ERCBH_2S

A Model for Calculating Emergency Response and Planning Zones for Sour Gas Wells, Pipelines, and Production Facilities

Volume 2: Emergency Response Planning Endpoints

Energy Resources Conservation Board

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ENERGY RESOURCES CONSERVATION BOARD
ERCBH2S: A Model for Calculating Emergency Response and Planning Zones for Sour Wells, Pipelines, and Production Facilities, Volume 2: Emergency Response Planning Endpoints

December 2010

Published by

Energy Resources Conservation Board
Centennial Place
Suite 1000, 250 – 5 Street SW
Calgary, Alberta T2P 0R4

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SUMMARY

Volume 2: Emergency Response Planning Endpoints provides a summary of the work done to-date to define the ERCB Emergency Planning Zone endpoint required for the computer model ERCBH2S. 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:

$$ERCB\,L50 = C^{3.5}t = 2.279 \cdot 10^{10}\ \text{ppm}^{3.5}\text{minutes} = \frac{4.557 \cdot 10^{11}}{20}$$

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

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_2S exposure endpoints:

H2S Exposure Endpoints			
Load Equation $L = tC^n$ with exponent n = 3.5			
Exposure Time (t minutes)	H_2S 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.

The H_2S 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 exposure to the ERCB EPZ Endpoint should not result in unconsciousness that would impair escape.

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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	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.
Overview	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 Version 1.20	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 Version 1.20	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_2S which could result in uncounsciousness. 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_2S 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_2S animal lethality data that received a moderate or higher grade in a recent review[1] (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_2S 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.

[1] Appendix 3 presents the results of work commissioned by the EUB to grade the quality of the H_2S toxicity studies used by others jurisdictions, and the basis for the EUB H_2S 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%

Entry	Authors	Study Code	Species (male, female)	Exposure Time (t, minutes)	H2S Concentration (C, ppm)	Number Tested	Number Killed	% Killed
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%
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%
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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%

Entry	Authors	Study Code	Species (male, female)	Exposure Time (t, minutes)	H2S Concentration (C, ppm)	Number Tested	Number Killed	% Killed
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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%
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 50th 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$^{(7/2)}$ (minutes*ppm$^{7/2}$)
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	**4.37E+11**
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^{11} is near the median of 4.37 10^{11} minutes*ppm$^{7/2}$. 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 = concentration, y = time). The bottom plot is the opposite, with concentration as the dependent variable and time as the independent variable (x = time, y = 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	$n = 3.58$	$n = 3.49$

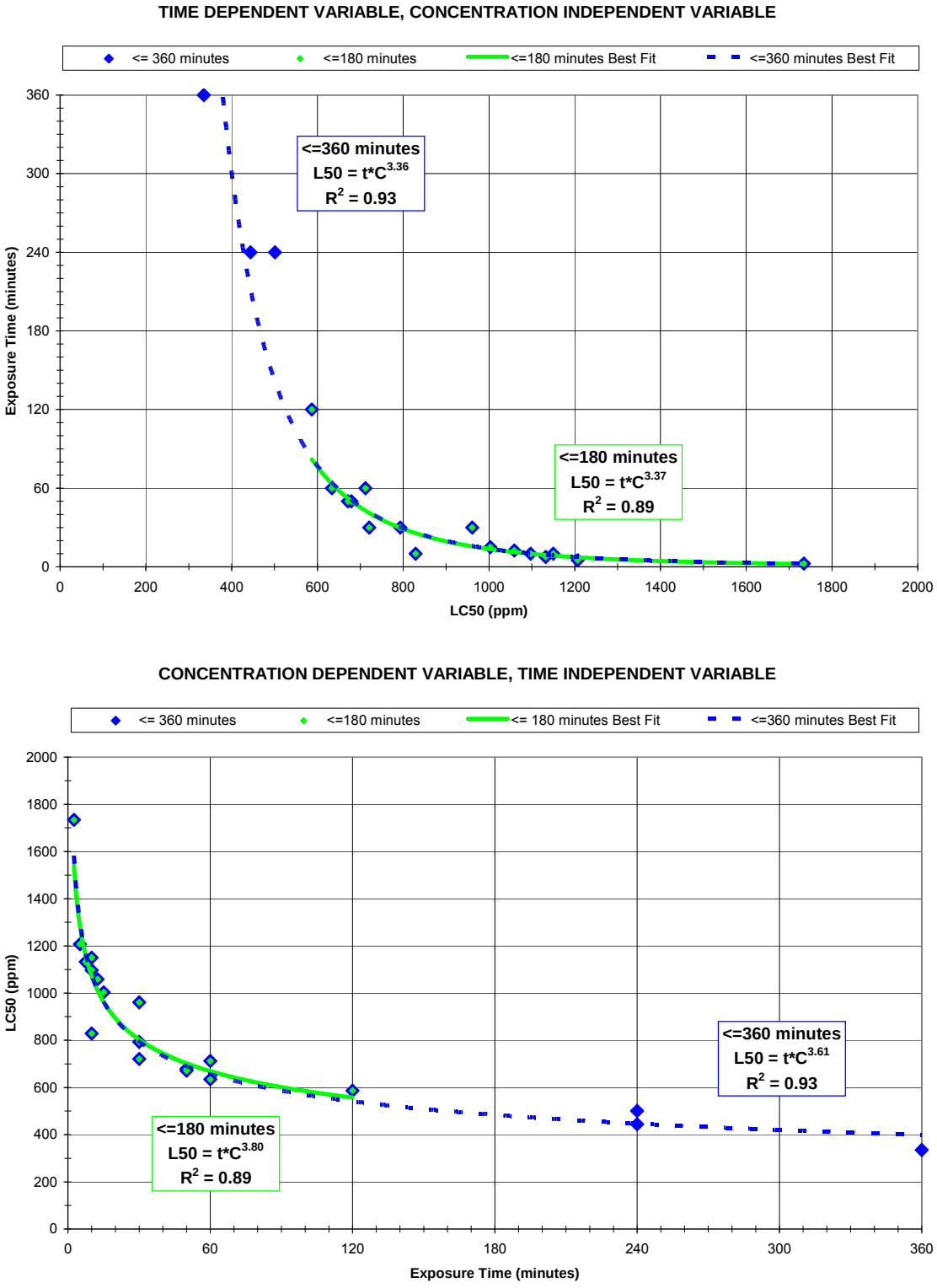


Figure 1 **LC50 and Time Pairs with Moderate Grading Presented Two Ways to Determine Exponent (the average *n* of top and bottom plot should be used)**

In the EPZ requirements, a maximum exposure duration of 3 hours has been set. Results for exposure times less than 3 hours (<=180 minutes) are compared to times under 6 hours (<=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 10^{11} minutes*ppm$^{7/2}$ and has a slope of -3.5, which is an exponent *n* of 3.5.

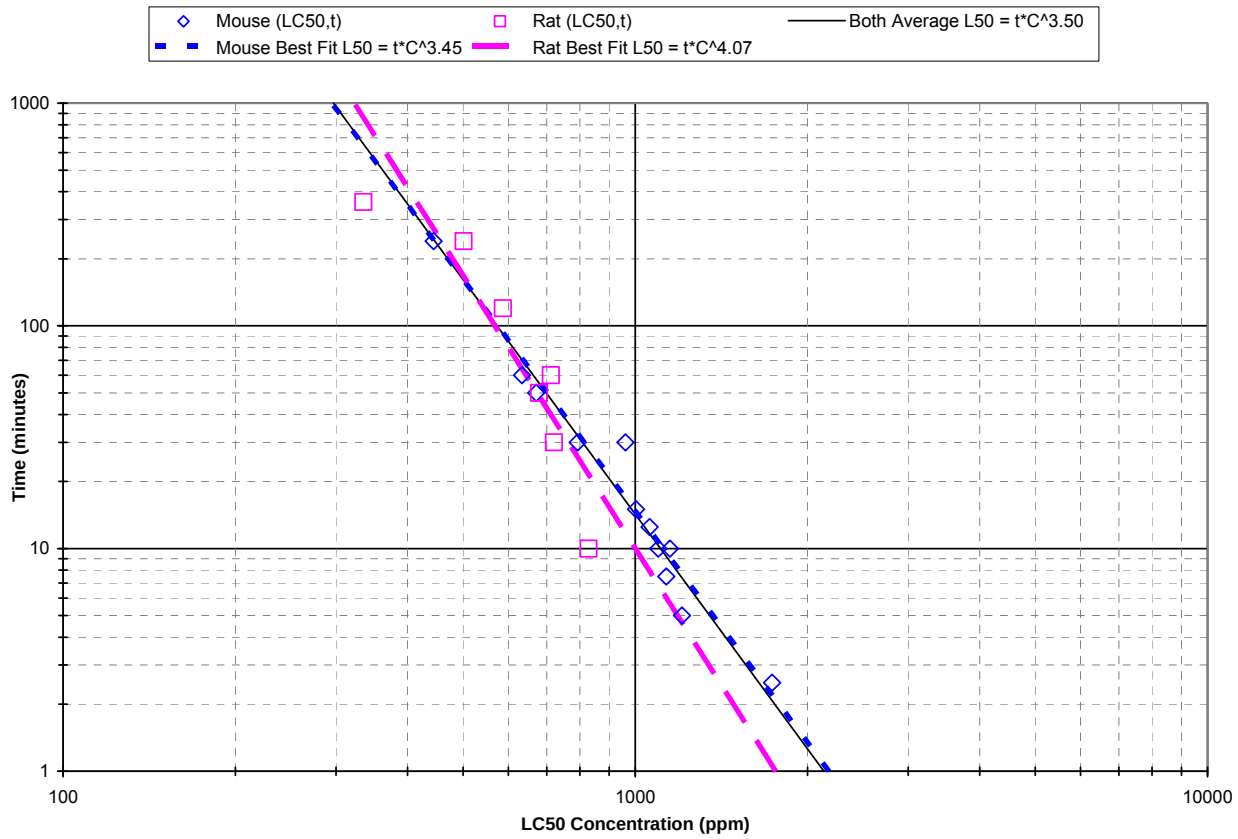


Figure 2 LC50 and Time Pairs with Moderate Grading showing *n* ≈ 3.5

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.

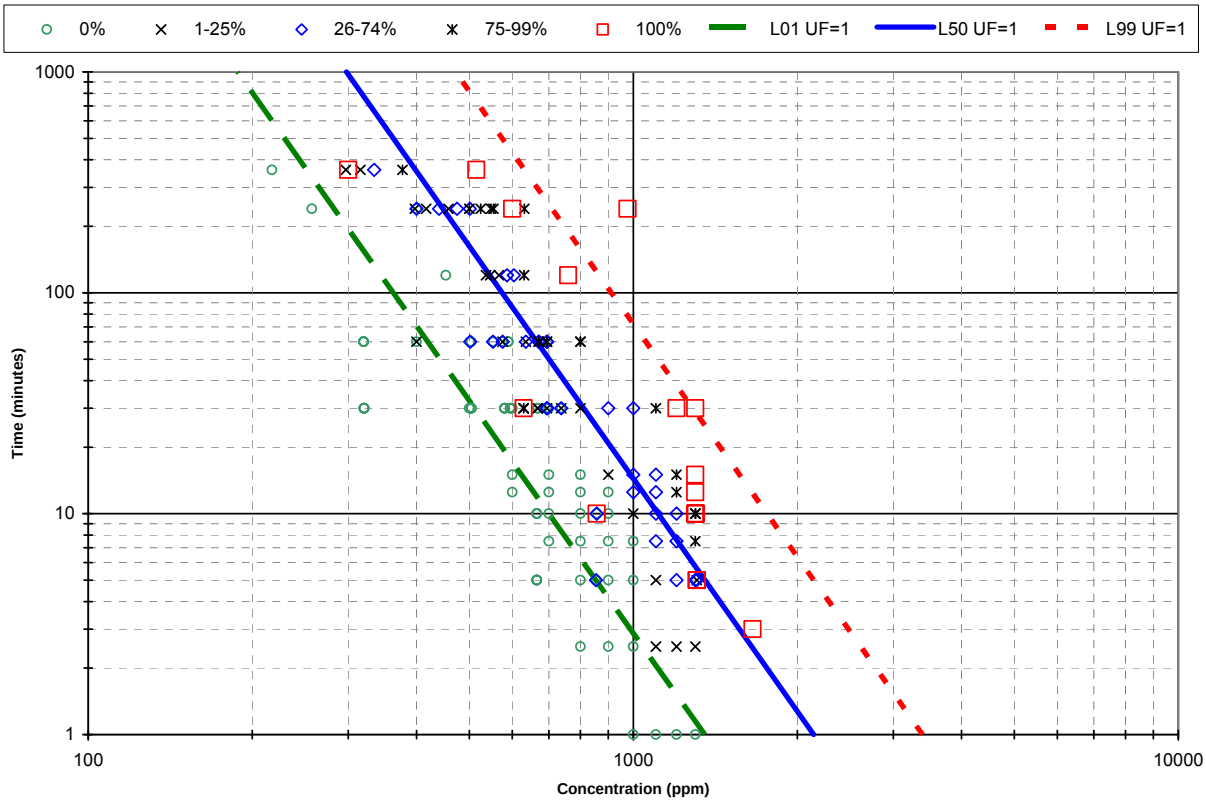


Figure 3 % Response, Concentration and Time Triplets with Moderate Grading showing Probit Analysis Results for L01, L50 and L99 with *n* of 3.5

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:

$$Y = a + b_1 \ln C + b_2 \ln t \tag{4.1}$$

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~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~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:

$$Y = a_c + b_c \ln C \tag{4.2}$$

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:

$$Y = a_t + b_t \ln t \tag{4.3}$$

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:

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

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^n) and E (ppm·minutes$^{1/n}$) have different units, and are related through:

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

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:

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

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:

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

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):

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

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 50th percentile, so

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

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),* …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 10th Percentile Lethal Concentration (LC10) or 90th Percentile

Lethal Concentration (LC90) than a 50th 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 (χ^2) test is used. The χ^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 χ^2 can be large. A value of χ^2 within the limits of random variation indicates satisfactory agreement between theory (the predicted line) and the observations. A significantly large χ^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 χ^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^2/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 then 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:

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

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

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* =*b1/b2*	Goodness of Fit X^2/df/ pass or fail	LC50 (ppm)
			a	*b1*	*b2*			
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)		
			Reported for *each* time	*Calculated* for *each* time	*Calculated* from *all* times
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^2/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^2/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^2/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 χ^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.

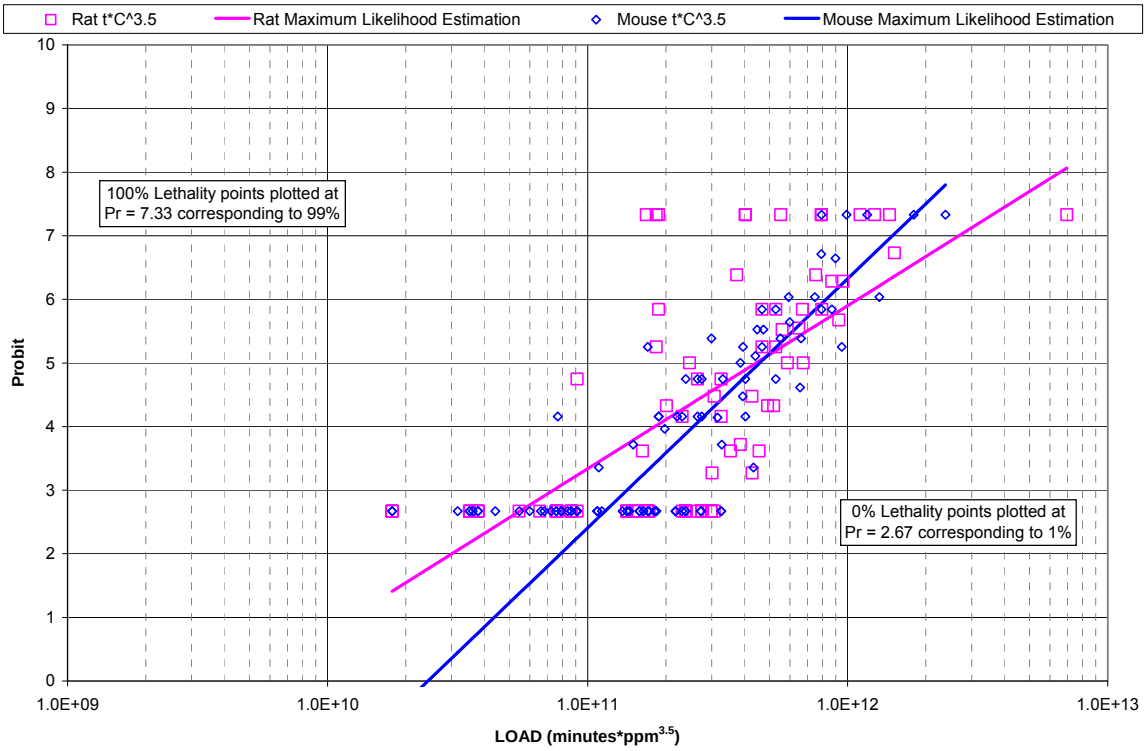
Notice that in all cases the goodness of fit test failed on χ^2. However it passed on the homogeneity test. b_2 ranges from 1.05 to 1.79 and is not sensitive to *n*.

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 $4.591\ 10^{11}$ minutes $ppm^{3.5}$ is slightly larger than for rats $4.454\ 10^{11}$ minutes $ppm^{3.5}$. 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 $4.557\ 10^{11}$ minutes $ppm^{3.5}$ and the b_2 is 1.44, in between the values when analyzed separately. This compares well to the simplified analysis average L50 of $4.50\ 10^{11}$ minutes $ppm^{3.5}$. The 95 % confidence interval is also shown. The L50 corresponds to a response of 50±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 $1.820\ 10^{11}$ minutes $ppm^{3.5}$ and the b_2 is 1.15. The 95 % confidence interval is also shown. The weighted results were not used in later analysis.



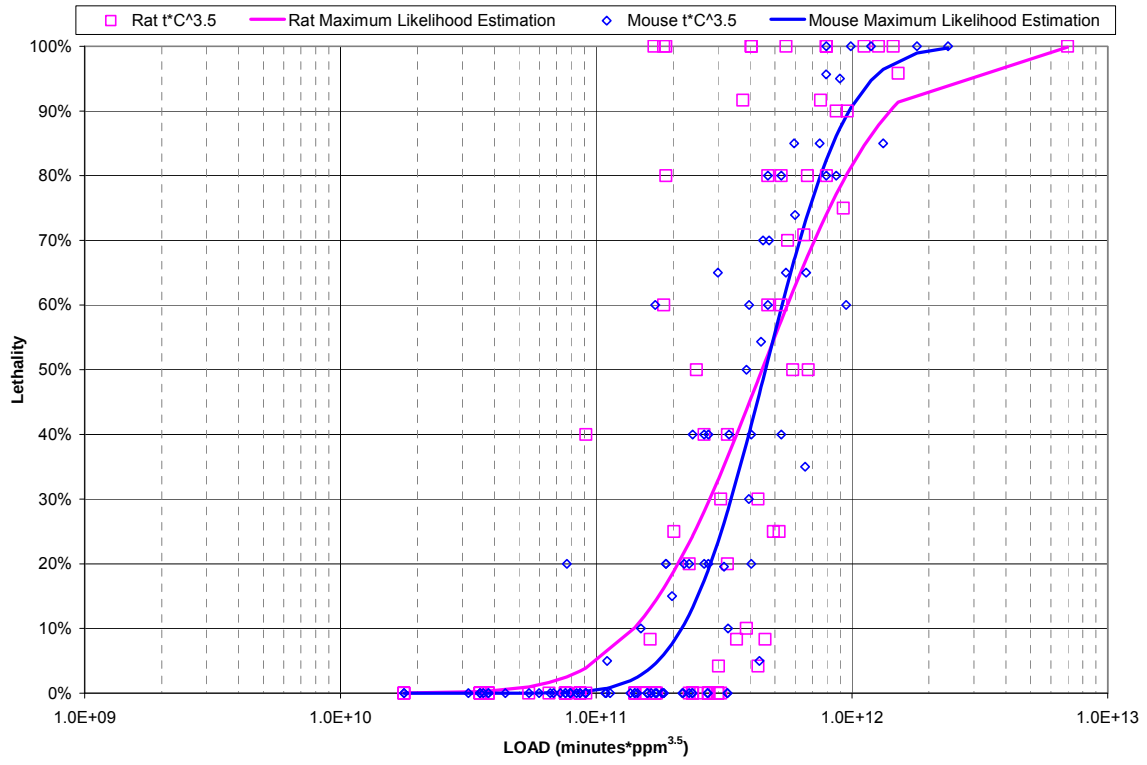
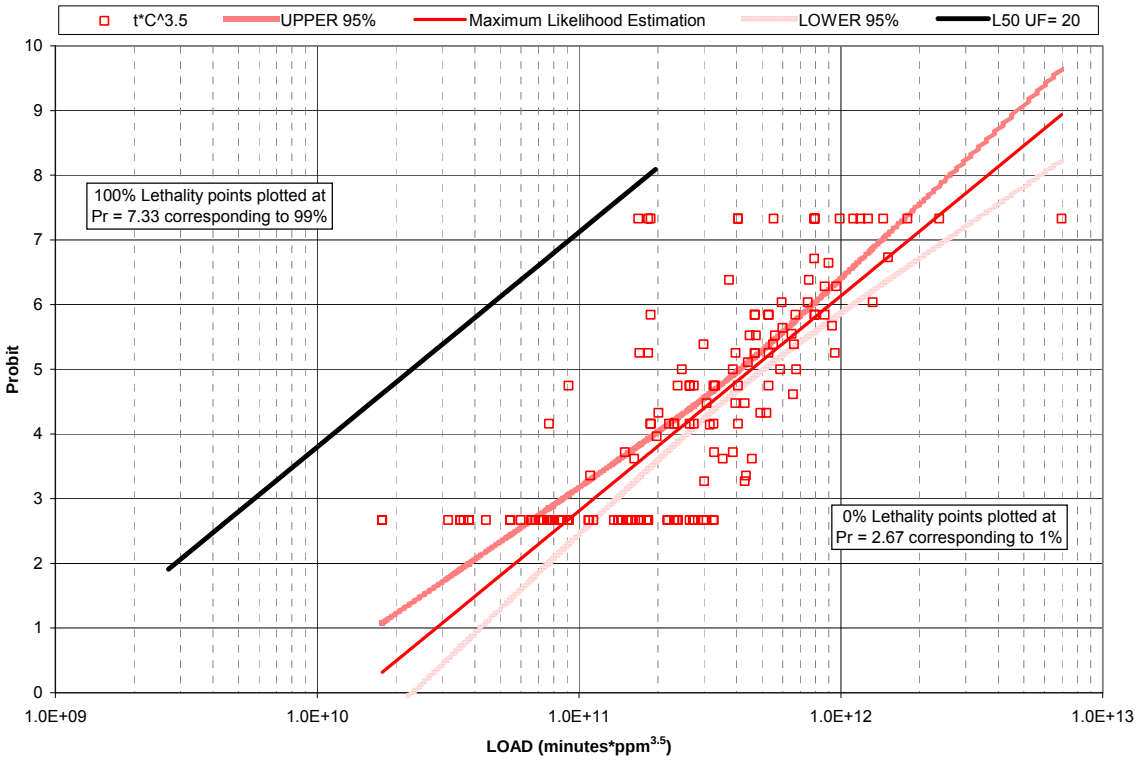


