3)	United Kingdom Health and Safety Executive (H2S SLOT)	Netherlands Committee for the Prevention of Disasters (H2S Lethality)	<i>Proposed</i> Energy Resources Conservation Board (H2S L50)	<i>Proposed</i> Energy Resources Conservation Board (H2S EPZ)
Observed Effect in Animals to Predicted Effect in Humans	No effect level for death to No Lethality	50% Lethality to 1% Lethality	50% Lethality to 50% Lethality	50% Lethality to 50% Lethality	50% Lethality to No Unconsciousness
<b>Interspecies Variability</b> (accounts for animals being physiologically different than people)	3	-	10	3	3
<b>Dosimetric Adjustment Factor</b> (ratio of load in human to load in animal to achieve same effect)	-	-	5.1 for rat 10.1 for mouse	-	-
<b>Intraspecies Variability</b> (accounts for differences in tolerability to exposure within species e.g. average to sensitive population)	3	-	-	3	3
<b>Inhalation Rate</b> (accounts for increased inhalation during emergency compared to animals at rest)	-	-	2	2	2
<b>Adequacy of Studies</b> (accounts for the inability of any single study to adequately address all possible adverse outcomes)	-	-	1 for one species, 0.5 for average of two or more species	-	-
<b>Nature/Severity of Effects</b> (changes endpoint e.g. L50 to L1, or LOAEL to NOAEL, or chronic to sub-chronic)	-	7.5	-	-	15
<b>Overall Factor</b>	10 on concentration	7.5 on load	4 rat, 2 mouse, less if both on concentration	<b>L50</b> <b>20</b> on load	<b>EPZ</b> <b>300</b> on load

## US EPA

In setting the Acute Exposure Guideline Level AEGL-3 for H<sub>2</sub>S, the US Environmental Protection Agency (US EPA) used a 'no observable adverse effect level' (NOAEL) from a single study and chose an uncertainty factor of 10. This was based on rounding upwards a factor of 3 for interspecies variability multiplied by a factor of 3 for intraspecies variability. These relatively low uncertainty factors were chosen because the rat and mouse data suggests little interspecies and intraspecies variability. A similar variability was therefore expected in humans.

## UK HSE

In setting the Specified Level of Toxicity for H<sub>2</sub>S, the United Kingdom Health and Safety Executive (HSE) did not apply interspecies uncertainty factors to the animal lethality data implying humans respond to H<sub>2</sub>S the same as a rat or mouse. The intraspecies uncertainty factor was not applied as sufficient data was available. The HSE does have a default nature and severity factor of 4 to change the 50% lethality animal data to 1% lethality for humans. For H<sub>2</sub>S a factor of 7.5 is applied instead of the default value to match the data.

## Dutch TNO

In determining probit equations for lethality, the Committee for Prevention of Disasters in the Netherlands provide an approach to extrapolate animal data to humans. In the Green Book they distinguish between locally acting irritants and systemically acting substances. H<sub>2</sub>S acts as an irritant at low concentrations but is a systemically acting substance at high concentrations that are fatal. For irritants the breathed-in dose per unit area on a rat and on a mouse are 3.3 and 5.5 times that on a man, respectively, based on physiological relations. For systemically acting substances the breathed-in dose per unit body weight on a rat and on a mouse are 5.1 and 10.2 times that on a man, respectively. This means that under conditions of rest, and by identical kinetics, dynamics, metabolism and sensitivity assumptions, the LC50 for a given time for humans will be higher than for the mouse or rat.

A safety factor of 5 is applied for irritants gases to account for uncertainty as to whether the same dose per unit area of lung has the same effect on humans and animals. A safety factor of 10 is applied for systemic gases to account for uncertainty as to whether the same dose per unit body mass has the same effect on humans and animals.

A further safety factor of 2 is applied to allow for increased inhalation rates during a toxic gas emergency. When these factors are combined and rounded the extrapolation factor is 0.25 for rats and 0.5 for mice for irritant and systemically acting substances. They conclude that there is no need to differentiate between irritants and systemically acting substances. The LC50 for humans is obtained by multiplying the LC50 for the test animal by the extrapolation factor. This corresponds to dividing by an uncertainty factor of 4 for rats and 2 for mice for lethal effects.

A further step is taken when there are data for more than one animal species. The average human LC50 obtained from 2 or more species is multiplied by a factor of 2 to obtain the human LC50. By having both rat and mouse data an uncertainty factor of 0.5 is introduced (1/0.5 is same as multiplying by 2). This has the effect of reducing the overall safety factor due to the additional confidence in the data. In concept this sounds reasonable but in setting the H<sub>2</sub>S LC50

for 30 minutes, one rat LC50 of 318 mg/m<sup>3</sup> and one mouse LC50 of 669 mg/m<sup>3</sup> were averaged to obtain 493.5 mg/m<sup>3</sup>, and then doubled to obtain an LC50 of 987 mg/m<sup>3</sup>. The final value is the sum of the two inputs which is twice the average. The procedure is not protective of the public and is an example of bad mathematics in the application of uncertainty factors.

## 7.5 Incorrect Applications of *UF*

Deriving an appropriate uncertainty factor is very important. It is as equally important to ensure that the factor is applied properly to the animal data otherwise unintended and extreme uncertainty factors could be introduced or conversely, give results that are not protective enough. To obtain the load or exposure for humans, the animal load or exposure is divided by the uncertainty factor. For toxic gases the observed lethal response is to a load ( $L = t * C^n$ ) or exposure ( $E = C * t^{1/n}$ ), as the data presented for H<sub>2</sub>S in the previous sections supports. The causative factor is the load  $L$  (the product of  $C^n$  and  $t$ ) or the exposure  $E$  (the product of  $C$  and  $t^{1/n}$ ).

However, some have applied the uncertainty factor to the concentration or time alone. For example, if an uncertainty factor of 10 is applied to the concentration and the exponent is 4,  $(C/10)^4$  results in an uncertainty factor of 10,000 on the load. If the exponent is 2, the uncertainty factor on the load is only 100. If the exponent is 1 the load becomes the dose and the uncertainty factor is 10. For the linear dose equation it does not make a difference if the uncertainty factor is applied to the concentration or the time as the load is the product of concentration and time. This certainly creates confusion if an uncertainty factor has different effects on the causative factor (the Load) depending on the exponent  $n$ . Several agencies have adopted the load model then misapplied the uncertainty factor in the traditional way to the concentration, thus perpetuating confusion.

The US EPA has applied the uncertainty factor to the concentration in setting the AEGL-3, as discussed by Hilderman *et al* in a conference paper. Based on an analysis of LC50 – time pairs, the toxic load equation is adopted with an exponent of 4.36. Then a NOAEL concentration of 504 ppm over 60 minutes is divided by an UF of 10 to obtain 50 ppm. The toxic load equation is then used to adjust the 50 ppm to other times. At these low concentrations the load equation with an exponent of 4.36 does not apply but the dose equation with an exponent of 1 would. The load uncertainty factor is  $(1/10)^{4.36}$  which equals 22,909. Hilderman *et al* note that the uncertainty factor should be applied to the load.

The Dutch TNO Green Book description of the approach used to adapt animal toxicity data to humans, the terms dose, load and LC50 are used. Dose and LC50 are as defined in this report. Load is not defined at all but implied to mean the same as in this report ( $L=tC^n$ ). The breathed-in dose per unit body weight or lung area is used to extrapolate LC50 data from animals to humans along with a safety factor. Quoting directly, “The difference in sensitivity between species is expressed as an extrapolation factor which influences the concentration  $C$  (or the exposure duration  $t$ ).” This is where the error is made, the load equation is used but the adjustment is made only to  $C$  not the load ( $L=tC^n$ ). The first step in the process is to take the LC50’s for times other than 30 minutes and adjust them to 30 minutes assuming  $tC^n = \text{constant}$ . The LC50 for 30 minutes for a human is then the extrapolation factor for that species times the LC50 for 30 minutes for that species. The table of extrapolation factors provides the ratio of (Load animal /

Load human) and safety factors used to derive the extrapolation factor but then the extrapolation factor is only applied to the concentration, not the load. For H<sub>2</sub>S, the LC50 for a human is 1/4 the LC50 of a rat and 1/2 the LC50 of a mouse.

The LC50 for humans is obtained by multiplying the LC50, not the L50, for the test animal by the extrapolation factor. Using the Dutch exponent  $n$  of 1.9 for H<sub>2</sub>S, this implies the human load is  $0.07 (=0.25)^{1.9}$  times the rat L50 not 0.25. For mice it is  $0.27 (=0.5)^{1.9}$  times the mouse L50, not 0.5. If an exponent of 4 is used, the difference and error is greater. If the exponent is 1 the approach is reasonable.

Locally, Alberta Health and Wellness used a 1000-fold uncertainty factor on load applied to an endpoint of moderate reversible respiratory distress in rats. The toxic load model with an  $n$  of 4.36 based on lethality was used. At concentrations of 20 ppm there is no evidence to support that an  $n$  of 4.36 for lethality is applicable to the endpoint. The dose model with an  $n$  of one should be used at low concentrations.

Applying the uncertainty factor to the concentration or time instead of the load (or exposure) is not supported by science or mathematics. *The uncertainty is not in the time or the concentration but in the effect, in this case the combination of time and concentration to cause 50 % lethality.* This error is attributed to using traditional dose approach to the non-linear toxic load. The data analysis confirms it is the load or exposure that the uncertainty factors should be applied to, but traditionally they were applied to the dose, thus creating the confusion. The net effect of this error is that the uncertainty factors become a function of the exponent  $n$ ; if this was intended it surely would have been discussed by the decision makers. However, the regulators who have made this error were unaware of their mistakes. Some may argue that this calculation error just contributes an additional safety factor. One could counter that it certainly creates uncertainty and shows a lack of understanding of what is trying to be accomplished. It is recommended that the ERCB correctly apply the uncertainty factor to the load.

## 7.6 Proposed ERCB UFs

Table 10 provided the proposed uncertainty factors for the ERCB Endpoints. It is recommended that an uncertainty factor of **20** be used to adjust the animal L50 to a human L50. This is based on rounding up the product of 3 for interspecies, 3 for intraspecies and 2 for inhalation rate. The ratio of the load in human to load in a rat and in a mouse to achieve same effect are set to one ( $DAF=1$ ). This is due to the uncertainty as to what it should be given what is done by other jurisdictions. It ranges from 1/5 to 1 for non-acute doses to 1-10 for acute doses. Most do not include it, implying that it is 1. These factors will be used on mouse and rat data to generate the probit parameters for lethality that will be used in risk assessments.