Figure 4 Separate Mice and Rats Probit Analysis for Load with *n* of 3.5



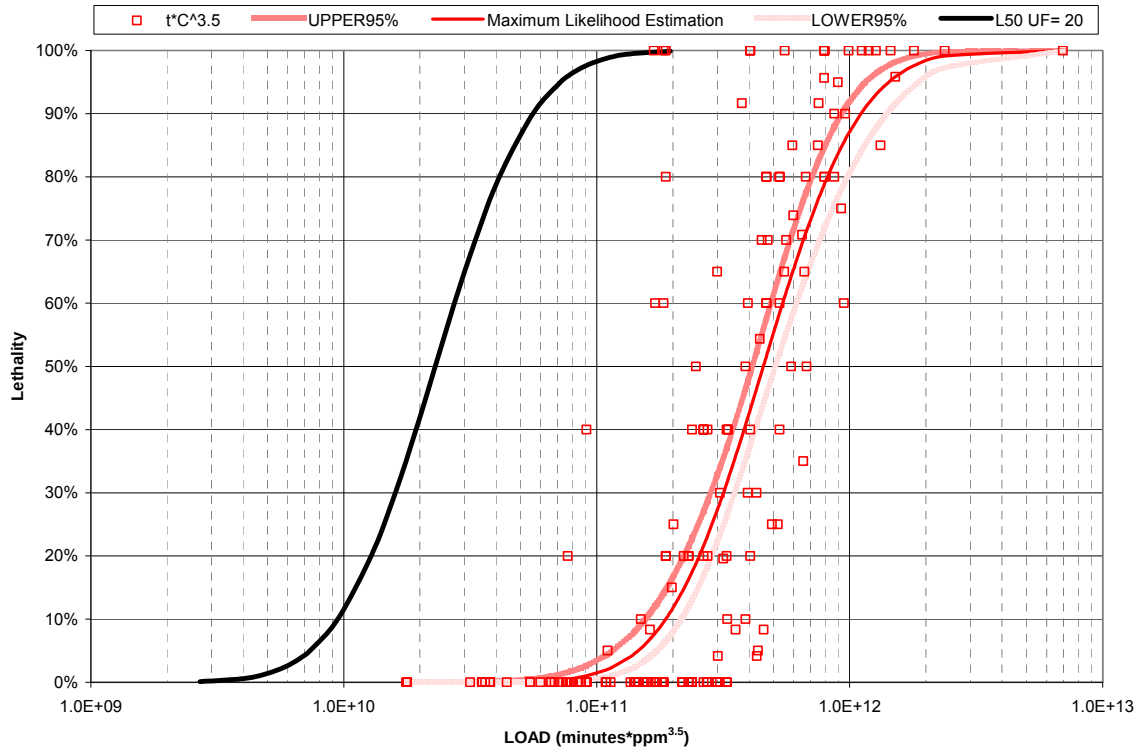


Figure 5 Combined Mice and Rats Probit Analysis for Load with *n* of 3.5

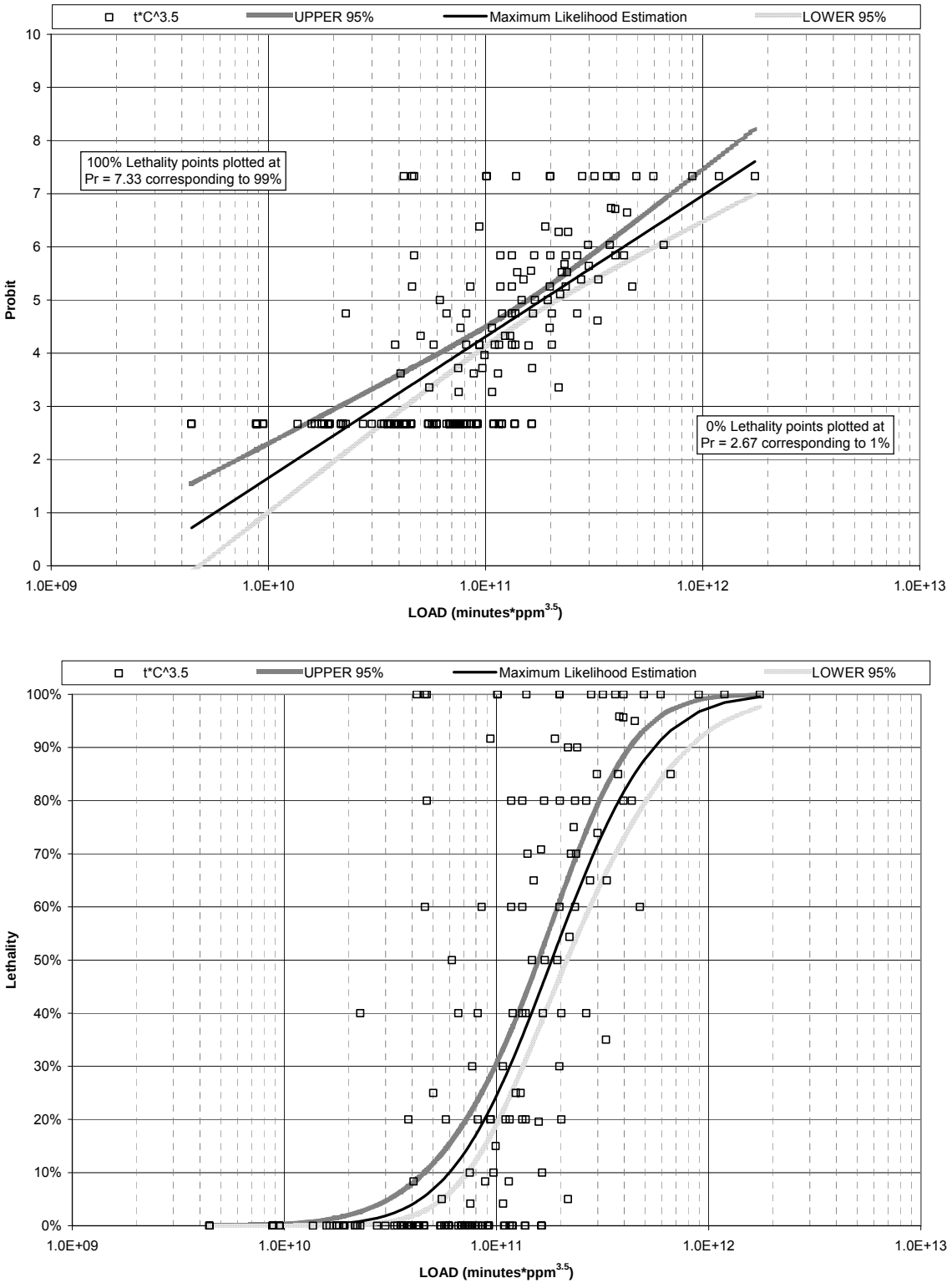


Figure 6 Weighted Mice and Rats Probit Analysis for Human Load with *n* of 3.5

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_2S for 1 minute and none died. The concentration was increased to 1100 ppm H_2S 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_2S 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 $5.54\ 10^{10}$, $2.01\ 10^{11}$ and $3.26\ 10^{11}$, minutes*ppm$^{3.5}$ respectively for the 22 studies. The L50 is $4.56\ 10^{11}$ minutes*ppm$^{3.5}$ 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 $L1=9.07\ 10^{10}$ minutes*ppm$^{3.5}$ (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$^{3.5}$ 4.662 10^{11}
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$^{3.5}$ 1.820 10^{11}
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^{11} minutes*ppm$^{3.5}$. This compares well to the L50 for all of the data of 4.56 10^{11} minutes*ppm$^{3.5}$. The 50th percentile righting reflex load RR50 is 1.82 10^{11} minutes*ppm$^{3.5}$. 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^{10} 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^{10} (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**.

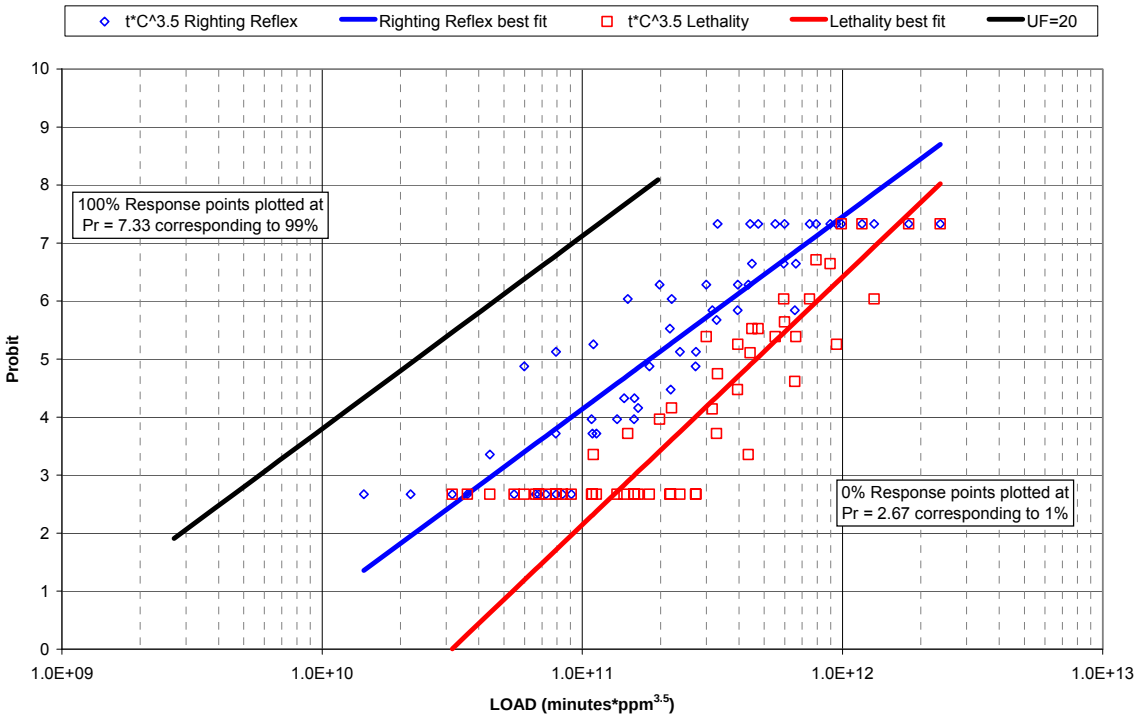
Table 8 provides the concentration-time-response data for unconsciousness in order of increasing load. A few entries may appear to be in error since for an exposure time the % response generally increases with the concentration. But there are a few exceptions, for example: at 7.5 minutes from 1100 to 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[1] (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[1] (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)



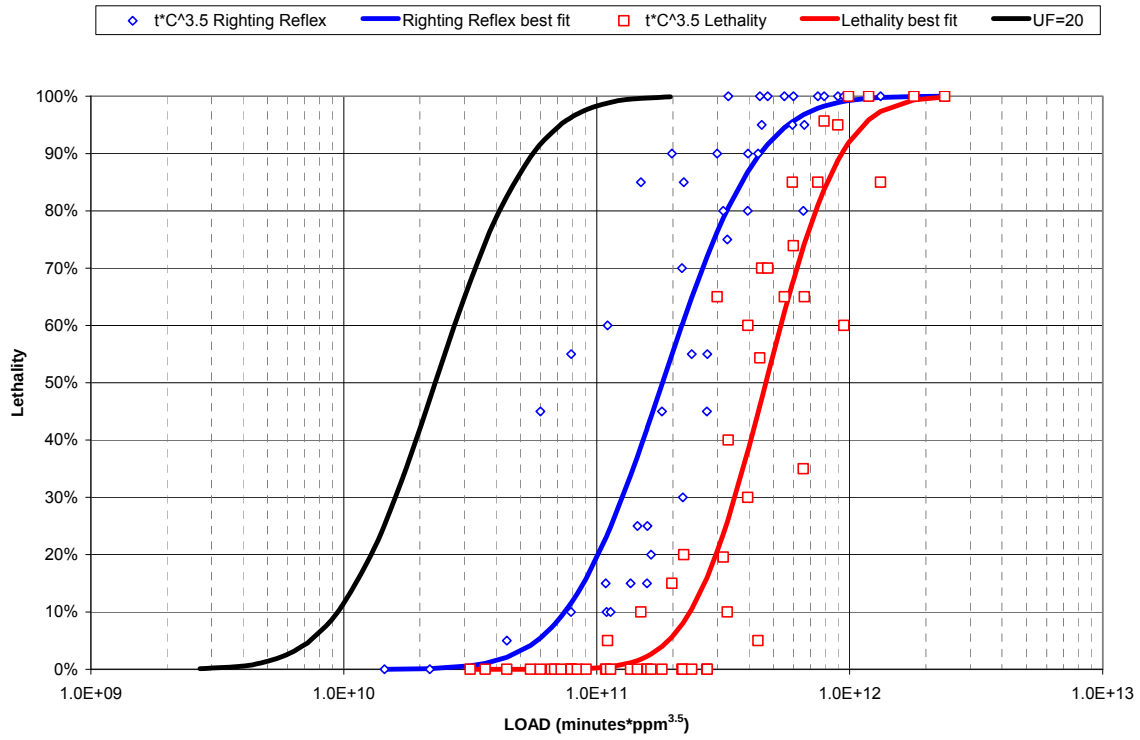


Figure 7 Unconsciousness and Lethality Data Probit Analysis for Load with n of 3.5

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_2S 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_2S is a systemically acting substance that is absorbed by the lungs and transported by the blood. The breathed-in dose is:

$$
\begin{aligned}
&D = \frac{V \cdot C \cdot t}{k_1}, \text{with} \\
&D = \text{breathed-in dose (mg)} \\
&V = \text{breathing minute volume (Litres/min)} \\
&C = \text{concentration (ppm)} \\
&t = \text{exposure duration (minutes)} \\
&k_1 = \text{unit constant}
\end{aligned}
\tag{7.1}
$$

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

$$
\begin{aligned}
&L = t \cdot C^n, \text{or} \\
&E = C \cdot t^{(1/n)}, \text{with} \\
&L = E^n \\
&L = \text{load } (\text{minutes} \cdot \text{ppm}^n) \\
&E = \text{exposure } (\text{ppm} \cdot \text{minutes}^{1/n}) \\
&n = \text{exponent}
\end{aligned}
\tag{7.2}
$$

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 *Cendpoint* for *tendpoint*. 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 *Cendpoint* for 0.5**tendpoint* 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 *tendpoint*, 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 *Cendpoint* for 0.5**tendpoint* 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 *tendpoint* 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 *tendpoint*, 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 = breathed-in load (mg) (7.3)

D_E = breathed-in exposure (mg)

k_L and k_E = unit constants dependent on k_1 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 *Cendpoint* at *tendpoint* is the same so the load can be used.

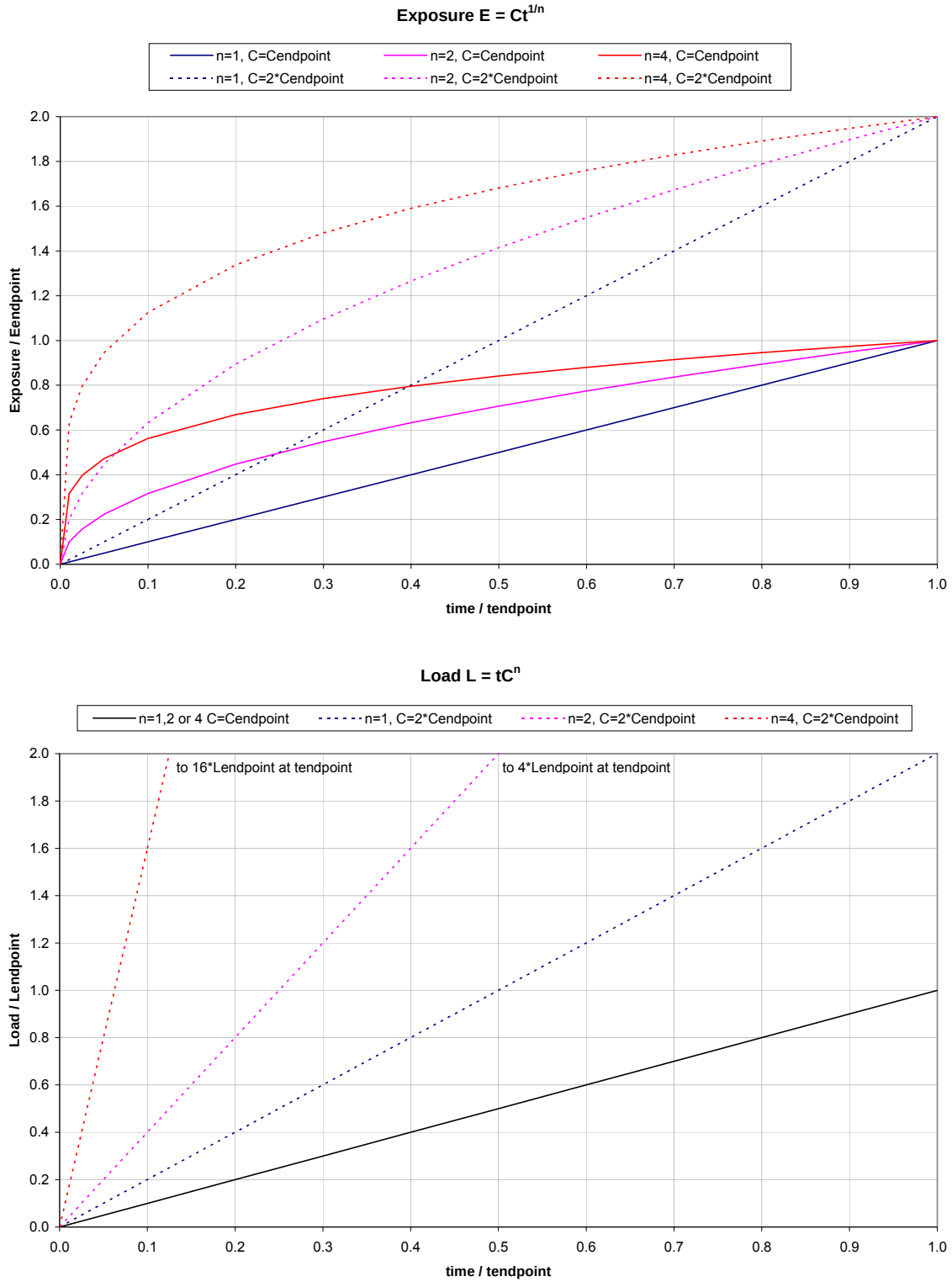


Figure 8 Exposure and Load Endpoint variation with Time

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.

$$
\begin{aligned}
&D_L^{'} = \frac{D_L}{X}, \text{or } D_E^{'} = \frac{D_E}{X}, \text{with} \\
&D_L^{'} = \text{adsorbed breathed-in load per unit X (mg/unit X)} \\
&D_E^{'} = \text{adsorbed breathed-in exposure per unit X (mg/unit X)} \\
&X = \text{parameter defining pathway with appropriate units}
\end{aligned}
\tag{7.4}
$$

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.

$$
\begin{aligned}
&\frac{D_{L1}^{'}}{D_{L2}^{'}} = DAF \ \frac{C_1^{n_1} \cdot t_1}{C_2^{n_2} \cdot t_2}, \text{or} \\
&\frac{D_{E1}^{'}}{D_{E2}^{'}} = DAF \ \frac{C_1 \cdot t_1^{1/n_1}}{C_2 \cdot t_2^{1/n_2}}, \text{where} \\
&DAF = \frac{V_1}{X_1} \Big/ \frac{V_2}{X_2}
\end{aligned}
\tag{7.5}
$$

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 $X_1=X_2$):

$$\frac{D'_{L1}}{D'_{L2}}=\frac{V_1\cdot C_1^n\cdot t_1}{V_2\cdot C_2^n\cdot t_2}\ \text{, or}$$
$$\frac{D'_{E1}}{D'_{E2}}=\frac{V_1\cdot C_1\cdot t_1^{1/n}}{V_2\cdot C_2\cdot t_2^{1/n}} \qquad (7.6)$$

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:

$$\frac{L2}{L1}=\frac{V_1\cdot D'_{L2}}{2\cdot V_1\cdot D'_{L1}}\ \text{, or}$$
$$\frac{E2}{E1}=\frac{V_1\cdot D'_{E2}}{2\cdot V_1\cdot D'_{E1}} \qquad (7.7)$$

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 ½ 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_2S. 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_2S, 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_2S 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

Uncertainty Factor Description	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
Interspecies Variability (accounts for animals being physiologically different than people)	1-10	10	3	10	-	3
Dosimetric Adjustment Factor (ratio of dose in human to dose in animal to achieve same effect)	-	-	0.184 for rat	-	-	-
Intraspecies Variability (accounts for differences in tolerability to exposure within species average to sensitive population)	1-10	10	10	10	3	3
Adequacy of Studies (accounts for the inability of any single study to adequately address all possible adverse outcomes)	1-10	-	-	5	-	-
Nature/Severity of Effects (changes endpoint e.g. L50 to L1, or LOAEL to NOAEL, or chronic to sub-chronic)	1-10	-	10	2	-	-
Uncertainty Factor	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_2S. 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_2S 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)	***Proposed*** Energy Resources Conservation Board (H2S L50)	***Proposed*** 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
Interspecies Variability (accounts for animals being physiologically different than people)	3	-	10	3	3
Dosimetric Adjustment Factor (ratio of load in human to load in animal to achieve same effect)	-	-	5.1 for rat 10.1 for mouse	-	-
Intraspecies Variability (accounts for differences in tolerability to exposure within species e.g. average to sensitive population)	3	-	-	3	3
Inhalation Rate (accounts for increased inhalation during emergency compared to animals at rest)	-		2	2	2
Adequacy of Studies (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	-	-
Nature/Severity of Effects (changes endpoint e.g. L50 to L1, or LOAEL to NOAEL, or chronic to sub-chronic)	-	7.5	-		15
Overall Factor	10 on concentration	7.5 on load	4 rat, 2 mouse, less if both on concentration	**L50** **20** on load	**EPZ** **300** on load

US EPA

In setting the Acute Exposure Guideline Level AEGL-3 for H_2S, 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_2S, the United Kingdom Health and Safety Executive (HSE) did not apply interspecies uncertainty factors to the animal lethality data implying humans respond to H_2S 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_2S 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_2S 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_2S LC50

for 30 minutes, one rat LC50 of 318 mg/m^3 and one mouse LC50 of 669 mg/m^3 were averaged to obtain 493.5 mg/m^3, and then doubled to obtain an LC50 of 987 mg/m^3. 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_2S 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= 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_2S, 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_2S, 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 to 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_2S 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_2S 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:

$$ERCB\,L50 = C^{3.5}t = 2.279 \cdot 10^{10}\ \text{ppm}^{3.5}\text{minutes} = \frac{4.557 \cdot 10^{11}}{20}$$
$$\text{Probit} = -29.415 + 1.443 \cdot \ln\left(C^{3.5}t\right) \tag{8.1}$$

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_2S 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_2S 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

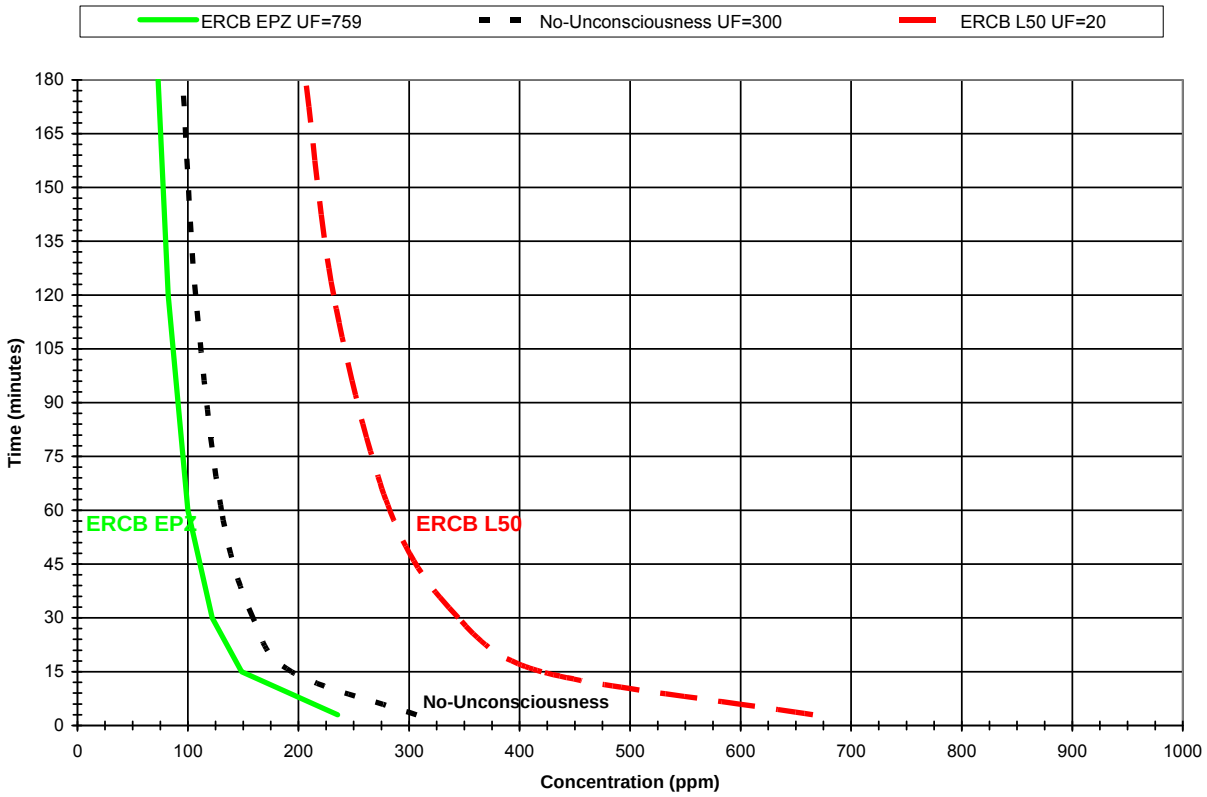


Figure 9 Concentrations and Exposure Times for ERCB Endpoints with $L=tC^{3.5}$

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_2S to the ERCB L50 Endpoint. The following table provides published probit equations for human lethality to H_2S. 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_2S

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[1] (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

[1](parameters for C in mg/m^3, 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.