For setting the emergency response and planning zones, it is recommended that the ERCB non-unconsciousness endpoint use an uncertainty factor of **300** to adjust the animal L50 to a load that is very unlikely to cause unconsciousness in susceptible humans during an emergency. The nature and severity effect uncertainty factor to go from 50% lethality to 1% unconsciousness is 15 based on the product of 3 for L50 to RR50 and 5 for RR50 to RR1 (see Sections 5 and 6).

## 8 ERCB ENDPOINTS

Two endpoints are required: the ERCB L50 with associated probit parameters for risk assessments and the ERCB EPZ load to define the emergency planning zone. Applying uncertainty factors is not ideal and is required when data is not directly applicable to the situation that is being assessed. Therefore the objective should *always* be to minimize uncertainty factors where the data allows.

### 8.1 ERCB L50

For acute exposure to H<sub>2</sub>S there is an abundance of animal data that can be used to extrapolate to the human population to account for:

- Intraspecies variability,
- Interspecies variability, and
- Emergency situations.

To extrapolate from rat/mouse lethality data to humans an uncertainty factor of **20** is recommended. This is more conservative than the uncertainty factors applied by the US EPA for the AEGL-3, UK HSE and Dutch TNO.

- A factor of three (3) is representative of intraspecies variability to capture the response of the sensitive individuals in the population. Test animals represent the average population of humans, an adjustment is made to account for the young and older members who are more susceptible and those that are more sensitive. A factor of 2.5 is used by the HSE, and TNO for ammonia and chlorine to adjust from the regular population to the vulnerable population and US EPA used a factor of 3.
- There appears little difference between mammalian species for acute exposure to H<sub>2</sub>S and it is judged that a factor of three (3) is reasonable to extrapolate between rat/mouse data and humans. The TNO uses a factor of 1 for mice and 2 for rats, the HSE uses a factor of 1 and US EPA uses a factor of 3.
- Laboratory animals are at rest during an exposure; during an emergency the breathing rate of humans' increases. A person will not remain passive during an emergency but will react with some form of physical activity such as seeking to escape or to obtain shelter. The inhalation rate increases and greater amounts of oxygen are required by the body. The base level of activity corresponds to rest. A standard level of activity corresponds to a normal mixture of sitting, standing and moving about for which the inhalation rate is twice that of the base level. TNO assumes the average breathing minute volume of an exposed population will increase to twice the value of the rest condition. A factor of two (2) is recommended for the ERCB.

The factor of 20 is based on multiplying and rounding upwards factors of three (3) for interspecies variability, three (3) for interspecies variability and two (2) for the increased inhalation rate during an emergency.

The L50 represent a toxic load for 50% lethality, including the susceptible population and is defined by the probit parameters:

$$\begin{aligned} \text{ERCB L50} &= C^{3.5}t = 2.279 \cdot 10^{10} \text{ ppm}^{3.5} \text{ minutes} = \frac{4.557 \cdot 10^{11}}{20} \\ \text{Probit} &= -29.415 + 1.443 \cdot \ln(C^{3.5}t) \end{aligned} \quad (8.1)$$

Table 11 and Figure 9 provide the ERCB L50 endpoint concentrations as a function of time.

## 8.2 ERCB EPZ

The ERCB EPZ criterion aims to prevent unconsciousness from significant exposure to sour gas, thus the L50 data must be scaled to some lower value. The nature and severity of effect uncertainty factor is used to adjust the toxic load to an acceptable outcome. Of particular interest are the exposure concentration-time data that results in *no deaths* and in *unconsciousness* in animals. In summary:

- L50 / LNOAEL = 2.27, and probit analysis provides a L50 / L1 = 5.02, round UF to 5 for no deaths. However a portion of the exposed population would be unconscious, as given by
- L50 / RR50 = 2.56, round UF to 3 for unconsciousness which is about the same as for no deaths above. At the LNOAEL, no deaths are expected but 50% of the population could be unconscious.
- RR50 / RRNOAEL = 2.59, however probit analysis provides a RR50 / RR1 = 5.04, round UF to 5 for no unconsciousness.

The endpoint scaling factor from rat/mouse L50 data to no deaths in animals is five (5) (L50/L1). The endpoint scaling factor from rat/mouse L50 data to no unconsciousness in animals is fifteen (15), based on multiplying factors of three (3) for RR50 (50% unconsciousness) from the L50 and five (5) for no unconsciousness from the 50% unconsciousness load (RR50/RR1).

To extrapolate from the rat/mouse L50 data to an endpoint that is *protective of death* in humans, an uncertainty factor of 100 (endpoint scaling factor of 5 multiplied by lethality uncertainty factor of 20) is needed. To extrapolate from the rat/mouse L50 data to an endpoint that is *protective of unconsciousness* in humans, an uncertainty factor of **300** (endpoint scaling factor of 15 multiplied by lethality uncertainty factor 20) is appropriate.

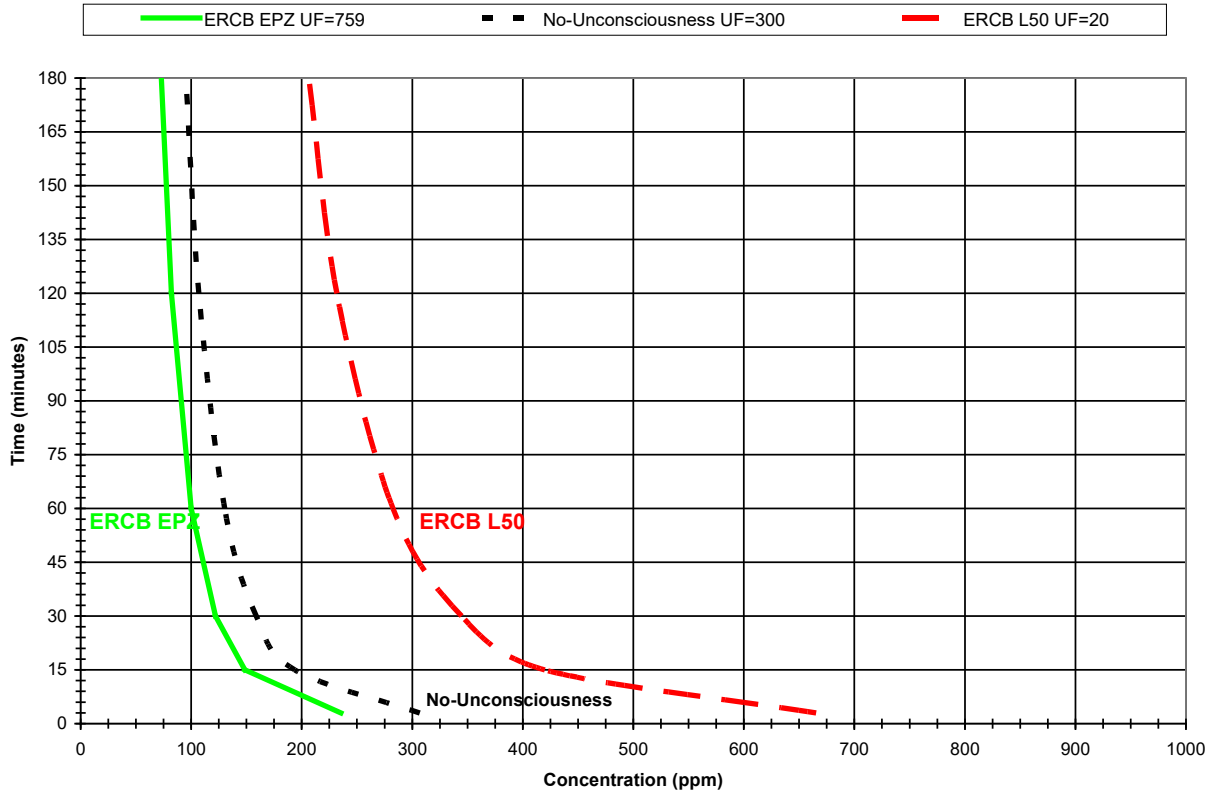
A **three hundred-fold** uncertainty factor is recommended for the ERCB non-unconsciousness endpoint to provide an adequate margin of safety. This accounts for adjusting animal lethality data to humans, people that might be more sensitive to H<sub>2</sub>S exposure (e.g. children and the elderly), increased inhalation during an emergency and unconsciousness that would prevent escape or sheltering.

The ERCB non-unconsciousness endpoint has been set at 130 ppm for 60 minutes with an exponent n of 3.5. By definition this endpoint will also be protective of lethality as it is set to a lower toxic load.

The ERCB Emergency Planning Zone (EPZ) endpoint has been set at 100 ppm for 60 minutes with an exponent  $n$  of 3.5 to provide a more conservative margin of safety. Table 11 and Figure 9 compare the concentrations and time pairs defined by the toxic load for various uncertainty factors.

**Table 11 Concentration and Exposure Time Pairs for ERCB Endpoints**

H2S Exposure Endpoints			
Load Equation $L = tC^n$ with exponent $n = 3.5$			
Exposure Time (t minutes)	H <sub>2</sub> S Concentration (C ppm)		
	ERCB EPZ UF=759	No Unconsciousness UF=300	ERCB L50 UF=20
3	235	307	665
15	149	194	420
30	122	159	345
60	100	130	283
120	82	107	232
180	73	95	207



**Figure 9** Concentrations and Exposure Times for ERCB Endpoints with  $L=tC^{3.5}$

The uncertainty factors required to produce the ERCB EPZ endpoint is 759, two and one half times the value of 300 supported by the unconsciousness data analysis. Using the probit parameters the predicted chance of lethality at the no-unconsciousness load is 0.005% (5 in 100,000). The ERCB EPZ endpoint results in a 0.000008% (8 in 10,000,000) chance of lethality. Note that response predictions are not reliable at less than 1%, but this does show the chance of lethality is extremely small. The proposed ERCB EPZ endpoint is protective of unconsciousness in humans.