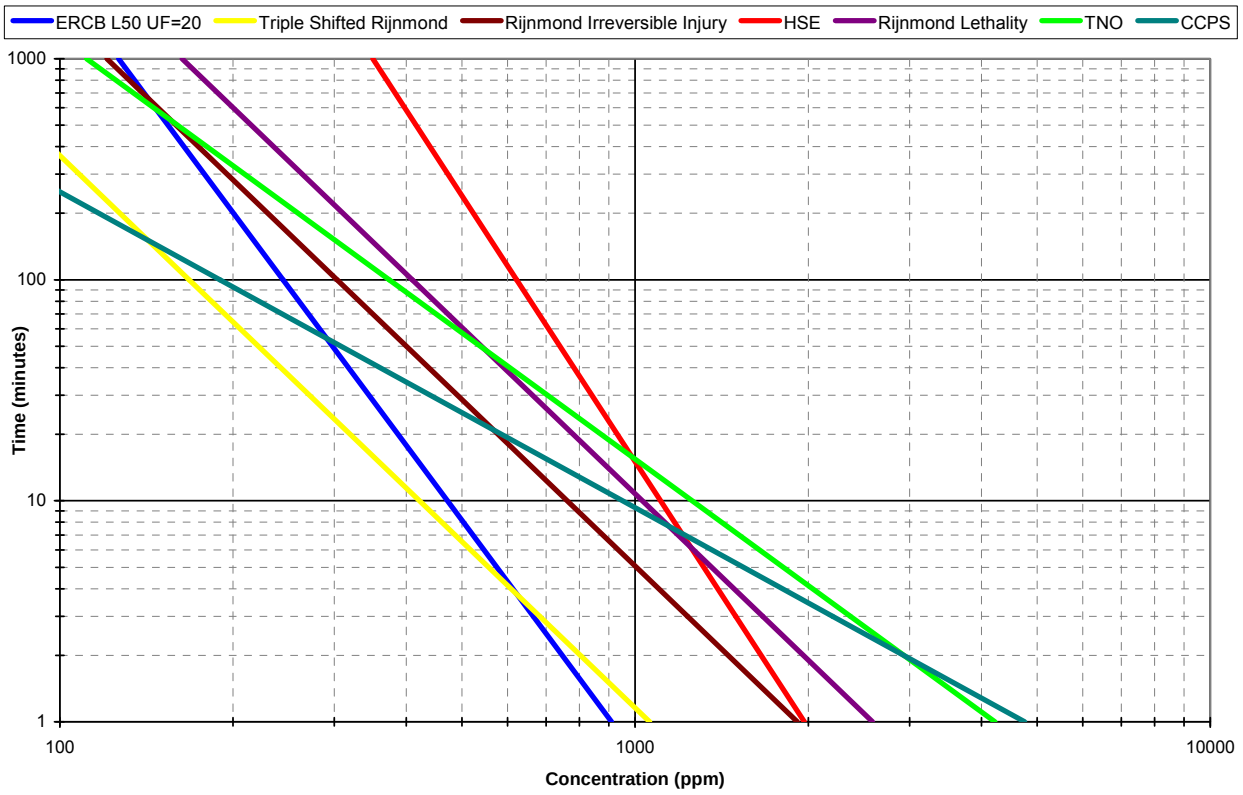


Figure 10 Comparison of Published $L50=t*C^n$ with Proposed ERCB Endpoints

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_2S 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 *b2* 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_2S 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_2S 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_2S to the ERCB Endpoints.

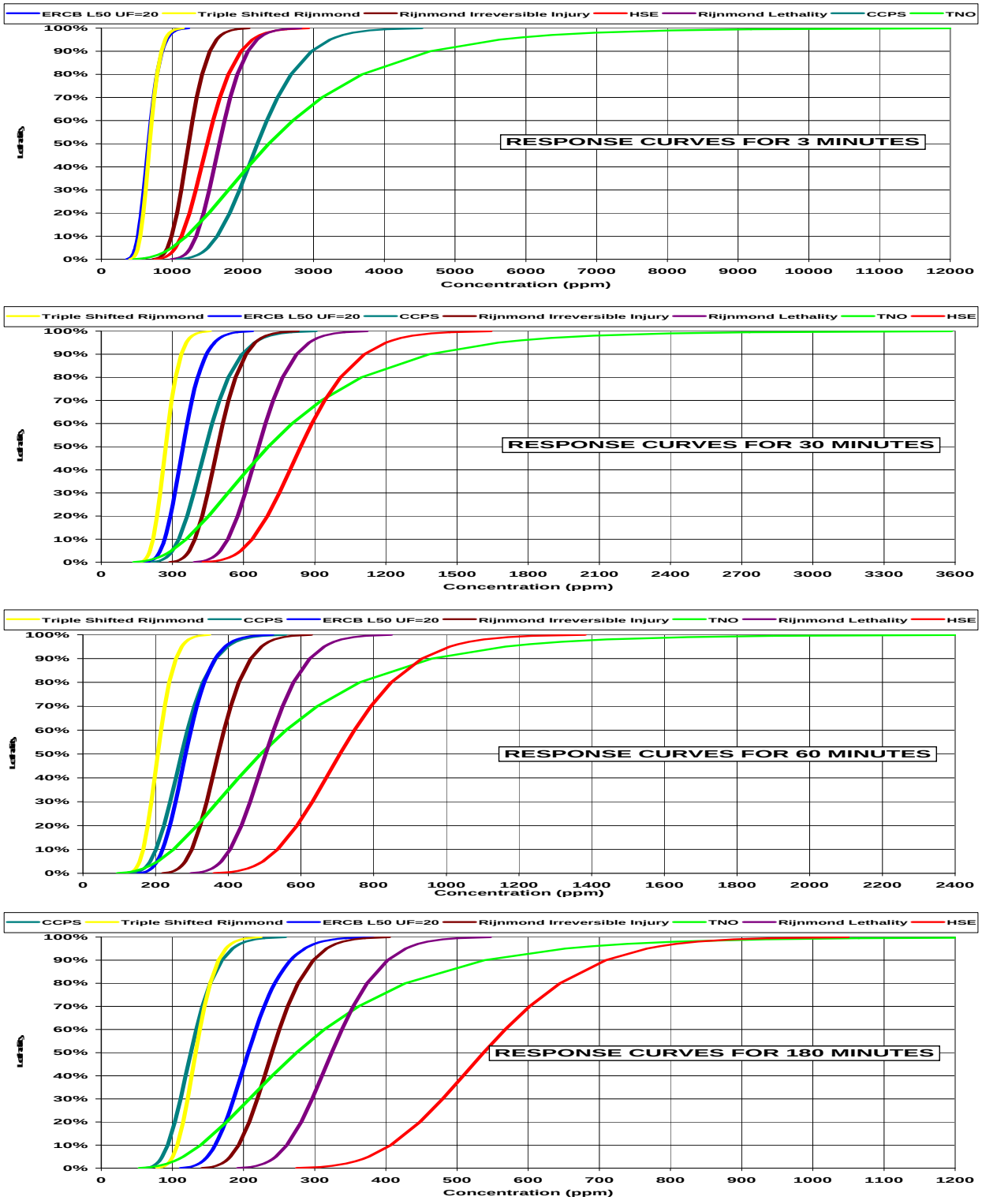


Figure 11 Lethality Response Sensitivity to Concentration and Time for Published Probit Parameters

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_2S 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 $4.56\ 10^{11}$ is one half of the highest no death human load of $9.07\ 10^{11}$ 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.

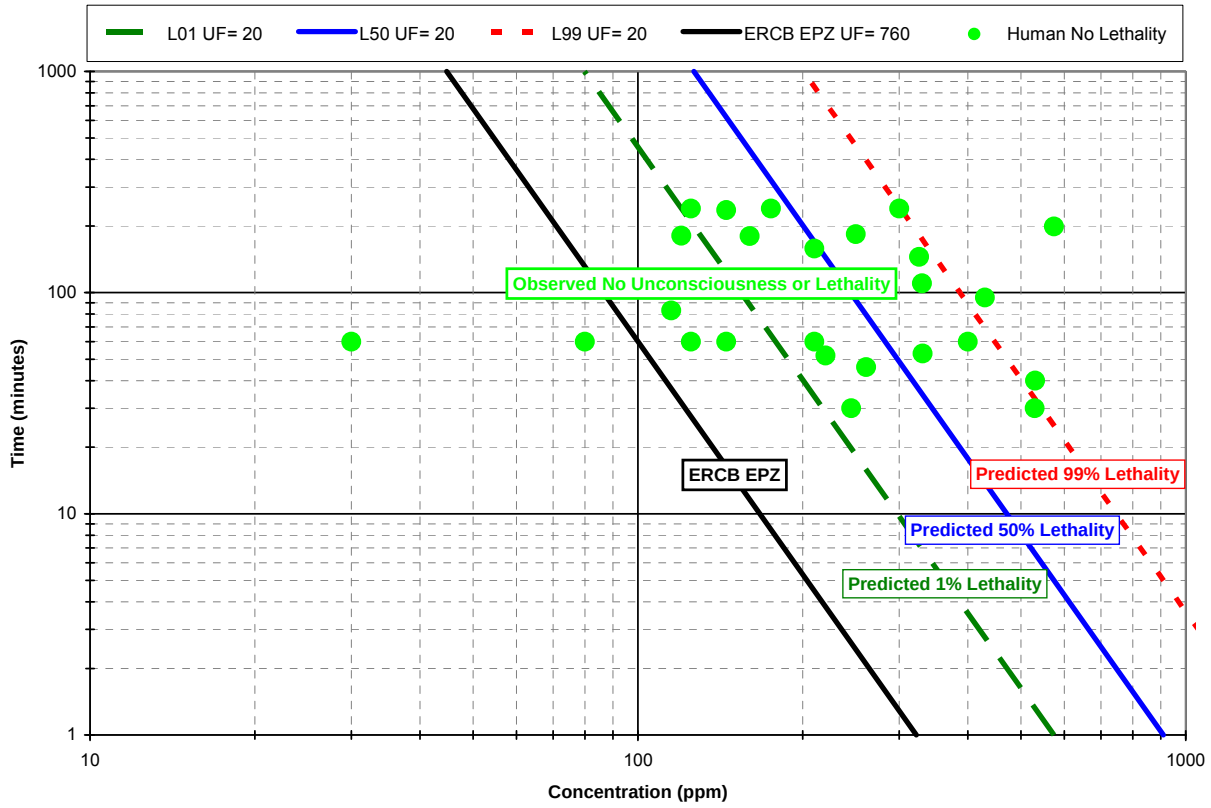


Figure 12 Human Exposures with Low Grading Compared to ERCB Endpoints

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

Stakeholder Engagement Process to Select the Hydrogen Sulphide Emergency Planning Zone Endpoint

This Appendix documents the stakeholder process undertaken by the EUB to select the EPZ endpoint.

The stakeholder engagement process began in December 2003 when the EUB published the draft requirements for calculating EPZs for sour wells, sour pipelines and sour production facilities. At that time the EUB also released the draft EUBMODELS (now renamed to EUBH_2S) software package containing a suite of computer programs (thermodynamics model, dispersion model and spreadsheet). EUBH_2S contains a principle input which is the EPZ endpoint. A draft value was proposed of $6.5x10^{10}$ (minutes*$ppm^{4.36}$). This is equivalent to 100 ppm for 60 minutes.

On May 6, 2004, the EUB hosted a multi-stakeholder workshop to discuss EPZ endpoints. Stakeholders from the EUB, provincial and local government, academia, health authorities and others were represented. The objective of the workshop was to allow opportunity for input into the EUB process. The goal was not to achieve consensus and it was stressed that the EUB would ultimately select a toxic load endpoint for the purpose of setting EPZs. Participants generally agreed that it was important to derive the criterion (i.e. the words) as to the purpose of the endpoint. There was also support of a multi-level approach for protecting public health and safety. Opinion on the actual value of the EPZ endpoint differed substantially.

On July 19, 2004, a focus group workshop comprised of EUB, industry, RHA and academic representatives was held. The objective was to discuss a draft EUB position report that presented the proposed endpoint which included suggestions from the workshop in May 2004. The group proposed the EPZ endpoint criterion for H_2S *as "the airborne exposure concentration of H_2S and exposure time that provides a conservative margin of safety to protect people from serious irreversible health effects including fatalities."* Agreement was not reached on selecting an actual endpoint value. The EUB at the workshop stated that a value would be picked and support obtained from Alberta Health and Wellness whose role it is to advise the EUB on health related matters. Following the focus group, the EUB met with Alberta Health and Wellness and presented a revised draft report that included input from the focus group and other stakeholders. Alberta Health and Wellness were supportive of the methodology and the value chosen.

Subsequent to the focus group and meeting with Alberta Health and Wellness:

- A presentation was made to the Provincial Advisory Committee on Public Safety and Sour Gas. Some stakeholders were still concerned with the method and EPZ endpoints values proposed.
- The Environmental and Non-Government Organization stakeholders (and members of the public) were concerned about the process undertaken to derive the endpoint.

To address these concerns, the EUB hosted a multi-stakeholder meeting for November 26 2004 to provide further opportunity for input into the process. In preparation of the meeting, EUB staff published a discussion report with revised EPZ endpoint values. The meeting was a formal process chaired by EUB Board Member Mr. Jim Dilay P.Eng and the proceedings recorded by a court reporter. The transcript from the meeting can be read free of charge at www.tscript.com. The EUB committed to summarize the views of the stakeholders whom participated to ensure that EUB staff had heard the views correctly, and then report to the Board for a decision on an appropriate EPZ endpoint and/or the next steps in the process.

Since the November 2004 meeting EUB staff continued to review literature associated with high concentration short term exposure to H2S. A supporting report by Cantox Environmental Ltd was commissioned to review the quality of the studies selected for determining the EPZ

endpoint. Meetings with Alberta Health and Wellness and Calgary Health Region have continued to determine the best path forward for implementation of EUBH2S until resolution on the EPZ endpoint is achieved. A scientific expert panel is planned under the leadership of Alberta Health and Wellness is planned.

APPENDIX 2

Overview of Hydrogen Sulphide Lethality Data and Exposure Criteria

Prepared for:
Public Safety Implementation Team
Alberta Energy Utilities Board

Prepared by:
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Public Safety and Air Quality Management

December 2004

Table of Contents

1. INTRODUCTION

The Alberta Energy and Utilities Board (EUB) has developed new requirements for calculating emergency planning zones (EPZ) for sour wells, sour pipelines and sour production facilities for the Albertan upstream petroleum industry (industry). The purpose of an EPZ is described in EUB Guide 71[1]. An EPZ is a priority area that ensures "*a quick, effective response to emergencies in order to protect the public from fatalities and irreversible health effects*".

This appendix provides the rationale, criterion and the numerical values of the hydrogen sulphide (H_2S) exposure endpoint used by other jurisdictions based on a review of literature. The goal of the paper is not to recommend an appropriate endpoint to protect the public, rather to improve the EUB's understanding of the endpoint setting process and the toxicology data used. The paper focuses on what is known on H_2S toxicity from the current scientific research as referenced by other jurisdictions. Answers are provided for the following questions:

1) What exposure criteria are available for H_2S?

2) What words are used to define the objective?

3) What process was used to set the endpoint?

4) What H_2S toxicity data were used in determining the endpoint?

5) What uncertainty factors were applied to the data?

6) Is an average concentration for a given time or a toxic load approach used?

7) What are the numerical values of exposure endpoints?

8) A comparison of the available criteria and exposure endpoints.

9) What exponent 'n' is appropriate for the toxic load equation over the range of concentrations and times used to derive the exposure endpoint

From this information and through a stakeholder process appropriate exposure endpoints will be selected by the EUB.

[1] Guide 71: Emergency Response and Preparedness Requirements for the Upstream Petroleum Industry.

2. H_2S LETHALITY DATA

Table 1 presents the H_2S lethality data gathered for this study. Only lethality data was reviewed as the endpoint criterion are serious irreversible health effects including fatalities. Serious irreversible health effects are difficult endpoint to classify but lethality is not. The table contains:

- the species (canary, cat, dog, dove, goat, guinea pig, human, monkey, mouse, pig, rabbit, rat),
- number of animals exposed,
- the percent fatalities,
- whether the value is calculated from several different exposures or is one observation,
- the exposure concentration and time,
- the original reference the data appeared in, and
- the exposure guidelines developed by regulatory agencies that reference the data:
    - **AEGL** - Acute Exposure Guideline Levels (AEGL) are currently under development by the National Research Council's Committee on Toxicology.
    - **ERPG** - Emergency Response Planning Guidelines (ERPG) developed by the American Industrial Hygiene Association.
    - **HSE** - Health and Safety Executive (UK).
    - **IDLH** - Immediately Dangerous to Life or Health concentrations developed by Institute for National Institute for Occupational Safety and Health (**NIOSH**).

This collection of data was not based on an exhaustive search. Most of the data was obtained from the thesis *Biological Variability in Risk Assessment Modelling of Industrial Gases* (Guo, B., 2001). The important data was added from the references used to support the regulatory criteria as indicated in the table. Each criterion will be discussed in subsequent sections.

The ERCB Technical Paper *Toxicological Justification of the Triple Shifted Rijnmond Equation* (Rogers, 1990) is provided as Appendix B. Much of the data referenced by Rogers is the same as in this previous section. No attempt was made to add data from Rogers to this database as in many cases the chance of lethality was not provided or there were disagreements in the data from the same source.

Figure 1 is a plot of all the data in the table with the lethality identified. The 50th percentile lethal load (L50) is highlighted. Longer exposure times and higher concentrations should be associated with higher chance of lethality. Notice that many of the lethality data points are inconsistent with each other; for example there are many 100% lethality points below and to the left of the $50^{\%}$ lethality line. This is due to different species and methods used. Some of the points are based on experiments done over a hundred years ago. This figure is similar to the scatter plot presented in the next section (Rogers 1992) in that all species and chance of lethality are presented together.

Due to the natural variability in a population the calculated median exposure value from many experiments is used to define the load that is lethal to 50% of the population. A response curve can be defined from the statistical analysis of the data.