## 9 HUMAN LETHALITY PROBIT PARAMETERS

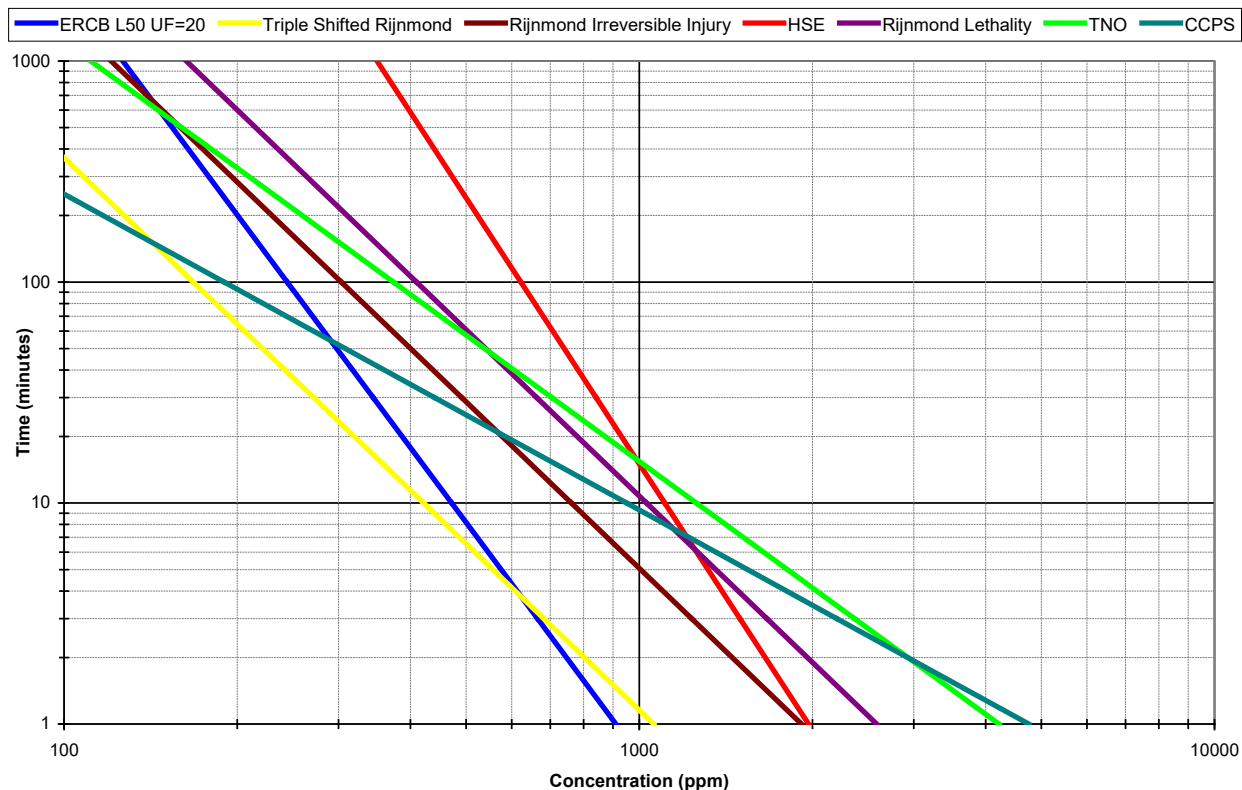
This section compares the published probit parameters for human lethality to H<sub>2</sub>S to the ERCB L50 Endpoint. The following table provides published probit equations for human lethality to H<sub>2</sub>S. These parameters are used in risk assessments performed in other countries to determine the chance of lethality.

**Table 12 Probit Parameters for Lethality to H<sub>2</sub>S**

Reference	$Y = a + b_2 \ln(tC^n)$			LC50 (ppm) for 60 minutes
	$a$	$b_2$	$n$	
Rijnmond Lethality (COVO 1982)	-41.48	2.366	2.5	503
Rijnmond Irreversible Injury (COVO 1982)	-39.70	2.366	2.5	372
Triple Shifted Rijnmond (ERCB 1990)	-36.20	2.366	2.5	206
Centre for Chemical Process Safety (Perry and Articola 1980)	-31.42	3.008	1.43	271
Committee for Prevention of Disasters <sup>1</sup> (TNO 1992)	-11.5	1	1.9	489
HSE (1990) (derived from L50 and L1)	-30.023	1.154	4.0	709
ERCB L50 with UF=20	-29.415	1.443	3.5	283

<sup>1</sup>(parameters for C in mg/m<sup>3</sup>, divided by 1.4 for ppm)

Note that the exponents  $n$  above for the older studies are lower than the value of 3.5 supported by this study and the 4 used by HSE. Figure 10 is a comparison of the L50 as a function of LC50 and LT50. The lines cross due to the differences in the exponent  $n$ . For example, the L50 at 1 minute has the proposed ERCB L50 resulting in the lowest concentration. As time increases the lines cross and at 100 minutes the Triple shifted Rijnmond results in the lowest concentration.



**Figure 10 Comparison of Published  $L50=t \cdot C^n$  with Proposed ERCB Endpoints**

Figure 11 shows how the predicted response changes with concentration for selected times. The curves depend on all three probit parameters and show that comparing the LC50 at one time (as in Table 12) can be misleading. The response curves may cross.

The American Institute for Chemical Engineers Centre for Chemical Process Safety values were based on estimates for hydrogen cyanide as no suitable data was available at the time (Lees, 1996). For H<sub>2</sub>S the lethal dose value for hydrogen cyanide was doubled and the constant *a* was adjusted for the probit equation.

The Committee for Prevention of Disasters of the Netherlands use a default value for *b*<sub>2</sub> of 1.0 for all gases as it corresponds to a high value for the ratio of LC95/LC05, and for concentrations below the LC50 is the conservative assumption (Lees, 1996). The *n* of 1.9 was based on the average of three published values instead of the default value of 2.

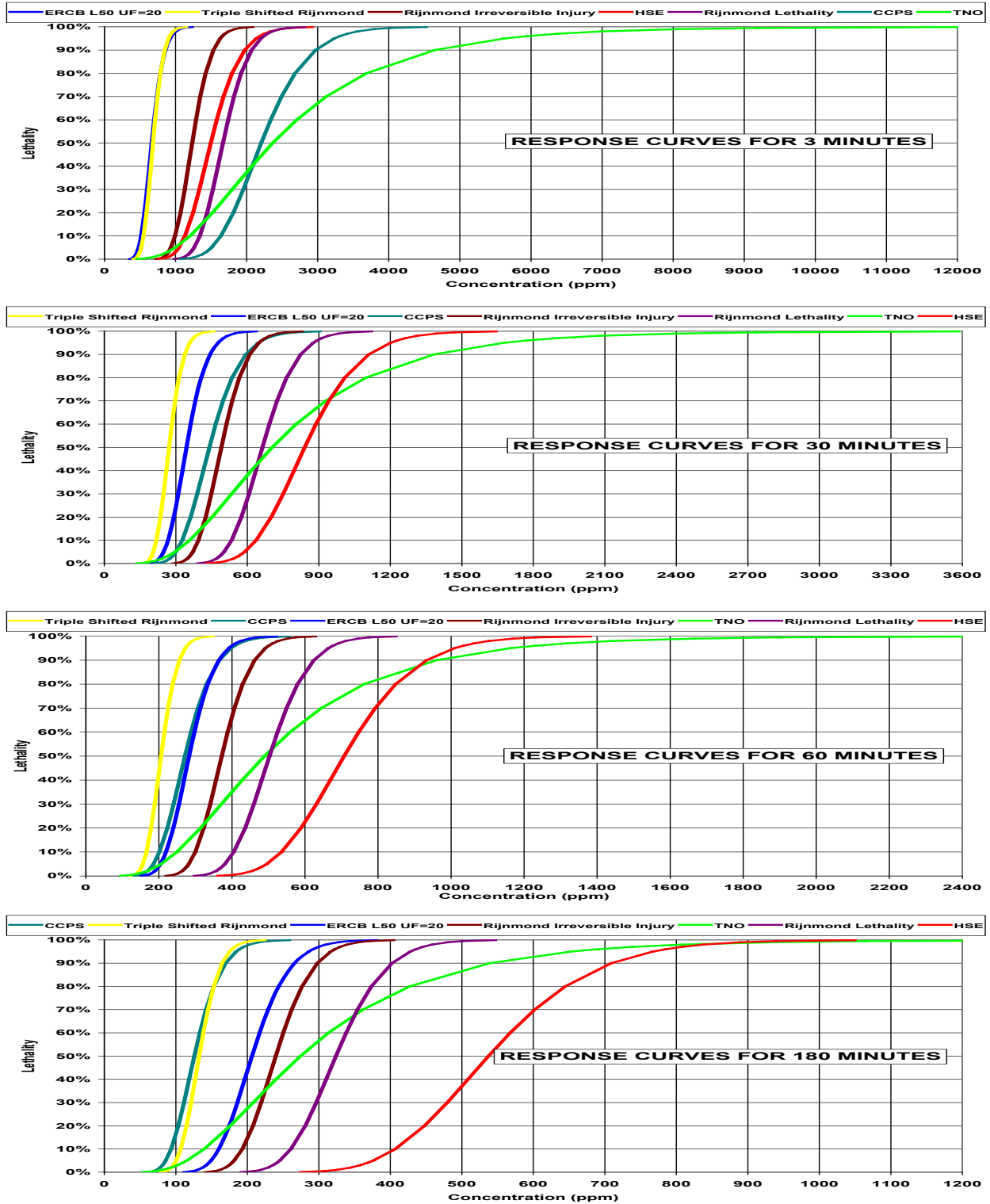
The probit parameters for humans incorporate varying degrees of safety factors. The ERCB 1990 triple shifted were adjusted three times before they were deemed acceptable at the time. The Triple Shifted Rijnmond parameters can be obtained from the Rijnmond lethality parameters by dividing the L50 by an uncertainty factor of 9.31. Likewise, the Rijnmond Irreversible Injury parameters can be obtained from the Rijnmond lethality parameters by dividing by an uncertainty factor of 2.13. The Triple Shifted Rijnmond Parameters define serious, irreversible

effects to an unknown degree. In the discussion of the Rijnmond parameters (COVO 1982) the following table of toxic effects were presented:

Effect	Time	H <sub>2</sub> S Concentration (ppm)
Odour detectable by most people	Any	0.1 to 0.4
Safe Exposure	8 hours	10
Maximum that can be inhaled without serious consequences	60 minutes	200
Lethal	Rapidly <30 minutes	>900 600-800

In comparison, the Rijnmond lethality parameters give an LC50 of 503 ppm for 60 minutes, the Rijnmond irreversible injury parameters give an LC50 of 372 ppm for 60 minutes and the Triple Shifted Rijnmond parameters give an LC50 of 206 ppm for 60 minutes. The Rijnmond parameters for lethality and irreversible injury are consistent with the above table but the Triple Shifted Rijnmond parameters are not as serious irreversible effects are predicted when serious consequences are not expected at 200 ppm for 60 minutes. The ERCB L50 parameters based on the moderately rated lethality data and an UF of 20 give an LC50 of 283 ppm for 60 minutes and are consistent with the above table. The ERCB EPZ based on an UF of 759 give an H<sub>2</sub>S concentration of 100 ppm for 60 minutes and is also consistent with the above table.