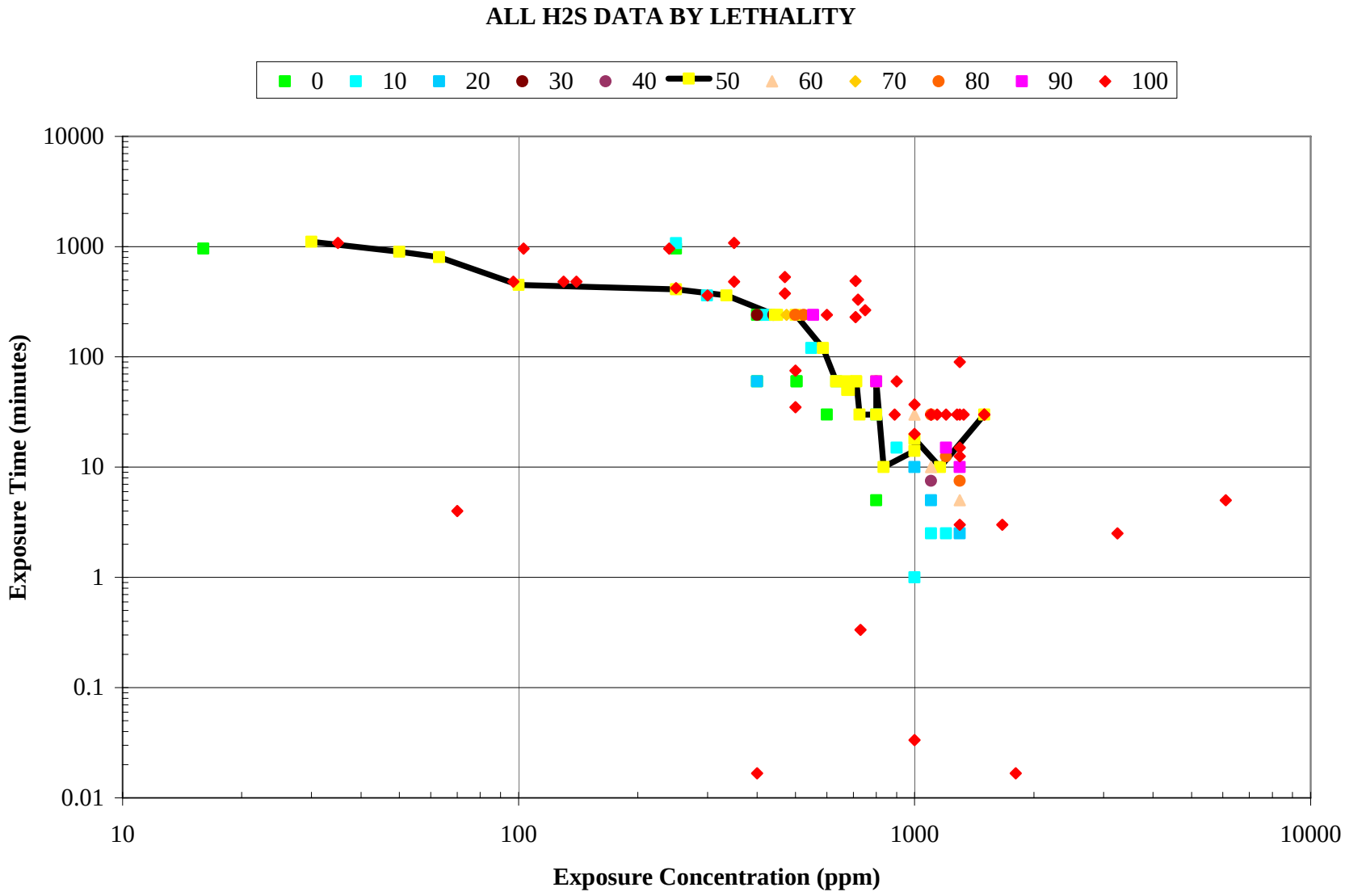


Figure 1 All H_2S Data by Lethality with L50 highlighted

Table 1 H_2S Lethality Data											
Record	Species	#Exposed	% Fatality	CALC	H_2S (ppm)	Time (min)	Original Reference	AEGL	IDLH	HSE	ERPG
1	Mouse	4	0		16	960	Weedon et al 1940			HSE	
2	Rat	8	0		250	960	Weedon et al 1940			HSE	
3	Rat	10	0		400	60	MacEwen & Vernot 1972	AEGL			
4	Rat	4	0		400	240	Lopez et al 1987			HSE	
5	Rat	10	0		504	60	MacEwen & Vernot 1972	AEGL			
6	Mouse	10	0		504	60	MacEwen & Vernot 1972	AEGL			
7	Human	1	0		600	30	Lefaux 1968		IDLH		
8	Human	1	0		800	5	Tab Biol Per 1933		IDLH		
9	Mouse	20	5		800	30	Clanechan 1979				
10	Mouse	20	5		1100	2.5	Clanechan 1979				
11	Rat	?	10	LC10	299	360	Prior et al 1988			HSE	
12	Rat	?	10	LC10	422	240	Prior et al 1988			HSE	
13	Rat	?	10	LC10	549	120	Prior et al 1988			HSE	
14	Rat	10	10		635	60	MacEwen & Vernot 1972				
15	Mouse	20	10		900	15	Clanechan 1979				
16	Human	10	10		1000	1	Prouza 1970				
17	Mouse	20	10		1200	2.5	Clanechan 1979				
18	Rat	8	12.5		250	1074	Weedon et al 1940				
19	Mouse	20	15		1300	2.5	Clanechan 1979				
20	Mouse	10	20		400	60	MacEwen & Vernot 1972				
21	Mouse	46	20		1000	10	Clanechan 1979				
22	Mouse	20	20		1100	5	Clanechan 1979				
23	Rat	10	30		400	240	Tansy et al 1981			HSE	ERPG
24	Rat	10	30		440	240	Tansy et al 1981			HSE	ERPG

Table 1 H_2S Lethality Data											
Record	Species	#Exposed	% Fatality	CALC	H_2S (ppm)	Time (min)	Original Reference	AEGL	IDLH	HSE	ERPG
25	Mouse	20	40		1100	7.5	Clanechan 1979				
26	Mouse	?	50	LT50	30	1110	Hays 1972			HSE	
27	Mouse	?	50	LT50	50	900	Hays 1972			HSE	
28	Mouse	4	50		63	804	Weedon et al 1940			HSE	
29	Mouse	?	50	LT50	100	450	Hays 1972			HSE	
30	Mouse	4	50		250	410	Weedon et al 1940			HSE	
31	Rat	?	50	LC50	335	360	Prior et al 1988	AEGL		HSE	
32	Rat	70	50	LC50	444	240	Tansy et al 1981	AEGL	IDLH	HSE	ERPG
33	Rat	2	50		450	240	Mitchell & Yant 1925			HSE	
34	Rat	?	50	LC50	501	240	Prior et al 1988	AEGL		HSE	
35	Rat	?	50	LC50	587	120	Prior et al 1988	AEGL		HSE	
36	Mouse	40	50	LC50	634	60	MacEwen & Vernot 1972	AEGL	IDLH	HSE	ERPG
37	Mouse	10	50		635	60	MacEwen & Vernot 1972				
38	Mouse	?	50	LC50	673	60	Back et al 1972		IDLH		
39	Mouse	?	50	LC50	676	50	Zwart et al 1990	AEGL			
40	Rat	?	50	LC50	683	50	Zwart et al 1990	AEGL			
41	Rat	40	50	LC50	712	60	MacEwen & Vernot 1972	AEGL		HSE	ERPG
42	Rat	?	50	LC50	713	60	Back et al 1972		IDLH		
43	Rat	?	50	LC50	726	30	Zwart et al 1990	AEGL			
44	Mouse	?	50	LC50	800	30	Zwart et al 1990	AEGL			
45	Dog	2	50		800	60	Mitchell & Yant 1925				
46	Rat	?	50	LC50	835	10	Zwart et al 1990	AEGL			
47	Rat	8	50		1000	14	Weedon et al 1940			HSE	
48	Mouse	4	50		1000	18	Weedon et al 1940			HSE	

Table 1 H_2S Lethality Data											
Record	Species	#Exposed	% Fatality	CALC	H_2S (ppm)	Time (min)	Original Reference	AEGL	IDLH	HSE	ERPG
49	Mouse	?	50	LC50	1160	10	Zwart et al 1990	AEGL			
50	Guinea Pig	2	50		1500	30	Mitchell & Yant 1925			HSE	
51	Mouse	46	54		1100	10	Clanechan 1979				
52	Mouse	20	60		1000	30	Clanechan 1979				
53	Mouse	20	60		1300	5	Clanechan 1979				
54	Rat	10	70		475	240	Tansy et al 1981			HSE	ERPG
55	Rat	10	80		500	240	Tansy et al 1981			HSE	ERPG
56	Rat	10	80		525	240	Tansy et al 1981			HSE	ERPG
57	Mouse	10	80		800	60	MacEwen & Vernot 1972				
58	Mouse	20	85		1100	30	Clanechan 1979				
59	Mouse	20	85		1200	12.5	Clanechan 1979				
60	Mouse	20	85		1300	7.5	Clanechan 1979				
61	Rat	10	90		554	240	Tansy et al 1981			HSE	ERPG
62	Rat	10	90		800	60	MacEwen & Vernot 1972				
63	Mouse	20	95		1200	15	Clanechan 1979				
64	Mouse	46	95		1300	10	Clanechan 1979				
65	Canary	2	100		35	1080	Mitchell & Yant 1925				
66	Dove	1	100		70	4	Eulenberg 1865				
67	Canary	6	100		97	480	Mitchell & Yant 1925				
68	Dog	2	100		103	960	Mitchell & Yant 1925			HSE	
69	Rabbit	1	100		130	480	Lehman 1892			HSE	
70	Canary	4	100		140	480	Mitchell & Yant 1925				
71	Dog	2	100		240	960	Mitchell & Yant 1925			HSE	
72	Mouse	4	100		250	420	Weedon et al 1940				

Table 1 H_2S Lethality Data											
Record	Species	#Exposed	% Fatality	CALC	H_2S (ppm)	Time (min)	Original Reference	AEGL	IDLH	HSE	ERPG
73	Rat	12	100		300	360	Alberta Environmental Centre 1986				
74	Dog	2	100		350	480	Mitchell & Yant 1925			HSE	
75	Guinea Pig	3	100		350	1080	Mitchell & Yant 1925			HSE	
76	Pig	1	100		400	0.02	O'Donoghue 1961				
77	Rabbit	1	100		470	375	Lehman 1892				
78	Guinea Pig	1	100		470	530	Lehman 1892			HSE	
79	Monkey	1	100		500	35	Lund & Wieland 1966			HSE	ERPG
80	Rabbit	1	100		500	75	Biefel & Polek 1880				
81	Rat	10	100		600	240	Tansy et al 1981			HSE	ERPG
82	Rabbit	1	100		710	230	Lehman 1892				
83	Cat	1	100		710	489	Lehman 1892				
84	Cat	1	100		720	330	Lehman 1892				
85	Canary	?	100		730	0.33	Mitchell & Yant 1925				
86	Rabbit	1	100		750	265	Lehman 1892				
87	Dog	3	100		890	30	Mitchell & Yant 1925			HSE	
88	Dog	1	100		900	60	Haggard 1925			HSE	
89	Human	1	100		1000	0.03	NIOSH 1977				
90	Mouse	4	100		1000	20	Weedon et al 1940				
91	Rat	8	100		1000	37	Weedon et al 1940				
92	Cat	1	100		1100	30	Eulenberg 1865				
93	Goat	4	100		1100	30	Mitchell & Yant 1925			HSE	
94	Dog	8	100		1140	30	Mitchell & Yant 1925			HSE	
95	Mouse	20	100		1200	30	Clanechan 1979				
96	Dog	4	100		1280	30	Mitchell & Yant 1925			HSE	

Table 1 H_2S Lethality Data											
Record	Species	#Exposed	% Fatality	CALC	H_2S (ppm)	Time (min)	Original Reference	AEGL	IDLH	HSE	ERPG
97	Rabbit	1	100		1300	3	Lehman 1892			HSE	
98	Mouse	20	100		1300	12.5	Clanechan 1979				
99	Mouse	20	100		1300	15	Clanechan 1979				
100	Mouse	20	100		1300	30	Clanechan 1979				
101	Guinea Pig	1	100		1300	90	Lehman 1892			HSE	
102	Goat	4	100		1330	30	Mitchell & Yant 1925			HSE	
103	Dog	1	100		1500	30	Haggard 1925			HSE	
104	Dog	9	100		1500	30	Mitchell & Yant 1925			HSE	
105	Rat	5	100		1665	3	Lopez et al 1989			HSE	
106	Dog	1	100		1800	0.02	Haggard 1925			HSE	
107	Rabbit	1	100		3250	2.5	Lehman 1892				
108	Human	1	100		6100	5	Winek et al 1968				

3. H_2S L50 DATA

Statistical methods have an important role in the design and interpretation 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. It can be shown that in experiments with small numbers of animals the confidence limits for 50% mortalities are wide and that those for other percentage mortalities are even wider. For 50% mortalities, 2 to 8 deaths in a group of 10 is the range for 95% confidence levels. For 10% mortalities, 0 to 3 deaths in a group of 10 is the range for 95% confidence levels. For 90% mortalities, 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^{th} Percentile Lethal Load (L10) or 90^{th} Percentile Lethal Load (L90) than a 50^{th} Percentile Lethal Load (L50). Alternatively, for a given number of animals the confidence in the L10 and L90 values is less than that in the L50.

L50 data has been used by other jurisdictions in setting exposure guidelines and is presented in Table 2. In the EPZ requirements, an upper bound exposure duration of 3 hours has been defined based on the persistence of the meteorological conditions. In other words, if a receptor (i.e. a person being exposed) was stationary and downwind of a sour gas plume, the maximum exposure time would be 3 hours because the dispersion conditions (stability class, wind speed and wind direction) are likely to change after that time to more favourable conditions for dispersing the sour gas plume. In addition, emergency response actions would have occurred by then. Therefore exposure times greater than 3 hours were excluded from the dataset. These are indicated as shaded data in Table 2.

Toxicologists use the term LC50 for the 50^{th} percentile Lethal Concentration for an exposure time, however the time is often ignored. In this study the abbreviation L for Load is used as it requires a pair of concentration and time data that defines the load for a given adverse effect. A straight line on a log concentration versus log time plot is represents the toxic load equation of:

$$\text{Toxic Load} = \text{Time} * \text{Concentration}^n$$

This is Haber's rule for toxic load which results in higher concentrations requiring less time to produce the same load and effect for an exponent n greater than 1. If exponent n=1 the equation is the linear dose relation.

Figure 2 is a plot of the L50 data from Table 2. The pink best-fit line for all of the L50 data has a lower slope (n=1.2) than the blue best-fit line for times less than 3 hours (n=3.8). Also the goodness of fit improves (r^2 increases from 0.75 to 0.88) with the smaller data set. Inspection of the data shows that the exponent changes with increased exposure time and decreased exposure concentrations. At lower levels, the human body processes H_2S, requiring longer exposure times for lethality. For comparison an exponential curve fit with a changing slope is shown as the black line. It has a better goodness of fit (r^2=0.92) but the theory has not been developed to support its use.

Figure 3 is the L50 concentration and time pairs for times under 6 hours (360 minutes). The data is presented two ways; the top plot shows time as the dependent variable and concentration as the

independent variable (x=concentration, y=time). The bottom plot is the opposite, with concentration as the dependent variable and time as the independent variable (x=time, y=concentration). The equations for the best fit lines are also provided. Notice the exponents for the equations 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 would be the same. This can create some confusion in determining the exponent.

To determine the 50^{th} percentile concentration LC50, exposure time is held constant (time is the independent variable) and the concentration is varied (concentration is the dependent variable) and the number of fatalities is recorded. All of the data in Figure 3 is LC50 data. In the bottom plot, the error is assumed to be in the concentration not the time measurements and it should be used. To determine the 50^{th} percentile time LT50, exposure concentration is held constant (concentration is the independent variable) and the time is varied (time is the dependent variable) and the number of fatalities is recorded.

Curve fits are also provided in Figure 3 for the data with exposure times less than 120 minutes. The exponent n increases for the shorter exposure times with higher concentrations. The goodness of fit also decreases for the smaller data set.

The method of Lichtfeld and Wilcoxson (1949) was applied to several data sets to determine the LC50 and confidence limits for comparison to published values.

Comparison of Published and Calculated LC50			
Species Reference	Exposure Time (minutes)	Published LC50 (95% confidence limits)	Calculated LC50 (95% confidence limits)
Mouse MacEwen & Vernot 1972	60	634 (576 – 698)	588 (474 – 730)
Rat MacEwen & Vernot 1972	60	712 (662 – 765)	713 (674 – 754)
Rat Tansy et al 1981	240	444 (416 – 473)	448 (420 – 478)

The published values could not be reproduced from the available data. This does not mean the published values are in error but demonstrates the variability in the statistical methods used. Note the 95% confidence limits range from +/- 5 to 20% of the LC50.

Each of these data sets used either an LC0 or LC100. In the MacEwen & Vernot 1972 mouse studies, the expected fatalities at 504 ppm is 36% compared to the 0% observed. In the MacEwen & Vernot 1972 rat studies, the expected fatalities at 504 ppm is 0.006% and at 400 ppm it is 0.00000001% which are the same as the 0% observed for both points. In the Tansy et al 1981 rat studies, the expected fatalities at 600 ppm is 96% compared to the 100% observed. The zero and 100% effect results can be used effectively in the data interpretation but care should be used in applying them directly.

Table 2		**H_2S L50 Data used in determining EUB Exposure Endpoints**								
Record	Species	Value	Concentration (ppm)	Time (min)	Original Reference	Referenced by:				
26	Mouse	LT50	30	1110	Hays 1972			HSE		NIOSH
27	Mouse	LT50	50	900	Hays 1972			HSE		NIOSH
29	Mouse	LT50	100	450	Hays 1972			HSE		NIOSH
31	Rat	LC50	335	360	Prior et al 1988	AEGL		HSE		
32	Rat	LC50	444	240	Tansy et al 1981	AEGL	IDLH	HSE	ERPG	
34	Rat	LC50	501	240	Prior et al 1988	AEGL		HSE		
35	Rat	LC50	587	120	Prior et al 1988	AEGL		HSE		
36	Mouse	LC50	634	60	MacEwen & Vernot 1972	AEGL	IDLH	HSE	ERPG	
38	Mouse	LC50	673	60	Back et al 1972		IDLH			
39	Mouse	LC50	676	50	Zwart et al 1990	AEGL				
40	Rat	LC50	683	50	Zwart et al 1990	AEGL				
41	Rat	LC50	712	60	MacEwen & Vernot 1972	AEGL		HSE	ERPG	
42	Rat	LC50	713	60	Back et al 1972		IDLH			
43	Rat	LC50	726	30	Zwart et al 1990	AEGL				
44	Mouse	LC50	800	30	Zwart et al 1990	AEGL				
46	Rat	LC50	835	10	Zwart et al 1990	AEGL				
49	Mouse	LC50	1160	10	Zwart et al 1990	AEGL				

Notes: **AEGL** - Acute Exposure Guideline Levels (AEGL) are currently under development by the National Research Council's Committee on Toxicology.
ERPG - Emergency Response Planning Guidelines (ERPG) developed by the American Industrial Hygiene Association.
HSE - Health and Safety Executive (UK).
IDLH - Immediately Dangerous to Life or Health concentrations developed by Institute for National Institute for Occupational Safety and Health (**NIOSH**).

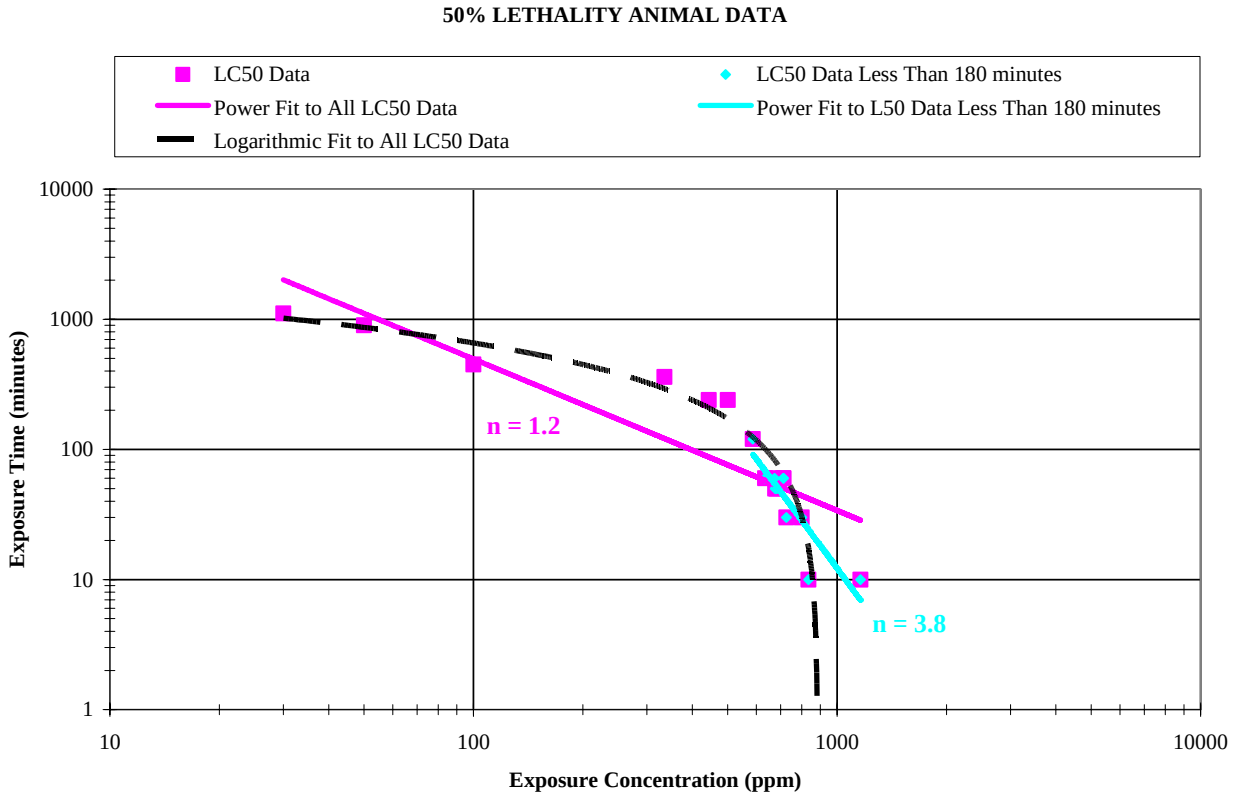


FIGURE 2 – PLOT OF L50 DATA

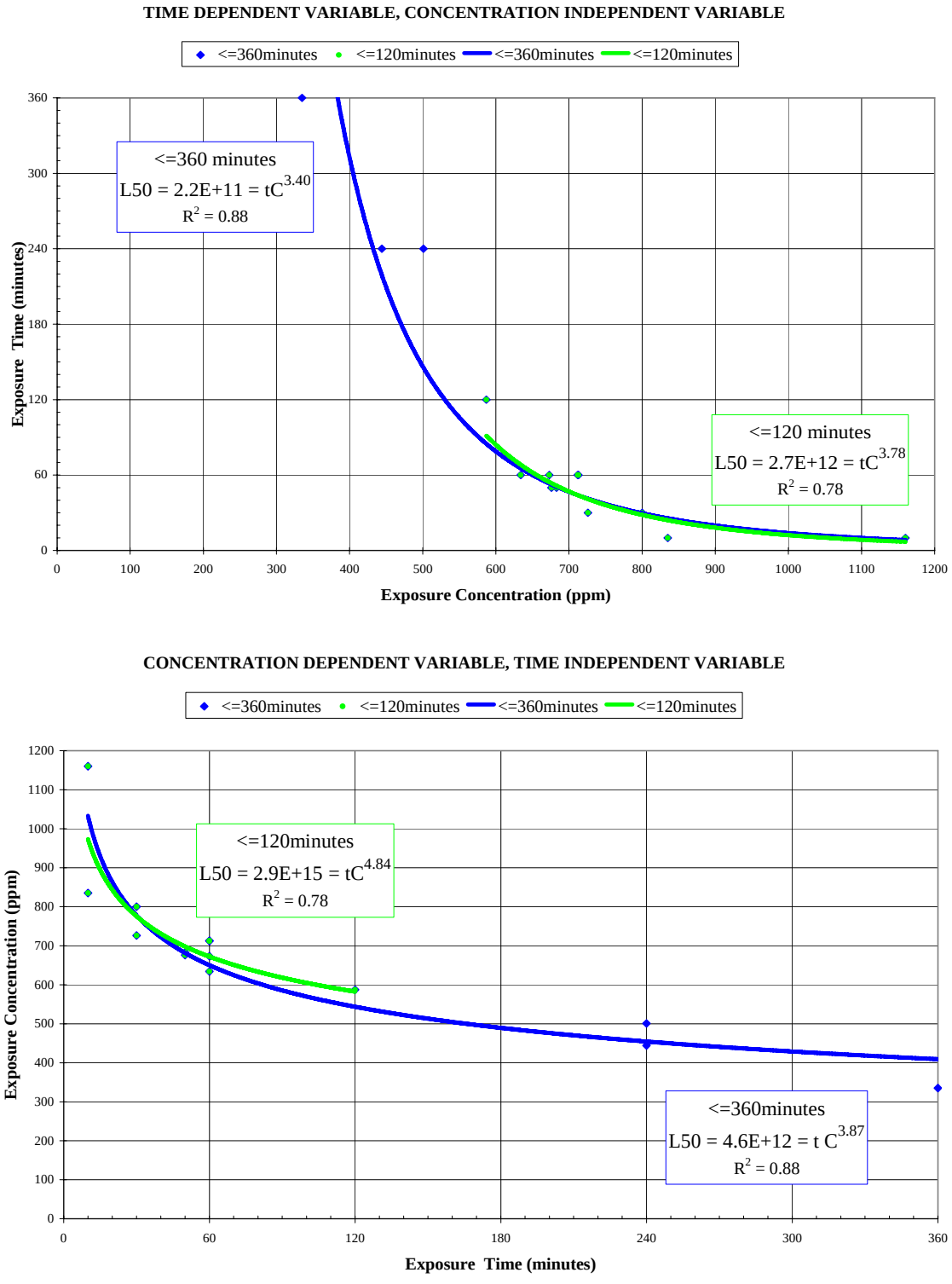


Figure 3 **50th Percentile H_2S Lethality Concentration and Time Pairs Presented Two Ways (the bottom plot should be used)**

4. PROBIT PARAMETERS

The probit equation can be derived from experimental data that provide the concentration, time and percentage of response. Population response to acutely toxic gases follows a lognormal distribution with toxic load which is expressed as:

$$Y = a + b_1 \ln C + b_2 \ln t$$

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,
C is the exposure concentration (ppm), and
t is the exposure duration (minutes).

The form of the equation used in hazard analysis is:

$$Y = a + b \ln C^n t$$

where: $n=b_1/b_2$ *and* $b=b_2$.

A probit Y of 5 corresponds to *L50*, so

$$L_{50} = \exp\left(\frac{5-a}{b}\right)$$

Similarly, a probit Y of 2.67 corresponds to *L1*. These can be converted to an LC_X for an exposure time t using:

$$LC_X = \left(\frac{L_X}{t}\right)^{\frac{1}{n}}$$

The following table compares probit equations for H_2S and the predicted LC1 and LC50 concentrations for a 60 minute exposure.

Probit Parameter Comparison of LC50					
Reference	*a*	*b*	*n*	LC1 (ppm) for 60 minutes	LC50 (ppm) for 60 minutes
Rogers AEUB 1990	-36.2	2.366	2.5	139	206
US Coast Guard 1980	-31.42	3.008	1.43	158	271
TNO[1] 1992	-11.5	1	1.9	144	489
HSE (derived from L50 and L1)	-30.0	1.154	4	427	707

[1](in mg/m3, divided by 1.4 for ppm)

Note that the exponents *n* above are lower than the values of about 4 plotted on Figure 3. The probit parameters for humans incorporate varying degrees of safety factors. The AEUB

parameters developed by Rogers provide the lowest LC50 values 60 minute exposures. Due to the differences in the exponent *n*, the lines will cross when plotted.

5. EMERGENCY EXPOSURE CRITERIA COMPARISON

Table 1 provides a summary of the H_2S emergency exposure criteria now in use in the world. The first three are from the United States. The last row is from the United Kingdom and is not used as an emergency exposure criterion, but is provided for comparison. Other European guidelines could not be found. The AEGL and IDLH are exposure guidelines in the event of an accidental release whereas the ERPG and SLOT are planning guidelines in preparation for an accidental release.

Table 1 Summary of Emergency Exposure Guidelines

Guideline	Target Group	Organization	Definition Purpose
AEGL	Public	U.S. EPA COT NRC	Acute Exposure Guideline Levels Three-tier guideline for emergency response
ERPG	Public	AIHA	Emergency Response Planning Guideline Three-tier planning guideline for emergency response planning
IDLH	Worker	NIOSH	Immediately Dangerous to Life and Health Highest concentration from which escape possible without permanent damage
SLOT	Public	U.K. HSE	Specified Level of Toxicity Dangerous Toxic Load used in context of land use planning

Each guideline is discussed in the following sections. Table 2 compares the criterion definition, the toxicity starting point, uncertainty factors used to adjust the starting point to the definition for human exposure and the toxic load exponent n for hydrogen sulphide. The EUB emergency planning criterion for hydrogen sulphide ***is the airborne exposure concentration of hydrogen sulphide and exposure time that provides a conservative margin of safety to protect people from serious irreversible health effects including fatalities.*** Note that in the three tier guidelines the third level is comparable to the EUB criterion.

The toxic load approach is used by most regulatory agencies, but as pointed out by Hilderman (2002) it is misused. Toxicologists have traditionally applied the uncertainty factors to the concentration which is consistent with the dose of C*t (Huber's law with an exponent of 1) and has led to an error. However if the toxic load approach is used, the uncertainty factor should be applied to the load. If it is applied to the concentration, the uncertainty factor is greatly increased for exponents greater than 1. Some toxic chemicals, like SO_2 have an exponent n less than 1. If the uncertainty factor was applied to the concentration for these chemicals the load uncertainty factor would decrease, an unintended result of doing the mathematics wrong.

The ERPG-3 does not use a toxic load approach or defined uncertainty factors, rather professional judgement is used to adjust the toxicity data for animals and humans to meet the criteria.

The AEGL-3 and IDLH used an uncertainty factor of 10 on the concentration to adjust the toxicity data to their criteria for humans. The AEGL-3 criterion starts with No Observed Adverse Effect Level for animals and then adjusts it to humans with a factor of 10 on concentration to meet their criteria of *"could experience life-threatening health effects or death"*. The wording is inconsistent in that no means nothing and could means something, so the chance of death is very low. The AEGL-3 factor of 10 is based on a factor of 3 for inter-species and 3 for intra-species variability. The IDLH uses a general safety factor of 10.

The HSE SLOT is the L1 (1% of the exposed people are not expected to survive). They have used an uncertainty factor of 7.5 to adjust L50 animal data to L1 human data. They have not allowed for inter-species and intra-species uncertainty factors.

Table 2 Comparison of Exposure Criterion and Uncertainty Factors

Criterion Definition	Toxicity Starting Point	Uncertainty Factor on Concentration	Uncertainty Factor on Load	Toxic Load Exponent n
AEGL-3 "*could experience life-threatening health effects or death"*	NOAEL animal	10	$10^{4.36}$ ~23,000	4.36
ERPG-3 "*without experiencing or developing life-threatening health effects"*	various	6-7 for L50 animal	not used	not used
IDLH "*exposure is likely to cause death or immediate or delayed permanent adverse health effects or prevent escape from such an environment"*	L50 animal and L0 human	10	$10^{2.2}$ ~160	2.2
SLOT "*Substantial fraction of exposed population requiring medical attention; Some people seriously injured, requiring prolonged treatment; Highly susceptible people possibly being killed"*	L50 animal	not used	7.5	4

Table 3 compares the exposure endpoints for hydrogen sulphide at different exposure times. The endpoint labelled ERCB-EPZ is provided for comparison. The common belief is that the EPZ formula and nomographs currently in use by the EUB are based on 100 ppm H_2S for a 3 minute averaging time. No documentation is available to confirm this; the range presented is based on the author's experience. The shaded rows are for the first two tiers of the AEGL and of the ERPG and are provided for the wide range in times and concentration is not surprising given the different objective of each criterion and the methods used to set the exposure endpoints.

Table 3 Comparison of Exposure Endpoints for H_2S

Guideline	H_2S (ppm) for Exposure Duration (minutes)								
Duration (minutes)	3	<5	10	15	30	60	120	240	480
ERCB-EPZ	?100 to 300?								
AEGL-3			76		59	50		37	31
ERPG -3						100			
IDLH					100				
SLOT	800	800	669	604	508	427	359	302	

6. IDLH

Reference http://www.cdc.gov/niosh/idlh/idlh-1.html

Highlighting has been added.

The "immediately dangerous to life or health air concentration values (IDLHs)" used by the National Institute for Occupational Safety and Health (NIOSH) as respirator selection criteria were first developed in the mid-1970's. The Documentation for Immediately Dangerous to Life or Health Concentrations (IDLHs) is a compilation of the rationale and sources of information used by NIOSH during the original determination of 387 IDLHs and their subsequent review and revision in 1994.

6.1. The Standards Completion Program

The definition for an IDLH that was derived during the SCP was based on the definition stipulated in 30 CFR 11.3(t). ***The purpose for establishing this IDLH was to determine a concentration from which a worker could escape without injury or without irreversible health effects in the event of respiratory protection equipment failure*** (e.g., contaminant breakthrough in a cartridge respirator or stoppage of air flow in a supplied-air respirator) and a concentration above which only "highly reliable" respirators would be required. ***In determining IDLHs, the ability of a worker to escape without loss of life or irreversible health effects was considered along with severe eye or respiratory irritation and other deleterious effects (e.g., disorientation or incoordination) that could prevent escape.*** Although in most cases, egress from a particular worksite could occur in much less than 30 minutes, ***as a safety margin, IDLHs were based on the effects that might occur as a consequence of a 30-minute exposure.*** However, the 30-minute period was NOT meant to imply that workers should stay in the work environment any longer than necessary following the failure of respiratory protection equipment; in fact, EVERY EFFORT SHOULD BE MADE TO EXIT IMMEDIATELY!

IDLHs were determined for each substance during the SCP on a case-by-case basis, taking into account the toxicity data available at the time. Whenever possible, IDLHs were determined using health effects data from studies of humans exposed for short durations. However, in most instances, a lack of human data necessitated the use of animal toxicity data. When inhalation studies of animals exposed for short durations (i.e., 0.5 to 4 hours) were the only health effects data available, IDLHs were based on the lowest exposure causing death or irreversible health effects in any species. When lethal dose (LD) data from animals were used, IDLHs were estimated on the basis of an equivalent exposure to a 70-kg worker breathing 10 cubic meters of air.

Since chronic exposure data may have little relevance to acute effects, these types of data were used in determining IDLHs only when no acute toxicity data were available and only in conjunction with competent scientific judgment. In a number of instances when no relevant human or animal toxicity data were available, IDLHs were based on analogies with other substances with similar toxic effects.

6.2. Current NIOSH Use of IDLHs

The current NIOSH definition for an immediately dangerous to life or health condition, as given in the NIOSH Respirator Decision Logic [NIOSH 1987], is a situation ***"that poses a threat of exposure to airborne contaminants when that exposure is likely to cause death or immediate or delayed permanent adverse health effects or prevent escape from such an environment".*** It is also stated that the purpose of establishing an IDLH is to ***"ensure that the worker can escape from a given contaminated environment in the event of failure of the respiratory protection equipment".*** The NIOSH respirator decision logic uses an IDLH as one of several respirator selection criteria. Under the NIOSH respirator decision logic, "highly reliable" respirators (i.e., the most protective respirators) would be selected for emergency situations, fire fighting, exposure to carcinogens, entry into oxygen-deficient atmospheres, entry into atmospheres that contain a substance at a concentration greater than 2,000 times the NIOSH REL or OSHA PEL, and for entry into immediately dangerous to life or health conditions. These "highly reliable" respirators include either a self-contained breathing apparatus (SCBA) that has a full facepiece and is operated in a pressure-demand or other positive-pressure mode, or a supplied-air respirator that has a full facepiece and is operated in a pressure-demand or other positive-pressure mode in combination with an auxiliary SCBA operated in a pressure-demand or other positive-pressure mode.

When the IDLHs were developed in the mid-1970's, only limited toxicological data were available for many of the substances. NIOSH has recently requested information on the current uses of IDLHs in the workplace and on the scientific adequacy of the criteria and procedures originally used for establishing them [Federal Register, Volume 58, Number 229, p. 63379, Wednesday, December 1, 1993]. The information received in response to the Federal Register announcement is being evaluated and will be used to establish future actions concerning IDLHs. In the interim, however, NIOSH decided to review the existing IDLHs, and revise them as appropriate.

6.3. Revised Criteria for Determining IDLHs

The criteria utilized to determine the adequacy of existing IDLHs were a combination of those used during the SCP and a newer methodology developed by NIOSH. These criteria form a tiered approach with acute human toxicity data being used preferentially, followed next by acute animal inhalation toxicity data, and then finally by acute animal oral toxicity data to determine an updated IDLH. When relevant acute toxicity data were insufficient or unavailable, then the use of chronic toxicity data or an analogy to a chemical with similar toxic effects was considered. In order to facilitate the revision process, secondary toxicological data were primarily used. Once a preliminary IDLH was developed, it was compared to the existing IDLH and to several other factors (e.g., existing short-term exposure guidelines and lower explosive limits).

The following "hierarchy" was followed to develop a "preliminary" value for the revised IDLH:

A. Human acute toxicity data were used if sufficient to determine a concentration that for up to 30 minutes does not cause death, serious or irreversible health effects, or does not impair or impede the ability to escape.

B. Animal acute lethal concentration (LC) data were considered next. The only animal lethal concentration data used involved mammals; the vast majority of the data was from studies of rats, mice, guinea pigs, and hamsters. It was decided to generally use the lowest reliable LC data, with LC50 data preferred. If acute LC data determined during a 30-minute period were not available, then the data, based on a study by ten Berge et al. [1986], were "adjusted" to an equivalent 30-minute value using the following relationship:

Adjusted LC50 (30 minutes) = LC50(t) * (t/0.5)** (1/n)

where: LC50(t) = LC50 determined over t hours

n = constant*

*Note: ten Berge et al. [1986] determined the relationship shown above based on experimental data. The constant "n" was determined by ten Berge et al. to be less than 3.0 for 18 of the 20 substances studied. Although the individual "n" values determined by ten Berge et al. [1986] were utilized when applicable during the review and revision of the original IDLHs, as a conservative estimate, an "n" = 3.0 was assumed when "adjusting" the LC data to 30 minutes for all other substances.

The LC values (after "adjusting" if necessary to 30 minutes) were divided by a safety factor of 10 to determine a "preliminary" IDLH for comparison purposes.

C. Animal lethal dose (LD) data were considered next. As was the case with the lethal concentration data, the only animal lethal dose data used involved mammals; the vast majority of the data were from studies of rats, mice, guinea pigs, and hamsters. It was decided to generally use the lowest LD data with oral LD50 data preferred. The LD data was used to determine the equivalent total dose to a 70-kg worker and, as was done during the SCP, the air concentration containing this dose was determined by dividing by 10 cubic meters. [Note: A worker breathing at a rate of 50 litres per minute for 30 minutes would inhale 1.5 cubic meters of air.] A "preliminary" IDLH for comparison purposes was determined by dividing these air concentrations by a safety factor of 10.

D. Chronic toxicity data were considered if no relevant acute toxicity data existed. However, the fact that chronic exposures may have limited relevance to acute effects was taken into consideration.

E. When relevant toxicity data applying specifically to the chemicals in question were lacking, and if it was determined to be justified, then analogies to substances with similar acute toxic effects were considered.

F. All "preliminary" IDLHs derived during this update were checked against the following factors prior to establishing the final "revised" IDLH:

1. Lower explosive limit (LEL): It was decided to restrict the "routine" entry into a possible explosive atmosphere to concentrations no greater than 10% of the LEL. [Note: SCP-derived IDLHs were set at 100% of the LELs if there were no known serious health hazards below these values. However, OSHA considers concentrations in excess of 10% of the LEL to be a hazardous atmosphere in confined spaces [29 CFR 1910.146(b)].]

2. RD50 data: An RD50 is defined as the 10-minute exposure concentration producing a 50% respiratory rate decrease in mice or rats and can be used to estimate severe respiratory irritation. Prolonged exposure to an RD50 concentration has been shown to produce respiratory tract lesions consistent with irritation [Alarie 1981; Buckley et al. 1984].

3. Other short-term exposure guidelines such as the American Industrial Hygiene Association's emergency response planning guidelines (ERPGs) and the National Research Council's emergency exposure guidance levels (EEGLs) and short-term public emergency guidance levels (SPEGLs), and occupational exposure standards or recommendations such as OSHA PELs, NIOSH RELs, or the American Conference of Governmental Industrial Hygienists (ACGIH) TLVs.

4. Based on the NIOSH respirator decision logic, the revised IDLHs could not be greater than 2,000 times the NIOSH REL (or OSHA PEL).

5. The revised IDLHs would not be greater than the original IDLHs derived during the SCP.

6.4. H_2S

The following is NIOSHs IDLH documentation for H_2S (http://www.cdc.gov/niosh/idlh/7783064.html).

CAS number: 7783064

NIOSH REL: 10 ppm (15 mg/m^3) 10minute CEILING

Current OSHA PEL: 20 ppm CEILING, 50 ppm 10minute MAXIMUM PEAK

1989 OSHA PEL: 10 ppm (14 mg/m^3) TWA, 15 ppm (21 mg/m^3) STEL

1993-1994 ACGIH TLV: 10 ppm (14 mg/m^3) TWA, 15 ppm (21 mg/m^3) STEL

Description of Substance: Colorless gas with a strong odor of rotten eggs.

LEL: 4.0% (10% LEL, 4,000 ppm)

Original (SCP) IDLH: 300 ppm

Basis for original (SCP) IDLH: The chosen IDLH is based on the statements by Patty [1963] that 170 to 300 ppm is the maximum concentration that can be endured for 1 hour without serious consequences; 400 to 700 ppm is dangerous after exposure of 0.5 to 1 hour [Henderson and Haggard 1943]. AIHA [1963] reported that 400 to 700 ppm caused loss of consciousness and possible death in 0.5 to 1 hour [MCA 1950].

ACUTE TOXICITY DATA:

Lethal concentration data:

Species	Reference	LC_{50} (ppm)	LC_{Lo} (ppm)	Time	Adjusted 0.5-hr LC (CF*)	Derived value
Rat	Back et al. 1972	713	-----	1 hr	977 ppm (1.37)	98 ppm
Mouse	Back et al. 1972	673	-----	1 hr	922 ppm (1.37)	92 ppm
Human	Lefaux 1968	-----	600	30 min	600 ppm (1.0)	60 ppm
Mouse	MacEwen & Vernot 1972	634	-----	1 hr	869 ppm (1.37)	87 ppm
Human	Tab Biol Per 1933	-----	800	5 min	354 ppm (0.44)	35 ppm
Rat	Tansey et al. 1981	444	-----	4 hr	1,141 ppm (2.57)	114 ppm

*Note: Conversion factor (CF) was determined with "n" = 2.2 [ten Berge et al. 1986].

Other human data: It has been reported that 170 to 300 ppm is the maximum concentration that can be endured for 1 hour without serious consequences [Henderson and Haggard 1943] and that olfactory fatigue occurs at 100 ppm [Poda 1966]. It has also been reported that 50 to 100 ppm causes mild conjunctivitis and respiratory irritation after 1 hour; 500 to 700 ppm may be dangerous in 0.5 to 1 hour; 700 to 1,000 ppm results in rapid unconsciousness, cessation of respiration, and death; and 1,000 to 2,000 ppm results in unconsciousness, cessation of respiration, and death in a few minutes [Yant 1930].

Revised IDLH: 100 ppm

Basis for revised IDLH: The revised IDLH for hydrogen sulphide is 100 ppm based on acute inhalation toxicity data in humans [Henderson and Haggard 1943; Poda 1966; Yant 1930] and animals [Back et al. 1972; MacEwen and Vernot 1972; Tansey et al. 1981].

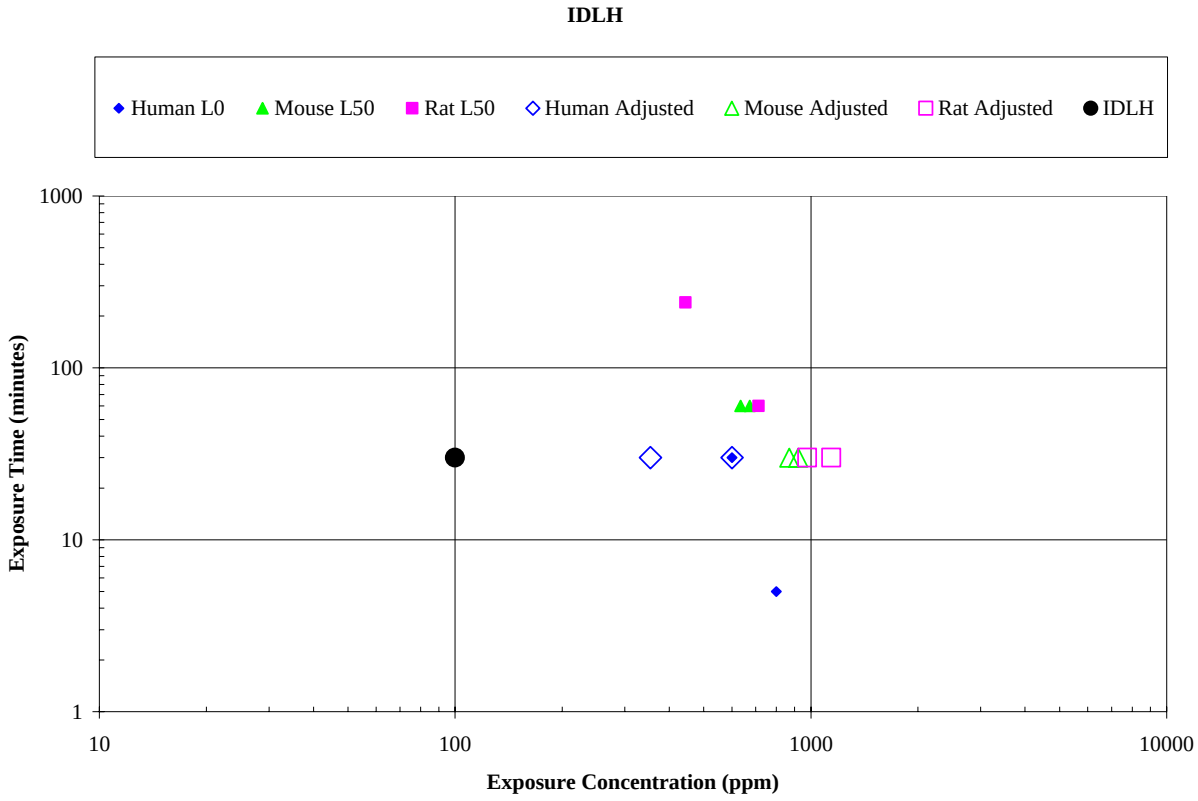
6.5. Discussion

a. Note that an exposure time is not explicitly provided in the IDLH definition but data was adjusted to 30 minutes using an exponent of 2.2 to determine the IDLH.

b. 30 minutes is the maximum exposure time to "ensure that the worker can escape from a given contaminated environment in the event of failure of the respiratory protection equipment".

c. Adjusted concentrations to 30 minute exposures based on safety or uncertainty factor of 10, as shown below.

d. Uncertainty factor equivalent to $10^{2.2}$~158 on load



7. ERPG

The Emergency Response Planning Guidelines (ERPGs) were developed by the ERPG committee of the American Industrial Hygiene Association. The ERPGs were developed as planning guidelines, to anticipate human adverse health effects caused by exposure to toxic chemicals. The ERPGs are three-tiered guidelines with one common denominator: a 1-hour contact duration. Each guideline identifies the substance, its chemical and structural properties, animal toxicology data, human experience, existing exposure guidelines, the rationale behind the selected value, and a list of references. The handbook that is updated annually provides an excellent summary of the History of Emergency Exposure Guidelines.