The next section compares the limited data on human exposures to H<sub>2</sub>S to the ERCB Endpoints.



**Figure 11 Lethality Response Sensitivity to Concentration and Time for Published Probit Parameters**

## 10 HUMAN EXPOSURE DATA

The proposed ERCB EPZ and ERCB L50 endpoints are compared to human exposure data in this section. There is very little human exposure data available for high concentration exposures. Two clinical studies involving controlled exposures of human subjects to H<sub>2</sub>S received a low grading by CANTOX. To receive a low grading:

- The study fails to meet the recommended guidelines, and serious weaknesses in experimental design, conduct and/or reporting are evident.
- Several aspects of the study are lacking when measured against the “quality benchmarks”.
- Significant departures from the recommended guidelines may be present, including errors in experimental conduct.
- Sufficient detail is lacking to permit meaningful interpretation of the findings.
- Study validity is questionable.
- Confidence in the findings and conclusions is low.

Table 13 lists the exposure concentration-exposure time combinations that were tested in each study and resulted in no mortality. The studies were published in 1892 and 1925; the low grading is due to the above concerns. The exposures are listed by increasing toxic load using the average concentration. Each test subject was exposed to increasing concentrations, the time between exposures is not provided. The maximum exposure concentration was 575 ppm and the maximum exposure time was 240 minutes.

These exposures are in the range that many would consider lethal to humans but there were no deaths. Complete details concerning the various combinations tested in each study are contained in the Document Review Forms found in Appendix A of the CANTOX study. The signs and symptoms listed are those reported to have occurred in the absence of mortality. Attention was given to signs and symptoms consistent with serious effects.

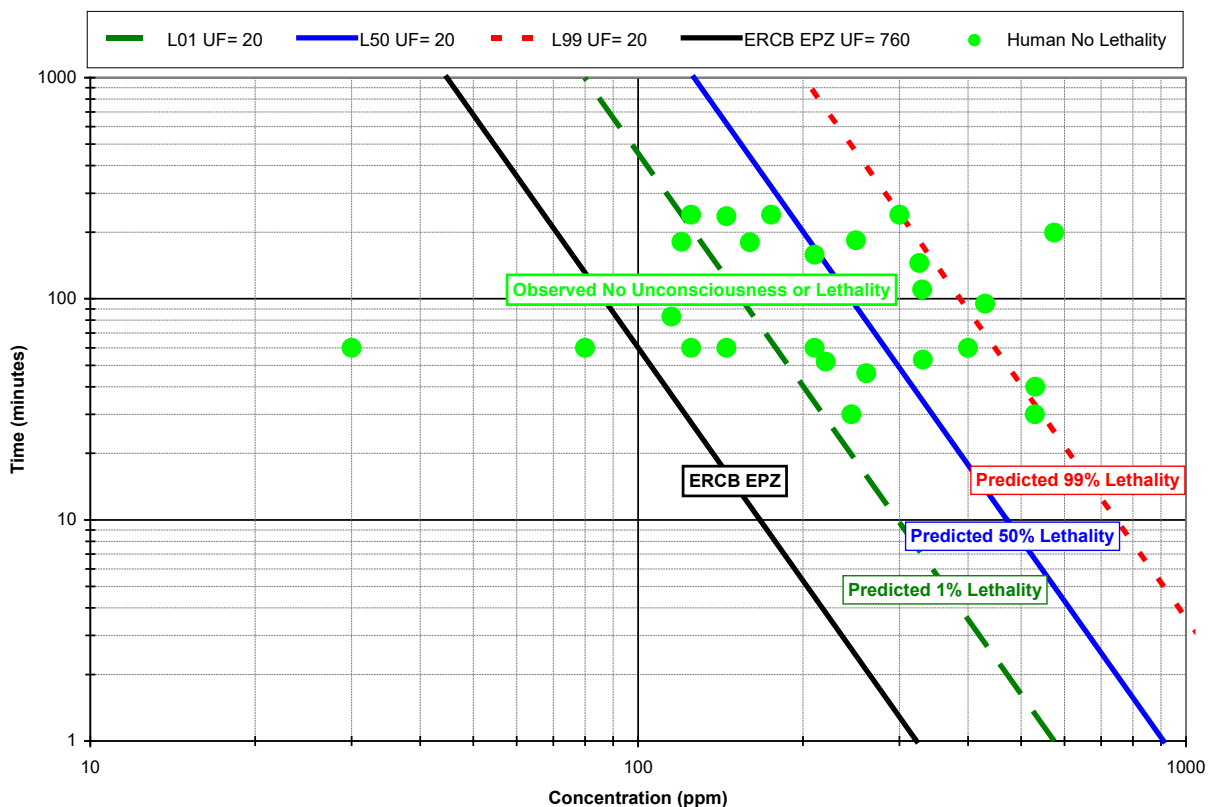
Based on physiological factors the Dutch determined that the L50 values for humans will be higher than for mice or rats. The predicted L50 for mice and rats of  $4.56 \cdot 10^{11}$  is one half of the highest no death human load of  $9.07 \cdot 10^{11}$  which caused headaches and persistent pain in the eyes.