The U.S. Department of Energy Subcommittee on Consequence Assessment and Protective Actions (SCAPA) provides the following summary of ERPG's (http://www.bnl.gov/scapa/erpgpref.htm).

The Emergency Response Planning Guideline (ERPG) values are intended to provide estimates of concentration ranges where one reasonably might anticipate observing adverse effects as described in the definitions for ERPG-1, ERPG-2, and ERPG-3 as a consequence of exposure to the specific substance.

- The ***ERPG-1*** is the maximum airborne concentration below which it is believed that nearly all individuals could be exposed for up to 1 hr without experiencing other than mild transient adverse health effects or perceiving a clearly defined, objectionable odor.

- The ***ERPG-2*** is the maximum airborne concentration below which it is believed that nearly all individuals could be exposed for up to 1 hr without experiencing or developing irreversible or other serious health effects or symptoms which could impair an individual's ability to take protective action.

- The ***ERPG-3*** is the maximum airborne concentration below which it is believed that nearly all individuals could be exposed for up to 1 hr without experiencing or developing life-threatening health effects.

It is recognized by the committee (and should be remembered by all who make use of these values) that human responses do not occur at precise exposure levels but can extend over a wide range of concentrations. The values derived for ERPGs should not be expected to protect everyone but should be applicable to most individuals in the general population. In all populations there are hypersensitive individuals who will show adverse responses at exposure concentrations far below levels where most individuals normally would respond. Furthermore, since these values have been derived as planning and emergency response guidelines, not exposure guidelines, they do not contain the safety factors normally incorporated into exposure guidelines. Instead, they are estimates, by the committee, of the thresholds above which there would be unacceptable likelihood of observing the defined effects. The estimates are based on the available data that are summarized in the documentation. In some cases where the data are limited, the uncertainty of these estimates is large. Users of the ERPG values are encouraged strongly to review carefully the documentation before applying these values.

In developing these ERPGs, human experience has been emphasized to the extent data are available. Since this type of information, however, is rarely available, and when available is only for low level exposures, animal exposure data most frequently forms the basis for these values. The most pertinent information is derived from acute inhalation toxicity studies that have included clinical observations and histopathology. The focus is on the highest levels not showing the effects described by the definitions of the ERPG levels. Next, data from repeat inhalation exposure studies with clinical observations and histopathology are considered. Following these in importance are the basic, typically acute studies where mortality is the major focus. When inhalation toxicity data are either unavailable or limited, data from studies involving other routes of exposure will be considered. More value is given to the more rigorously conducted studies, and data from short-term studies are considered to be more useful in estimating possible effects from a single 1-hr exposure. Finally, if mechanistic or dose-response data are available, these are applied, on a case by case basis, as appears appropriate.

It is recognized that there is a range of times that one might consider for these guidelines; however, it was the committee's decision to focus its efforts on only one time period. This decision was based on the availability to toxicology information and a reasonable estimate for an exposure scenario. Users who may choose to extrapolate these values to other time periods are cautioned to review the documentation fully since such extrapolations tend to hold only over very limited time frames, it at all.

The ERPG guidelines do not protect everyone. Hypersensitive individuals would suffer adverse reactions to concentrations far below those suggested in the guidelines. In addition, ERPGs, like other exposure guidelines, are based mostly on animal studies, thus raising the question of applicability to humans. The guidelines are focused on one period of time: 1 hour. Exposure in the field may be longer or shorter. However, the ERPG committee strongly advises against trying to extrapolate ERPG values to longer periods of time.

The most important point to remember about the ERPGs is that they do not contain safety factors usually incorporated into exposure guidelines such as the TLV. Rather, they estimate how the general public would react to chemical exposure. Just below the ERPG-1, for example, most people would detect the chemical and may experience temporary mild effects. Just below the ERPG-3, on the other hand, it is estimated that the effects would be severe, although not life-threatening. The TLV, on the other hand, incorporate a safety factor into their guidelines, to prevent ill effects. The ERPG should serve as a planning tool, not a standard to protect the public. To review the current ERPG list, check the ERPG Working List. For a more detailed discussion of the level of concern (LOC) , check the references available on our Level of Concern page.

In comparison to other LOCs, the ERPG guidelines are clearly defined and are based on extensive, current data. The rationale for selecting each value is explained, and other pertinent information is also provided. But, at the present time, ERPG guidelines have been developed for fewer than 100 chemicals.

7.1. H_2S

The following are the recommended ERPGs and the rationales from the AIHA (2004) ERPG summary sheet for H_2S.

The ***ERPG-3*** is the maximum airborne concentration below which it is believed that nearly all individuals could be exposed for up to 1 hr without experiencing or developing life-threatening health effects is 100 ppm. This value is based on human experience, e.g., a report of unconsciousness and decreased blood pressure in an otherwise healthy individual exposed to an estimated concentration of 230 ppm H_2S for 20 min. In addition, after exposure to 200 to 300 ppm for 1 hr, individuals experienced marked conjunctivitis and respiratory tract irritation, but no deaths occurred. In an animal study, an LC50 of 712 ppm (1 hr) was reported.

The ***ERPG-2*** is the maximum airborne concentration below which it is believed that nearly all individuals could be exposed for up to 1 hr without experiencing or developing irreversible or other serious health effects or symptoms which could impair an individual's ability to take protective action is 30 ppm. This value is based on animal studies where no deaths occurred when rats were exposed to 45 ppm for 5 hrs, but unconsciousness and cardiac irregularities were reported in rabbits exposed to 72 ppm for 1.5 hrs.

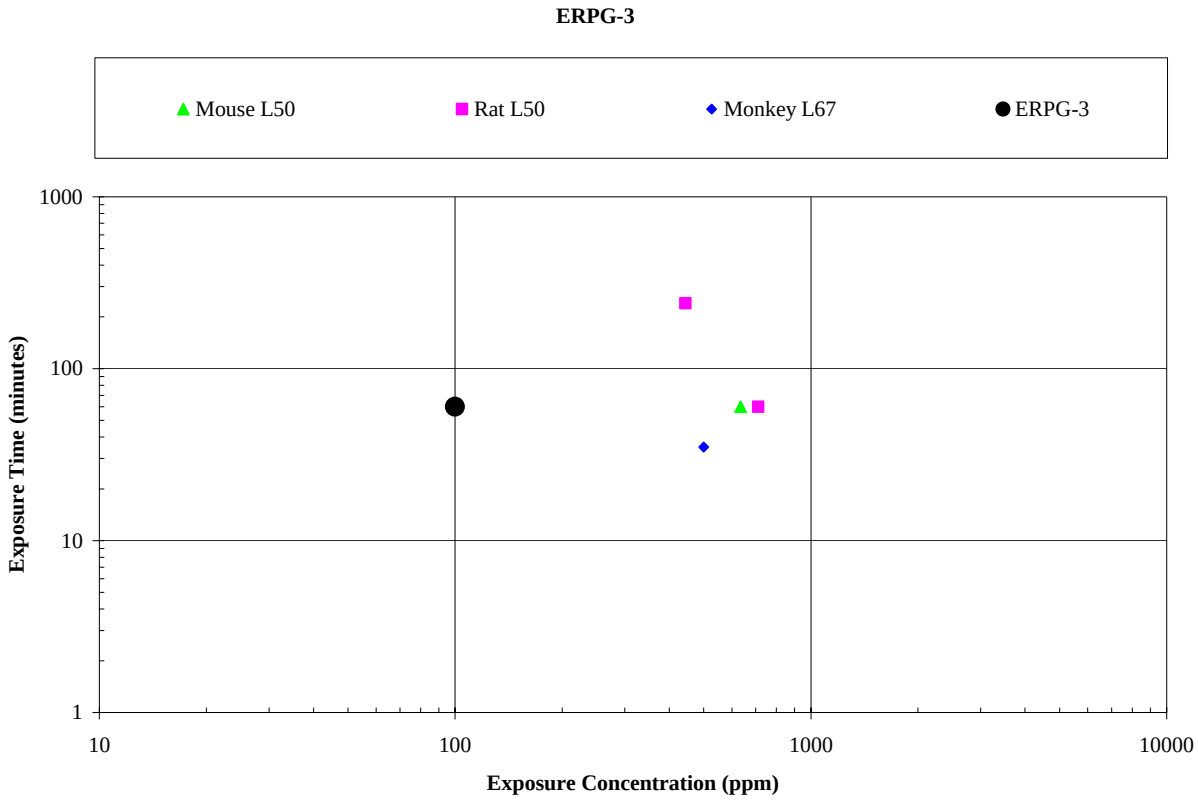
The ***ERPG-1*** is the maximum airborne concentration below which it is believed that nearly all individuals could be exposed for up to 1 hr without experiencing other than mild transient adverse health effects or perceiving a clearly defined, objectionable odor is 0.1 ppm. This value is based on the fact that the (objectionable) odor of H_2S is distinct at 0.3 ppm.

7.2. Discussion

a. Rat data from Tansy eta l (1981) to determine L50 for 4 hour exposure provided, but not the L50 (as plotted below).

b. Uncertainty factors based on professional judgement but not specified. Calculated as between 6 and 7 on concentration for adjusting L50 of animal data.

c. Toxic load approach not used, thus exponent n not specified.


ERPG-3
Mouse L50
Rat L50
Monkey L67
ERPG-3
1000
100
10
1
Exposure Time (minutes)
10
100
1000
10000
Exposure Concentration (ppm)

8. AEGL

The following was obtained from the United States Environmental Protection Agency website at http://www.epa.gov/opptintr/aegl.

Under the authority of the Federal Advisory Committee Act (FACA) P. L. 92-463 of 1972, the National Advisory Committee for Acute Exposure Guideline Levels for Hazardous Substances (NAC/AEGL Committee) was established to identify, review and interpret relevant toxicological and other scientific data and develop AEGLs for high priority, acutely toxic chemicals.

AEGLs represent threshold exposure for the general public and are applicable to emergency exposure periods ranging from 0 minutes to 8 hours. AEGL-2 and AEGL-3, and AEGL-1 levels as appropriate, will be developed for each of five exposure periods (10 and 30 minutes, 1 hour, 4 hours, and 8 hours) and will be distinguished by varying degrees of severity of toxic effects. It is believed that the recommended exposure levels are applicable to the general population including infants and children, and other individuals who may be susceptible. The three AEGLs have been defined as follows:

> **AEGL-1** is the airborne concentration (expressed as parts per million or milligrams per cubic meter [ppm or mg/m^3]) of a substance above which it is predicted that the general population, including susceptible individuals, could experience notable discomfort, irritation, or certain asymptomatic, non-sensory effects. However, the effects are not disabling and are transient and reversible upon cessation of exposure.
>
> **AEGL-2** is the airborne concentration (expressed as ppm or mg/m^3) of a substance above which it is predicted that the general population, including susceptible individuals, could experience irreversible or other serious, long-lasting adverse health effects or an impaired ability to escape.
>
> **AEGL-3** is the airborne concentration (expressed as ppm or mg/m^3) of a substance above which it is predicted that the general population, including susceptible individuals, could experience life-threatening health effects or death.

Airborne concentrations below the AEGL-1 represent exposure levels that could produce mild and progressively increasing but transient and non-disabling odour, taste, and sensory irritation, or certain asymptomatic, non-sensory effects. With increasing airborne concentrations above each AEGL, there is a progressive increase in the likelihood of occurrence and the severity of effects described for each corresponding AEGL. Although the AEGL values represent threshold levels for the general public, including susceptible subpopulations, such as infants, children, the elderly, persons with asthma, and those with other illnesses, it is recognized that individuals, subject to unique or idiosyncratic responses, could experience the effects described at concentrations below the corresponding AEGL.

8.1. Development Process

The process that has been established for the development of the AEGL values is the most comprehensive ever used for the determination of short-term exposure limits for acutely toxic

chemicals. A summary of the overall process is presented in diagram form in AEGL Development Process. The development of AEGL values through the Federal Advisory Committee and stakeholder concept strives to accomplish the following process objectives:

1. Development of scientifically valid AEGL values for use in chemical emergency planning, prevention and response programs.

2. Comprehensive identification of published and unpublished information sources used to set AEGLs.

3. Sharing resource burdens by stakeholder members.

4. Adoption of consistent emergency planning both domestically and internationally.

5. Transparency of program methods (Standard Operating Procedures) and information through public participation at meetings and by commenting on Federal Register notices.

6. Inclusion of National Academy of Sciences peer review and final arbitration of AEGL values and methods.

The process consists of four basic stages in the development and status of the AEGLs, and they are identified according to the review level and concurrent status of the AEGL values. They include (1) draft AEGLs, (2) proposed AEGLs, (3) interim AEGLs, and (4) final AEGLs. The entire development process can be described by individually describing the four basic stages in the development of AEGL values.

8.2.Stage 1: Draft AEGLs

This first stage begins with a comprehensive search of the published scientific literature. Attempts are made to mobilize all relevant unpublished data through industry-trade associations and from individual companies in the private sector. A more detailed description of the published and unpublished sources of data and information utilized is provided which addresses search strategies. The data are evaluated by following the published NRC guidelines (NRC, 1993a) and this SOP manual, and selected data are used as the basis for the derivation of the AEGL values and the supporting scientific rationale. Data evaluation, data selection, and development of a technical support document (TSD) are all performed as a collaborative effort among the staff scientists at the organization drafting the TSDs, the chemical manager, and two chemical reviewers. This group is called the AEGL Development Team. Specific NAC/AEGL Committee members are assigned to a team for each chemical under review. Hence, a separate team comprising different committee members is formed for each chemical under review. The product of this effort is a TSD that contains draft AEGLs. The draft TSD is subsequently circulated to all other NAC/AEGL Committee members for review and comment prior to a formal meeting of the committee. Revisions to the initial TSD and the draft AEGLs are made up to the time of the NAC/AEGL Committee meeting scheduled for formal presentation and discussion of the AEGL values and the documents. At the committee meeting, the committee deliberates and, if a quorum is present, attempts to reach a consensus or a two-thirds majority vote to elevate the draft AEGLs to "proposed" status. A quorum of the NAC/AEGL Committee is defined as 51% or more of the

total NAC/AEGL Committee membership. If agreement cannot be reached, the committee conveys its issues and concerns to the AEGL Development Team and further work is conducted by this group. After completion of additional work, the chemical is resubmitted for consideration at a future meeting. If a consensus or a two-thirds majority vote of the committee cannot be achieved because of inadequate data, no AEGL values will be developed until adequate data become available.

8.3. Stage 2: Proposed AEGLs

Once the NAC/AEGL Committee has reached a consensus or a two-thirds majority vote on the AEGL values and supporting rationale, they are referred to as “proposed” AEGLs and are published in the Federal Register for a 30-day review and comment period. Following publication, the committee reviews the public comments, addresses and resolves relevant issues, and seeks a consensus or a two-thirds majority vote of those present on the original or modified AEGL values and the accompanying scientific rationale.

8.4. Stage 3: Interim AEGLs

Following resolution of relevant issues raised through public review and comment and subsequent approval of the committee, the AEGL values are classified as "interim." The interim AEGL status represents the best efforts of the NAC/AEGL Committee to establish exposure limits, and the values are available for use as deemed appropriate on an interim basis by federal and state regulatory agencies and the private sector. The interim AEGLs, the supporting scientific rationale, and the TSD, are subsequently presented to the NRC/AEGL Subcommittee for its review and concurrence. If concurrence cannot be achieved, the NRC/AEGL Subcommittee will submit its issues and concerns to the NAC/AEGL Committee for further work and resolution.

8.5. Stage 4: Final AEGLs

When concurrence by the NRC/AEGL Subcommittee is achieved, the AEGL values are considered "final" and published by the NRC. Final AEGL values may be used on a permanent basis by all federal, state and local agencies, and private organizations. It is possible that new data will become available from time to time that challenges the scientific credibility of final AEGLs. If that occurs, the chemical will be resubmitted to the NAC/AEGL Committee and recycled through the review process.

8.6. H_2S

The summary of the H_2S interim AEGLS (November 2002) follows.

Hydrogen sulphide is a colorless, flammable gas at ambient temperature and pressure. It has an odour similar to that of rotten eggs and is both an irritant and asphyxiant. The air odour threshold ranges between 0.008 and 0.13 ppm, and olfactory fatigue, may occur at l00 ppm. Paralysis of the olfactory nerve has been reported at 150 ppm (Beauchampet al., 1984).

Controlled human data were used to derive AEGL-l values. Three of ten asthmatic volunteers exposed to 2 ppm H_2S for 30 minutes complained of headache and eight of ten experienced [non-significant] increased airway resistance (Jappinen et al.,1990). Since there were no clinical symptoms of respiratory difficulty and there were no significant changes in FVC or FEV_1, the AEGL-l was based exclusively upon increased complaints of headache in the three volunteers (Jappinen et al., 1990). A modifying factor of 3 was applied to account for. the wide variability in complaints associated with the foul odour of H_2S and the shallow concentration-response at the relatively low concentrations that are consistent with definition of the AEG-1. The 30-minute experimental value was scaled to the 10-minute, 1-,4-, and 8-hour time points, using $C^{4.4} \times t = k$. The exponent of 4.4 was derived from rat lethality data ranging from 10-minutes to 6-hours exposure duration.

The level of distinct odour awareness (LOA) for hydrogen sulphide is 0.01 ppm. The LOA represents the concentration above which it is predicted that more than half of the exposed population will experience at least a distinct odour intensity, about. 10 % of the population will experience a strong odour intensity. The LOA should help chemical emergency responders in assessing the public awareness of the exposure due to odour perception. Thus, the derived AEGL-l values are considered to have warning properties.

The AEGL-2 was based on the induction of perivascular edema in rats exposed to 200 ppm hydrogen sulphide for 4 hours (Green et al., 1991; Khan et al., 1991). An uncertainty factor of 3 was applied since rat and mouse data suggest little interspecies variability. An intraspecies uncertainty factor of 3 was applied to account for sensitive individuals. The intraspecies uncertainty factor of 3 is considered sufficient because application of the default uncertainty factor of 10 would result in a total uncertainty factor of 30 which would yield AEGL-2 values inconsistent with the total database for hydrogen sulphide. AEGL-2 values derived with larger uncertainty factors are essentially identical to or below the 10 ppm concentration causing no ad¥erse health effects in humans exercising to exhaustion for up to 30 minutes (Bhambhani and Singh, 1991; Bhambhani et al., 1994, 1996a, 1996b, 1997). Therefore, the total uncertainty factor is 10. The 4-hour experimental value was then scaled to the 10-, and 30 minute, 1-, and 8-hour time points, using $C^{4.4} \times t = k$. The exponent of 4.4 was derived from empirica1 rat lethality data ranging from 10 minutes to 6 hours exposure duration.

The AEGL-3 was based the highest concentration causing no mortality in the rat after a 1- hour exposure (504 ppm) (MacEwen and Vernot, 1972). An uncertainty factor of 3 was used to extrapolate from animals to humans since rat and mouse data suggest little interspecies variability .An uncertainty factor of 3 was applied to account for sensitive individuals. The intraspecies uncertainty factor of 3 is considered sufficient because application of the default results in AEGL-3 values inconsistent with the total database. AEGL-3 values derived with larger uncertainty factors were equal to or less than twice the concentration that failed to produce adverse health effects in humans exercising to exhaustion for up to 30 minutes (Bhambhani and Singh, 1991; Bhambhani et al., 1994, 1996a, 1996b, 1997). Increased mortality or irreversible medical conditions consistent with the definition of AEGL-3 are unlikely at such concentrations. Therefore, the total uncertainty factor is 10. The value was then scaled to the 10-, and 30 minute, 1-,4-, and 8-hour time points, using $C^{4.4} \times t = k$. The exponent of 4.4 was derived from rat lethality data ranging from 10 minutes to 6 hours exposure duration.

In summary the interim AEGL values are:

Hydrogen sulphide 7783-06-4 (Interim)					
ppm					
	10 min	30 min	60 min	4 hr	8 hr
AEGL 1	0.75	0.60	0.51	0.36	0.33
AEGL 2	41	32	27	20	17
AEGL 3	76	59	50	37	31

* Level of Odour Awareness = 0.01 ppm

8.7.Discussion

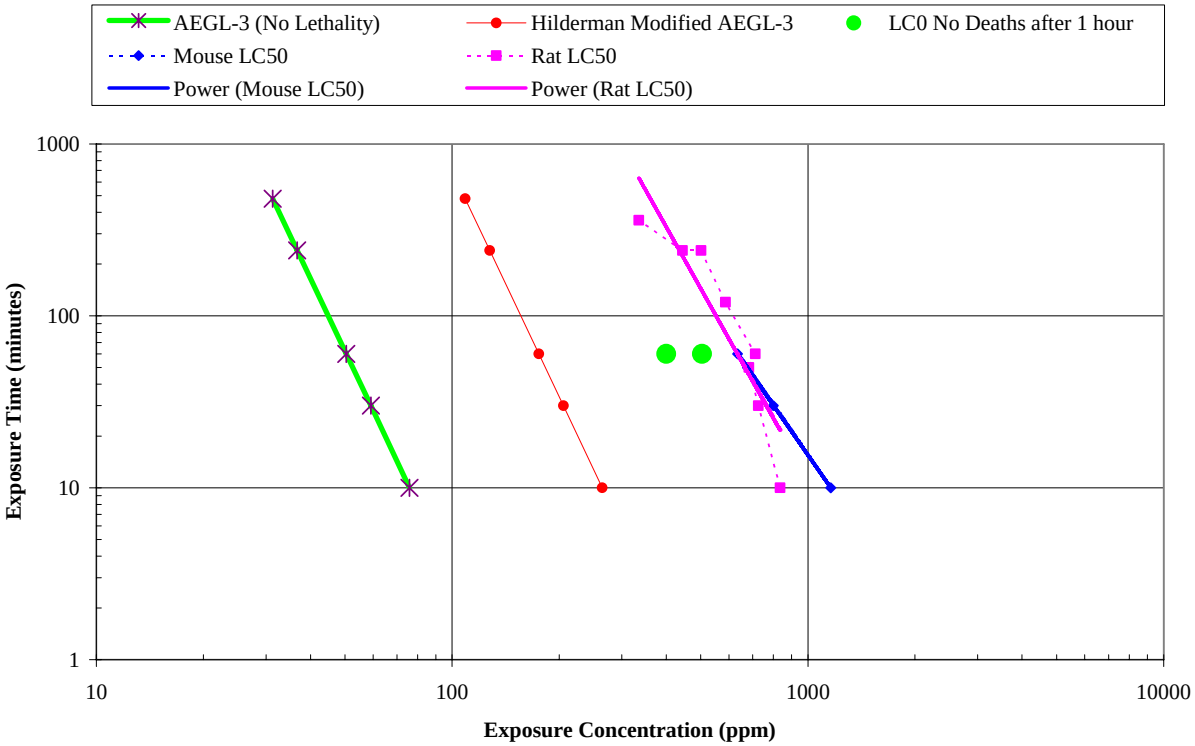
a. Rats and mice L50 data considered, but AEGL-3 based on NOAEL in rats and mice.

b. Uncertainty factor of 10 on NOAEL concentration of 504 ppm for 60 minute exposure is $10^{4.36}$~23,000 on Load.

c. The severity and effect factor to adjust the L50 to the Load for NOAEL can be determined from the data to be about 3.

d. Hilderman (2002) study suggests using uncertainty factor of 100 on Load, as plotted below.

US EPA Acute Exposure Guideline Level - AEGL-3



9. HSE

The following was obtained from the United Kingdom Health and Safety Executive (HSE) website at http://www.hse.gov.uk/hid/haztox.htm. This is one of many pages provideing access to information relating to the Control of Major Accident Hazards Regulations 1999 (COMAH) which came into force on 1 April 1999.

The Dangerous Toxic Load (DTL) describes the exposure conditions, in terms of airborne concentration and duration of exposure, which would produce a particular level of toxicity in the general population. One level of toxicity used by HSE in relation to the provision of land use planning (LUP) advice is termed the **Specified Level of Toxicity (SLOT)**. HSE has defined the LUP SLOT as:

1. Severe distress to almost every one in the area
2. Substantial fraction of exposed population requiring medical attention
3. Some people seriously injured, requiring prolonged treatment
4. Highly susceptible people possibly being killed

As discussed in by Turner and Fairhurst (1993), these criteria are fairly broad in scope, reflecting the fact that:

> 1) there is likely to be considerable variability in the responses of different individuals affected by a major accident;
>
> 2) there may be pockets of high and low concentrations of a toxic substance in the toxic cloud release, so that not everyone will get exactly the same degree of exposure; and
>
> 3) the available toxicity data are not usually adequate for predicting precise dose-response effects.

Importantly, the criteria are also relatively easy for non-scientists to understand in terms of the overall health impact.

9.1. The Basis of the Toxicology Assessment

The toxicity expressed by a given substance in the air is influenced by two factors, the concentration in the air (c) and the duration of exposure (t). A functional relationship between c and t can be developed, such that the end product of this relationship is a constant:

$f(c,t) = \text{constant}$

This constant is known as the Toxic Load. In HSE, the Toxic Load relating to the LUP SLOT is known as the **SLOT Dangerous Toxic Load** or **SLOT DTL**. For a number of gases the relationship between c and t is simple:

Toxic Load = c x t

This relationship is sometimes known as the Haber law. As an example, animal toxicity data for methyl isocyanate indicates that the LUP SLOT is produced by each of these c and t pairs:

t (min)	5	10	30	60	120
c (ppm)	150	78	25	12	6

In this example the constant, or SLOT DTL, is 750 ppm.min (that is 150 x 5, 25 x 30, etc.).

However, the equation c x t = constant does not apply to all substances, so the following general equation has been developed:

Toxic Load = $c^n.t$

For methyl isocyanate, n in the $c^n.t$ relationship is 1. In the case of sulphur dioxide, n = 2 and animal toxicity data suggest that the following pairs of c and t will each produce the LUP SLOT:

t (min)	5	10	30	60	120
c (ppm)	965	682	394	279	197

Here, the constant, or SLOT DTL, is 4.6×10^6 ppm^2.min (that is 965^2 x 5, or 394^2 x 30).

9.2.Determination of the SLOT and SLOD DTLs

How does HSE determine the c and t relationship, or DTL, which would produce the LUP SLOT for a given substance? In general, the absence of human data means that we rely heavily on animal data. If information is available concerning accidental chemical exposures to humans causing severe toxicity (comparable to the LUP SLOT), it usually lacks any quantification of the duration of exposure and associated inhalation conditions. Unfortunately the available, directly relevant animal data is also usually very limited. So, a pragmatic approach, based on the data that are most likely to be available, is adopted. This involves single exposure mortality data (usually LC_{50} tests over a known duration) designed to identify exposure conditions that produce mortality in 50% of a group of animals. The methodology is presented in detail in the Turner and Fairhurst (1993) paper, but some key points are noted here.

The starting point is to work from single, short-term (i.e. up to 4 hours duration) inhalation exposure studies in animals. In a real-life major accident situation, residents in the vicinity of a COMAH site might be exposed for a matter of minutes as the toxic cloud might be dispersed rapidly by wind. However, in some weather conditions, people could be exposed for a matter of hours. Looking at the SLOT criteria, it can be seen that they reflect exposure conditions just on the verge of causing a low percentage of deaths in the exposed population. Hence, we take conditions producing around 1% mortality in animals as being representative of SLOT conditions. To directly observe 1% mortality (LC_1) a group size of at least 100 animals is needed, whereas group sizes of 5 or 10 rats or mice are typically used in routine toxicity tests. In deriving the DTL, the available acute toxicity data from different species is compared and the data from the most sensitive animal species is used, unless there are good grounds to consider that this would be inappropriate.

Where there are sufficient dose-response data points it might be possible to derive the 1% mortality conditions using probit analysis or estimate the values by judgement. Where insufficient data are available to do this, then we take a default approach of simply dividing the LC_{50} by 4. We should now have one value of t and one value of c, which when taken together represent an estimate of the exposure conditions producing the LUP SLOT.

The next step is to determine the value of n in the $c^n t$ = DTL equation. If the LC_{50} has been experimentally determined for several time periods, preferably within the same study, then n can be calculated using a linear regression approach. If there are no data to derive n, then n is usually taken to be 1, as a default position.

We can now insert the pair of c and t values representing one set of exposure conditions predicted to produce the LUP SLOT together with the value of n into the $c^n t$ = DTL equation. The DTL equation can be used to calculate all sets of exposure conditions that would produce the LUP SLOT.

A similar procedure can be followed to derive a toxic load equation to predict exposure conditions producing any other specified level of toxicity that may be of interest. For example a DTL relating to the mortality of 50% of an exposed population, a specified level known as the **SLOD DTL**, can be determined.

There are many limitations to the approach described above, such as difficulties extrapolating animal data to humans, lack of relevant toxicity data, the use of animal data of poor or unknown quality, frequent use of the default assumption that n in the $c^n t$ = DTL equation is equal to 1 and uncertainties about the universal applicability of the $c^n t$ concept. However, the described approach is probably the best that can be achieved with the available data and current state of scientific knowledge. HSE believes that it is important in regulatory toxicology to use consistent and transparent methodology, and this approach remains central to our DTL assessments.

Sometimes there is a need for a DTL for a substance with no acute toxicity data. One way around this problem is to base the DTL assessment on the known toxic properties of a structurally related substance- known as a read-across, or SAR approach. This is an uncertain process that requires a high level of professional judgement. Alternatively, it may be recommended that data relating to an exemplar substance be used. Exemplar substances are usually the most toxicologically potent substances among those that have previously been assessed by HSE. The exemplar should have similar physical properties (e.g. solid, liquid or gas) to the substance for which a DTL cannot be determined.

9.3. The Use of Toxicology Data in COMAH Safety Reports

When preparing Safety Reports under the COMAH Regulations, authors are required to provide estimates of the extent (i.e. hazard ranges and widths) and severity (i.e. how many people are affected, including the numbers of fatalities) of the consequences of each identified major accident hazard. For an evenly distributed population, the number of fatalities resulting from a toxic release may be approximated by estimating the number of people inside the concentration contour leading to an LD_{50} dose (i.e. SLOD DTL). This approximation results from the assumption that those people inside the SLOD contour who do not die (due to factors such as

physiology, fitness levels, etc) will be balanced by an approximately equal number outside the SLOD contour who do die (again, due to factors such as physiology, state of health etc.)

Further, the number of people injured (serious and minor) by the release may be approximated by the number people estimated to be between the SLOD and SLOT DTL contours (i.e. the SLOT DTL contour is taken as a pragmatic limit for injuries).

When estimating the numbers of people affected, authors should bear in mind that a proportion of the population will be indoors. This will provide a degree of protection against the effects of the release as compared to being outdoors. The level of protection is related to the rate at which air and toxic material enters the building and may be measured in air changes per hour (ACH). Models exist (see Davies and Purdy, 1986) to determine the outdoor concentration required to give an indoor SLOT or SLOD DTL dose. This (usually higher) outdoor concentration effectively defines the hazard range for people inside buildings.

9.4.H_2S

The following is from the Derivation of Exposure Conditions for Land-Use Planning SLOT (Specified level of Toxicity) in the Toxicology of Substances in Relation to Major Hazards – Hydrogen Sulphide (Turner and Fairhurst 1990)

As indicated earlier, two distinct mechanisms of toxicity are operating under single, high exposure conditions - inhibition of cytochrome oxidase leading to respiratory arrest, and production of pulmonary oedema. Under each of the many sets of exposure conditions of interest, each of these mechanisms will contribute, to varying degrees, in the overall extent of toxicity observed. The relationship between atmospheric concentration (c) and exposure period (t) for each mechanism of toxicity will probably be different, such that no one consistent relationship between c and t, in terms of the overall extent of toxicity seen, will be evident. Also, many acute inhalation studies on H_2S in animals have been of the 'time-to-effect', rather than 'severity of effect observed post-exposure' type. Such studies should be examined with considerable caution when attempting to derive a relationship between c and t for severity of effect, as is required in this assessment. Furthermore, only one study has examined the c/t relationship directly, and even then relatively long (2-6 hours) exposure periods were used and no clear relationship emerged.

The above factors suggest that the general step-wise approach to deriving the 'dangerous toxic load', outlined in Assessment of the Toxicity of Major Hazard substances(1) and used in previous papers on other substances, should be modified in this particular case. .The most appropriate approach would appear to be to consider whether a general pattern of responsiveness emerges from scrutiny of the data as a whole.

(a) 'SLOT' conditions predicted from animal data

In the studies on H_2S conducted in animals no one species or strain clearly emerges as being the most sensitive. Beginning with the fixed-duration/post- exposure observation studies, of which there are relatively few, two recent and apparently well-conducted studies in rats exposed for 4 hours yielded LCso values of 444 and 501 ppm. In each study the exposure- response curve was

steep. In the study yielding an LC50 of 501 ppm, the LC10 was 422 ppm; in the study for which an LC50 of 444 ppm was obtained, 30% mortality was observed at 400 ppm, the lowest concentration tested. Another rather old (1925) study in rats indicated that in atmospheres of 310-350 ppm H_2S, rats died in 1-8 hours. These and other data available from rat studies suggest that exposure to 300 ppm for four hours would produce pronounced eye, nose and respiratory tract irritation, respiratory distress and a low percentage mortality (perhaps around 1 %) in rats. Moving from this point to shorter exposure times, the data available in rats, mainly involving observations during exposure, suggest that a similar level of toxicity would be produced in rats exposed to about 400 ppm for one hour. At 800 ppm, exposure for only a period of between seconds and a few (~5) minutes would be predicted to produce unconsciousness in the more susceptible rats.

The data available from other animal species suggest a generally similar degree of sensitivity - only relatively small interspecies differences are evident. Rabbits and cats appeared somewhat less sensitive than rats, and the responsiveness of mice, guinea pigs and dogs was similar to that of rats, although in mice and guinea pigs longer exposures of around 8 hours could be expected to produce serious toxic effects with occasional deaths at around 100 ppm. The one study available in Rhesus monkeys, involving only two animals, suggests that exposure to 500 ppm H_2S for 20-30 minutes should be viewed with great concern.

Overall, the available animal data lead to the following predictions for land-use planning application SLOT conditions:

- 300 ppm for 4 hours
- 400 ppm for 1 hour
- 500 ppm for 30 minutes
- 800 ppm as an exposure time-independent 'ceiling' concentration, ie a concentration of concern irrespective of exposure duration.

(b) Comparison of predicted 'SLOT' conditions with available human data

Looking at the information on the effects of single exposure to hydrogen sulphide in humans, although few reliable measures of exposure concentration and duration are available, the data do appear to be consistent with the above set of SLOT c and t values. The one exception is a report of a man being rendered unconscious by exposure to 230 ppm for 'at least 20 minutes'. However, there is clearly uncertainty about the length of exposure and the exposure concentration was estimated from measurements made after the incident. Thus there is some doubt as to the precise exposure conditions encountered in this one case.

(c) SLOT conditions and derived DTL relationship

From the above discussion it is suggested that the set of c and t values given above in (a) represent a reasonable prediction of conditions resulting in the land-use planning application SLOT. In order to predict all combinations of atmospheric concentration and exposure period resulting in this SLOT it is highly desirable that, if possible, a dangerous toxic load (DTL)

relationship is derived. In fact, a plot of the values 300 ppm/4 hours, 400 ppm/1 hour, 500 ppm/30 minutes reveals a close fit with the relationship c^4t. Therefore, for practical purposes it is suggested that this DTL relationship is used in combination with an exposure time-independent 'ceiling' concentration of 800 ppm. Substitution of the values 300 ppm/4 hours into the above c/t relationship gives the following DTL constant:

- DTL = 2×10^{12} $ppm^4 min$

Using this equation, the value of c reaches the 'ceiling' of 800 ppm when t is equal to 5 minutes or less. Therefore, some examples of predicted land-use planning application SLOT conditions are:

	Exposure Period (minutes)						
	0-5	10	15	30	60	12	240
Atmospheric concentration (ppm)	800	669	604	508	427	359	302

The following summary is available from the web page link:

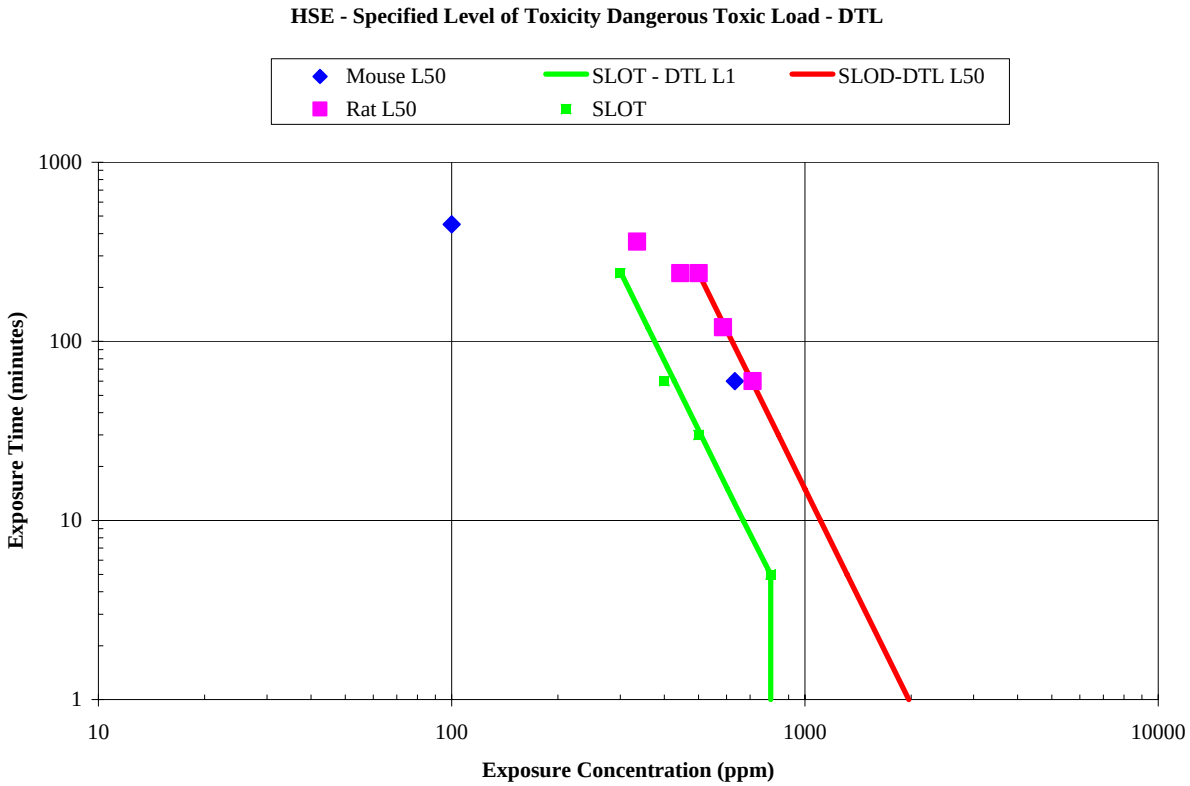
Substance	**'n' value**	**SLOT DTL**	**SLOD DTL**
Hydrogen Sulphide	4	2×10^{12}	1.5×10^{13}

9.5. Discussion

a. Pragmatic approach used, for example rounding of exponent to 4, eyeball best fit lines, see figure below

b. No Uncertainty Factor applied from rat and mouse L50 to human SLOD-L50

c. The default severity and effect factor of 4 used to adjust the L50 to L1 was not used for H_2S, rather a value of 5.5 was used based on the data.


HSE - Specified Level of Toxicity Dangerous Toxic Load - DTL
Mouse L50
SLOT - DTL L1
SLOD-DTL L50
Rat L50
SLOT
1000
100
10
1
Exposure Time (minutes)
10
100
1000
10000
Exposure Concentration (ppm)

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Appendix B

Toxicological Justification of the Triple Shifted Rijnmond Equation

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TOXCON Consulting Ltd.
Edmonton Alberta

April 1990

ERCB Technical Paper

from ERCB Report 90-B
Volume 7

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Toxicological Justification of the Triple Shifted Rijnmond Equation

The GASRISK model uses probit analysis to estimate the probability of lethality of H_2S in a population of humans. This method was determined by Concord Scientific Corporation (CSC) as being the simplest way of incorporating existing toxicological data into the computational model. By this method, the probit function relates population response to the inhaled dose of H_2S. The latter parameter is calculated using the concept of "toxic load" as defined by Equation 1 below:

$$L = \chi^n \cdot t_E \tag{1}$$

where L = toxic load (units = $ppm^n \cdot min$)

χ = H_2S concentration (units = ppm)

t_E = exposure time

n = constant exponent (usually > 1.0)

The toxicological outcome of the combination of χ^n and t_E is non-linear with the value of n ranging from 2.0 - 3.0 for a variety of toxic gases including H_2S (ten berge *et al*, 1986).

In biological populations, the probability of a severe adverse effect such as lethality is assumed to be log-normally distributed. This is created by the differential susceptibility of individuals within the population, i.e. some are very sensitive to the same toxic load while others are very resistant. The corresponding probit function defining this phenomena is given in Equation 2.

$$Y = k_2 ln(L) + k_1 \tag{2}$$

In order to employ the probit approach to estimate probability of lethality, values for k_1, k_2 and n must be derived from the toxicological literature. CSC undertook a limited review of existing toxicological information on humans and animals in order to derive these variables. From this analysis, the Triple-Shifted Rijnmond equation was generated by CSC. Using the values of k_1 = -36.2, k_2 = 2.366 and n = 2.5, CSC then calculated fatal H_2S concentrations (ppm) for selected exposure times. Their data is presented in Table 5.4 of their report.

A more extensive review was undertaken by Dr. R. Rogers of known cases of animal and human lethality in the H_2S literature. The results of this study (summarized in Figures B-1, B-2, B-3, Table B-1 and B-2) clearly validate CSC's conclusion that the Triple-Shifted Rijnmond equation more accurately fits human and animal lethality data reported in the literature.

An examination of the family of curves for different species in Figure B-1 reveals that different species vary in their sensitivity to lethal concentration-time combinations of H_2S exposure. Birds (e.g. canaries, doves) appear to be the most sensitive species while mice, rats, guinea pigs, dogs and goats are more resistant. In fact, the data suggests that these species respond very similarly to different concentration-time concentrations, i.e. there is no clear separation of curves for each species. The experience for man is more variable as evidenced by the greater scatter of the data points. For all species, however, there is a general sigmoidal distribution on the log-log plot of exposure of time versus concentration.

The curves illustrate that lower concentrations of H_2S will produce lethality at long exposure times while high concentrations of H_2S will produce lethality in short periods of time for all species. This general relationship implies that H_2S is affecting the physiological response of each species in a similar fashion, perhaps through the inhibition of cytochrome oxidase.

In Figure B-2, the probit plots have been overlaid on the original data. An examination of the original L_{50} Rijnmond plot suggests that an H_2S concentration of 1000 ppm would require an exposure time of approximately 12 minutes to produce lethality in 50 percent of the exposed population. For the Triple-Shifted Rijnmond plot, this same concentration would require only 1.5 minutes to produce lethality. Experience with acute H_2S exposures in the oil and gas industry within Alberta suggests that exposure to levels of H_2S at 1000 ppm is rapidly fatal. Thus, the Triple-Shifted curve appears to more accurately reflect human experience in Alberta. A comparison of the original Rijnmond plot to the Triple-Shifted plot suggests that the latter is more conservative in its prediction of lethality. This is best understood if one notes that at exposure times greater than 5 minutes, most of the data points fall to the right of the Triple-Shifted plot, i.e. this plot will predict lethality when the data would suggest that minimal lethality would occur. This leads to the conclusion that long exposures (e.g. > 3 hr) appear to be safe by a factor of 2 with respect to the H_2S concentration.

The fact that the Triple-Shifted L_{50} curve lies close to the canary curve suggests that in order for this curve to be applicable to the human situation, humans would have to be as sensitive to H_2S as canaries. This clearly is unlikely for the average individual. But what about the so-called hypersusceptibles within the population, i.e. asthmatics, the elderly and those with severe respiratory disease? In this case, the Triple-Shifted curve is probably a more accurate predictor of their response.

One other factor that appears to have a direct bearing on the selection of the most appropriate probit plot is the level of activity of the individuals. Withers and Lees (1985).

Figure B-3 is an enlargement of the more congested portion of the data set. It shows certain data points more clearly.