**Table 13 Human Exposures with Symptoms**

Author(s)	Study Code	H2S Concentration (ppm)	Exposure Time (minutes)	Symptoms
Lehmann (1892)	CL011	20 to 40	60	None reported.
Lehmann (1892)	CL011	70 to 90	60	No symptoms other than slight local irritation.
Lehmann (1892)	CL011	100 to 130	83	No symptoms other than slight nasal irritation.
Lehmann (1892)	CL011	100 to 150	60	No symptoms other than local irritation.
Lehmann (1892)	CL011	140 to 150	60	No symptoms other than slight to unpleasant local irritation.
Lehmann (1892)	CL011	100 to 140	181	Transient difficulty in breathing, pain in eyes, intolerance to light ... symptoms eased by end of exposure, but local irritation had not completely cleared by 4 days post-exposure ... latent headache.
Mitchell and Yant (1925)	CL010	100 to 150	240	Cough, disturbed respiration, accompanied by pain in eyes and throat irritation.
Lehmann (1892)	CL011	145	236	Persistent headache, pain in eyes
Lehmann (1892)	CL011	210 to 280	30	No symptoms other than local irritation.
Lehmann (1892)	CL011	210	60	Headache and eye irritation ... continuing for several hours post-exposure.
Lehmann (1892)	CL011	120 to 200	180	Transient difficulty in breathing, slight irritation of eyes and throat ... latent headache, slight bronchitis.
Lehmann (1892)	CL011	210 to 230	52	Progressive local irritation, otherwise no symptoms ... latent diarrhoea.
Lehmann (1892)	CL011	261	46	No symptoms other than local irritation of eyes and trachea ... rapid recovery.
Mitchell and Yant (1925)	CL010	150 to 200	240	Cough, difficult respiration, irritation of eyes and throat, light intolerance.
Lehmann (1892)	CL011	210	158	Headache, pain in eyes ... symptoms persisted for 24 hours
Lehmann (1892)	CL011	331	53	Local irritation and latent headache.
Lehmann (1892)	CL011	250	184	Light headache, inflammation of eyelids ... recovery within 2.5 hours post-exposure
Lehmann (1892)	CL011	250 to 410	110	Difficult respiration, pain in eyes, light intolerance ... latent diarrhoea, slight bladder pain.
Mitchell and Yant (1925)	CL010	350 to 450	60	Headache, cough, difficult respiration, irritation of eyes and nasal passages.
Lehmann (1892)	CL011	326	145	Pain in head and eyes ... rapid recovery.
Mitchell and Yant (1925)	CL010	250 to 350	240	Headache, difficult respiration, weariness, irritation of eyes and nasal passages, light intolerance.

Author(s)	Study Code	H2S Concentration (ppm)	Exposure Time (minutes)	Symptoms
Lehmann (1892)	CL011	530	30	Headache, unsteadiness, giddiness, trembling of the extremities, accompanied by local irritation... latent diarrhoea, headache, pain in bladder.
Lehmann (1892)	CL011	531	40	Persistent headache and local irritation of eyes and trachea.
Lehmann (1892)	CL011	370 to 490	95	Cough, pain in eyes, swelling of eyelids, light intolerance ... latent diarrhoea.
Lehmann (1892)	CL011	575	199	Headache and persistent pain in eyes.

Figure 12 compares the no lethality human exposures to the ERCB L50 with an UF of 20 (L1 and L99 are also provided) and the proposed ERCB EPZ with an UF of 759. Notice that many of the plotted no lethality concentration time pairs are within the range where lethality is predicted to occur using the ERCB probit parameters. The comparison confirms that the selected uncertainty factors are cautious and protective.



**Figure 12 Human Exposures with Low Grading Compared to ERCB Endpoints**

The no-lethality human exposure data has a low grading partially due to the uncertainty in the concentrations. If the concentrations were high by a factor of two they would be shifted to the left to lower concentrations (the distance from 200 to 100 ppm). With this adjustment, the conclusion about the cautiousness of the endpoints remains the same; the proposed ERCB L50 probit parameters are based on reasonable uncertainty factors and the ERCB EPZ is protective of unconsciousness.

## **APPENDIX 1: Public Consultation**

(file attached as Appendix 1.pdf)

## **APPENDIX 2: Overview of Hydrogen Sulphide Lethality Data and Exposure Criteria**

(file attached as Appendix 2.pdf)

**APPENDIX 3: Review and Assessment of the Technical Quality of Lethality Data Proposed for Use in “Toxic Load” Calculations in Support of Hydrogen Sulphide Exposure Endpoints for Emergency Planning Purposes**

(file attached as Appendix 3.pdf)