FIGURE B-1

TOXIC LOAD - HYDROGEN SULPHIDE

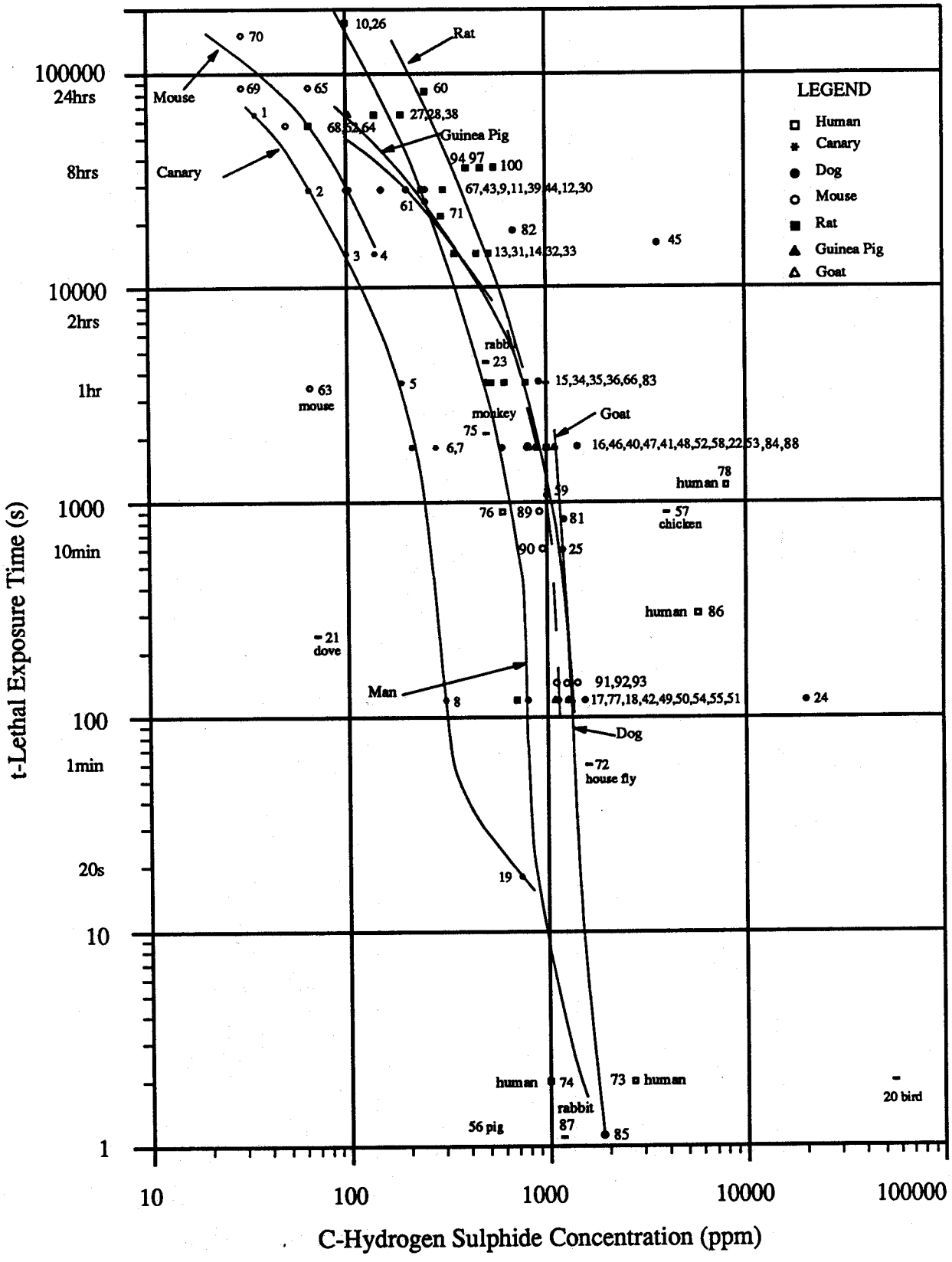

FIGURE B-2

TOXIC LOAD - HYDROGEN SULPHIDE

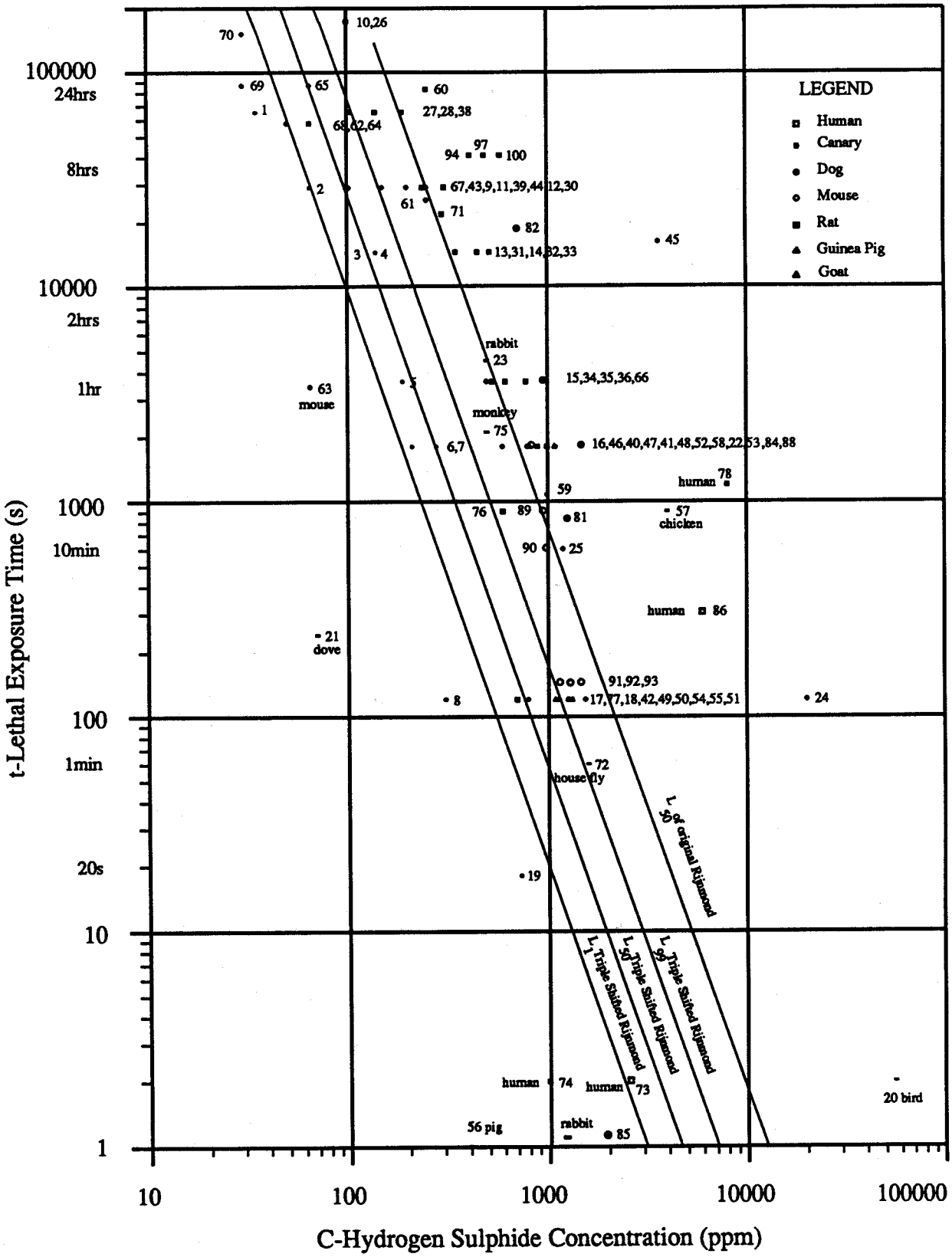

FIGURE B-3

TOXIC LOAD-HYDROGEN SULPHIDE

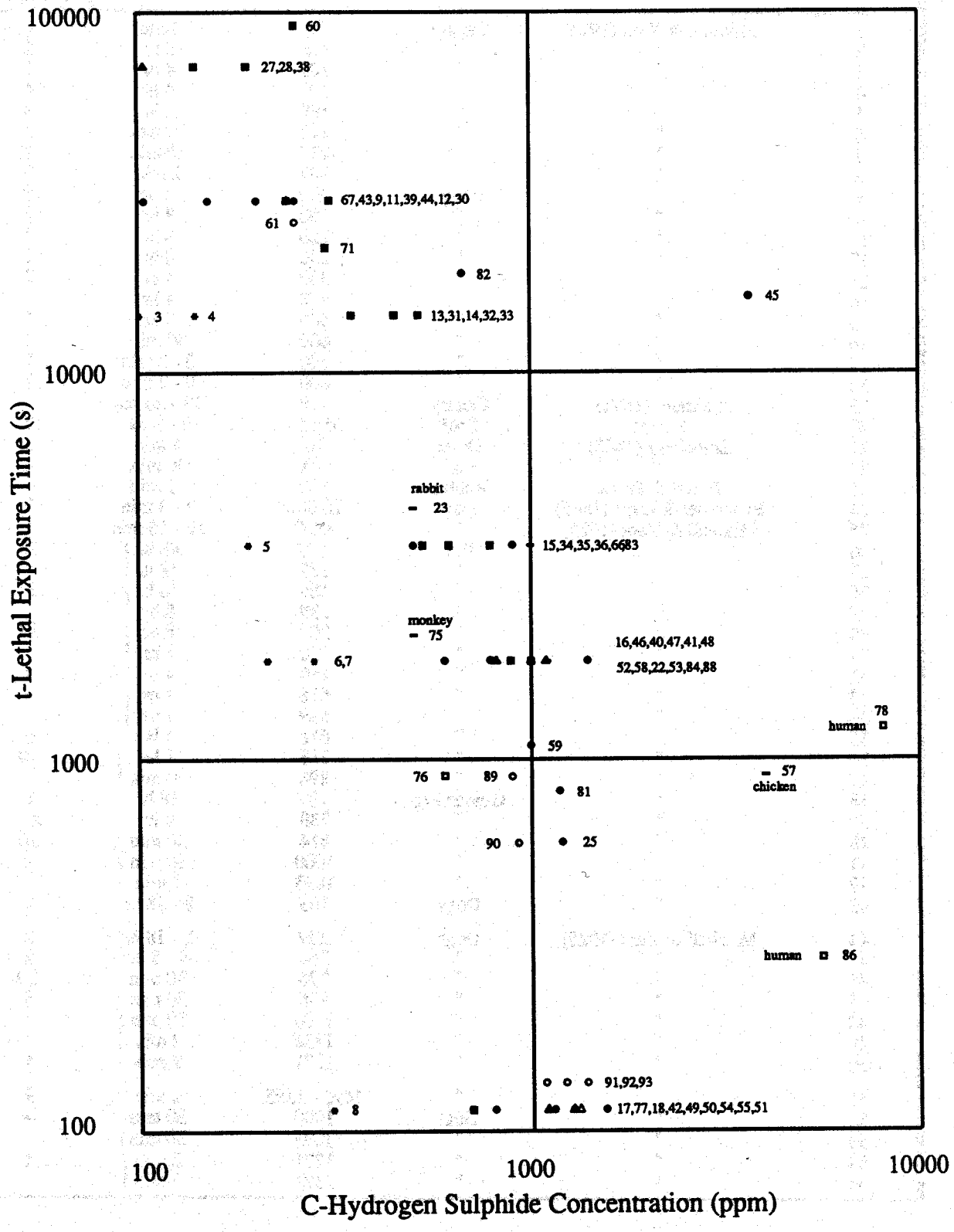

Table B-1

Lethality Data

Point #	Source	Species	Conc (ppm)	t_e	# of humans or animals
1	Mitchell & Yant (1925)	Canary	35	18 hr	2
2	"	"	65	8 hr	2
3	"	"	100	4 hr	6
4	"	"	139	4 hr	4
5	"	"	189	1 hr	4
6	"	"	211	30 min	3
7	"	"	278	30 min	?
8	"	"	307	2 min	?
9	"	Dog	100	48 hr	?
10	"	"	150	8 hr	?
11	"	"	200	8 hr	?
12	"	"	250	8 hr	?
13	"	"	350	4 hr	?
14	"	"	450	4 hr	?
15	"	"	500	1 hr	?
16	"	"	600	30 min	?
17	"	"	700	0 - 2 min	?
18	"	"	800	0 - 2 min	?
19	Lehman (1892)	Canary	729	18 - 20 sec	
20	Barker	Bird	55,555	0 - 2 sec	?
21	Eulenberg (1865)	Dove	70	4 min	1
22	"	Cat	1100	30 min	1
23	Biefel & Polek	Rabbit	500	75 min	1
24	Brouardel & Loye (1885)	Dog	20,000	2 - 3 min	?
25	Mitchell & Yant (1925)	"	1200	10 - 15 min	?
26	"	Rat	100	48 hr	19
27	"	"	139	18 hr	
28	"	"	189	18 hr	17
29	"	"	239	8 hr	
30	"	"	307	8 hr	13
31	"	"	350	4 hr	
32	"	"	450	4 hr	2
33	"	"	518	4 hr	3
34	"	"	529	1 hr	
35	"	"	618	1 hr	3
36	"	"	786	1 hr	40
37	"	"	896	30 min	
38	"	Guinea Pig	103	18 hr	2
39	"	"	239	8 hr	2/3
40	"	"	814	30 min	10
41	"	"	1000	30 min	2
42	"	"	1093	2 min	
43	"	Dogs	103	8 - 18 hr	2
44	Mitchell & Yant (1925)	Dogs	239	8 - 18 hr	2
45	"	"	350	4 - 8 hr	2
46	"	"	796	30 min	1/2
47	"	"	886	30 min	3
48	"	"	1000	30 min	8
49	"	"	1136	2 min	
50	"	"	1271	2 min	4
51	"	"	1493 - 1593	2 min	9
52	"	Goat	1000	30 min	4
53	"	"	1093	30 min	
54	"	"	1271	2 min	4
55	"	"	1321	2 min	

Table B-1 (Continued)

Lethality Data

56	O'Donoghue (1961)	Pig	400	1 sec	1
57	Klentz & Fedde (1976)	Chicken	4000	15 min	?
58	Weedon et al (1940)	Rat	1000	29 - 37 min	8
59	"	Mice	1000	18 - 20 min	4
60	"	Rat	250	23 hr	3/8
61	"	Mouse	250	7 hr	4/4
62	"	Rat	65	16 hr	1/8
63	"	Mouse	65	57 min	1/4
64	"	"	65	16 hr	3/4
65	"	"	65	24 hr	1/4
66	"	House Fly	1000	1 hr	87/100
67	Hays (1972)	Mouse	100	8 hr	3/8
68	"	"	50	16 hr	
69	"	"	30	24 hr	3/8
70	"	"	30	42 hr	2/8
71	Alta. Envt. Centre (1986)	Rat	300	6 hr	12/12
72	Evans	House Fly	1600	1 - 2 min	90/100
73	Prouza (1970)	Humans	1000	< 1 min	1/10
74	Niosh (1977)	"	1000	2 sec	1/1
75	Milby (1962)	Monkey	500	35 min	1/3
76	"	Man	600	15 min	
77	"	"	700	2 min	
78	McCabe & Clayton (1952)	"	~8000	20 min	22/320
79	Mitchell & Yant (1925)	"	50 - 100	8 - 48 hr	0/1
	"	"	100 - 150	8 - 48 hr	?
	"	"	150 - 200	8 - 48 hr	?
	"	"	250 - 350	4 - 8 hr	?
	"	"	350 - 450	4 - 8 hr	?
	"	"	500 - 600	15 - 60 min	?
	"	"	700	0 - 2 min	?
	"	"	700 - 785	0 - 2 min	?
	"	Dogs	1200	10 min	?
80	Sandage (1961)	Rat	20	90 days @ 24 hrs/day	20/100
81	Haggard (1921)	Dog	1000	15 min	?
82	Haggard (1925)	"	500 - 700	several hrs	?
83	"	"	900	< 1 hr	?
84	"	"	1500	15 - 30 min	?
85	"	"	1800	immediate	?
86	Winek et al (1968)	Human	6100	< 5 min	1/1
87	O'Donoghue (1961)	Rabbit	1000	1 sec	1/3
88	Clanechan (1979)	Mouse	800	30 min	1/20
89	"	"	900	15 min	2/20
90	"	"	1000	10 min	3/46
91	"	"	1100	2.5 min	1/20
92	"	"	1200	2.5 min	2/40
93	"	"	1300	2.5 min	3/20
94	Tansy et al (1981)	Rat	400		3/10
95	"	"	440		/10
96	"	"	475		/10
97	"	"	500		8/10
98	"	"	525		8/10
99	"	"	554		9/10
100	"	"	600		10/10

Table B-2

Reports of Lethality for H_2S

Reference	Details
20 Barker	1 part H_2S/18 parts air---kills birds immediately (55,000 ppm) 1 part H_2S/210 parts air---asphyxiated dogs (4761 ppm) (no time given)
21 & 22 Eulenberg (1865) (see Mitchell, 1924 for reference)	1000 ppm---fatal for cats, rabbits & doves "within a short time" dove killed in 4 min @ 0.007% (70 ppm) 140 ppm for 10 min---no effect on cat but; 70 ppm for 25 min---asphyxia (slower death) 1100 ppm for 30 min---death (more immediate)
23 Biefel & Polek (1880)	500 ppm for 75 min---death of rabbit
24 Brouardel & Loye (1885)	dogs---20,000 ppm---death 2 - 3 min
67 to 70 Hays (1972)	mice (3/8 female mice) died for each of 100 ppm amd 30 ppm/8 hr exposure. Modified lethal concentration duration 50 = 7.5 hr
72 Evans	house flies (90% killed) after 1 - 2 min. exposure/1600 ppm
78 McCabe	Poza Rica, Mexico 160,000 ppm H_2S 22 deaths/320 hospitalized exposure duration ~ 20 minutes (not known if instantaneous, intermediate or continuous) 22 deaths.....9 dead on arrival .....4 dead within 2 hours .....4 dead within 6 hours .....1 @ 24 hours .....1 @ 48 hours .....1 @ 5 days .....1 @ 6 days .....1 @ 9 days H_2S ~ 31,000 ppm
79 Mitchell & Yant (1925)	A. Man 50 - 100 ppm 8 - 48 hr--no effect 100 - 150 ppm 8 - 48 hr--death 150 - 200 ppm 8 - 48 hr--death 250 - 350 ppm 4 - 8 hr--death 350 - 450 ppm 4 - 8 hr--death 500 - 600 ppm 15 - 60 min--death 700 ppm 0 - 2 min--death 700 - 785 ppm 0 - 2 min--death B. Dogs: 1200 ppm for 10 min--death (10 - 15 min)
94 - 100 Tansy et al (1981)	Sprague-Dawley rats (male & female) LC_{50} = 444 ppm (4 hr)
Kleinfeld (1964)	89 people exposed to H_2S; 12 people severe - 2/12 died First man - ~ 30 min exposure; conc. unknown Second man - same

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

PREPARED FOR:

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Attention: Gary Neilson

July 07, 2005

Project No. 88070

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ACKNOWLEDGEMENTS

In preparing this report entitled *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*, CANTOX ENVIRONMENTAL INC. ("the consultant") relied on the assistance of a number of individuals.

The consultant first wishes to acknowledge the contributions of Ms. Angela Jones and her fellow librarians at the Alberta Energy and Utilities Board for their assistance in obtaining copies of the various scientific papers that formed the basis of the review. Their help in retrieving the papers in an organized and timely manner allowed the review to proceed on schedule in its entirety.

The consultant also wishes to acknowledge the assistance received from Mr. Michael Gressel of the U.S. National Institute for Occupational Safety and Health, Centre for Disease Control and Prevention in retrieving and providing English versions of certain of the scientific papers published in foreign-language journals. His assistance helped to further the review.

The consultant also wishes to thank Mrs. Rhona Taylor for her assistance and advice in performing the review. Her objectivity and attention to detail helped to ensure that the review met the requisite standards demanded of this type of work.

Finally, the consultant wishes to acknowledge the contributions of Mr. Gary Neilson of the Alberta Energy and Utilities Board for coordinating many aspects of the review. His support and encouragement allowed the review to proceed smoothly with full respect for the need for consistency, objectivity and scientific rigour.

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July 2005

EXECUTIVE SUMMARY

In December 2003, the Alberta Energy and Utilities Board (AEUB) published a series of proposed requirements for calculating Emergency Planning Zones (EPZs) for "sour" gas wells, pipelines and production facilities.[1] The proposed requirements were developed in response to recommendations concerning emergency planning and preparedness made by the Provincial Advisory Committee on Public Safety and Sour Gas.[2] In order to facilitate meaningful stakeholder consultation with respect to the proposed requirements, the AEUB subsequently issued a Discussion Paper outlining the basis and substance of the proposed methodology to be used for determining EPZs.[3] The methodology included the calculation of an EPZ "endpoint" for hydrogen sulphide (H_2S), with the endpoint defining a hypothetical exposure at which serious irreversible health effects, including fatalities, would not be expected among the general public in the event of an emergency involving the release of "sour" gas, with a conservative margin of safety incorporated. The calculation embraced mathematics that considered both exposure concentration and exposure time, and relied on health effects data on H_2S published in the scientific literature, with an emphasis on lethality data.

Comments on the Discussion Paper were invited from a number of different stakeholders, and a workshop was held to gather input concerning the proposed approach.[4] In response to the comments received, the AEUB elected to commission a review of the technical quality of the health effects information that served as the basis of the proposed EPZ endpoint(s) for H_2S. The intent was to ensure that:

- The health effects data were both representative and technically sound.
- The technical quality of the health effects data was such that the information could be used with confidence to develop a scientifically defensible EPZ endpoint(s).
- The health effects data were appropriate for the determination of the concentration-time-response characteristics of H_2S in terms of serious, irreversible outcomes, most notably lethality.

The review was performed by CANTOX ENVIRONMENTAL INC. ("the consultant"), and completed in accordance with the Terms of Reference developed for the work. A total of 21 papers comprising 25 original health effects studies and/or summaries of health effects data on H_2S were reviewed. Each of the papers was cited in the Discussion Paper and given consideration as part of the EPZ endpoint calculations (… albeit the endpoint calculations ultimately relied on a small subset of papers only, with emphasis on exposure concentration-exposure time combinations corresponding to LC_{50} values). The papers included non-clinical studies involving controlled exposures of test animals to H_2S, clinical investigations involving controlled exposures of human subjects, case reports describing accidental exposures in the workplace, and review articles summarizing health effects data

[1] See www.eub.gov.ab.ca/BBS/new/Projects/sgr.htm.
[2] Provincial Advisory Committee of Public Safety and Sour Gas. 2000. Findings and Recommendations – Final Report, December 2000.
[3] Alberta Energy and Utilities Board. 2004. Proposed Hydrogen Sulphide Endpoints for Emergency Response Planning – A Discussion Paper for the November 26 Stakeholder Meeting, October 2004.
[4] A multi-stakeholder workshop was convened by the AEUB on November 26, 2004 in Calgary, AB.

gathered by others. Much of the information reviewed concerned the health effects associated with short-term inhalation exposures to H_2S, with an emphasis on exposures causing death.

The review consisted largely of comparison of the design, conduct and reporting features of each study against a series of "quality benchmarks". The benchmarks were based on the recommendations of a number of leading scientific and regulatory authorities for the proper design, execution and reporting of health effects studies, including the U.S. Environmental Protection Agency (US EPA), the Organization for Economic Co-operation and Development (OECD), the European Union (EU), and the Society of Toxicology (SOT). Each study was graded in terms of how well the design, conduct and reporting features matched the recommendations. A grading system was developed to distinguish between low *vs*. moderate *vs*. high quality studies as well as to identify any studies having no practical value. The grading system was intended principally to gauge the adequacy and usefulness of each study in terms of advancing understanding of the concentration-time-response characteristics of H_2S vis-à-vis lethality following short-term exposure.

The principal findings that emerged from the work were:

- None of the studies received a "high" rating, signifying that each of the studies suffered from one or more weaknesses that detracted from its usefulness and limited the level of confidence that could be assigned to its findings and conclusions. The lack of high grades was due, in part, to the age of most of the studies, with many pre-dating the recommended testing guidelines (circa 1980). Some of the studies were performed in the late 1800's using archaic designs, makeshift equipment, and poor reporting standards. The absence of high grades also may have resulted from the strict application of the "quality benchmarks" throughout the review, which demanded that each study meet very stringent and exacting standards. In some cases, the weaknesses were modest, allowing a "moderate-to-high" grade to be assigned.
- A number of the studies (≈40%) received a "low" grade, signalling significant deficiencies in experimental design, conduct and reporting that seriously detracted from their usefulness. Weaknesses common to these studies included: inadequate description of equipment, including the exposure chamber, gas delivery system and/or metering devices; use of makeshift and "dated" instrumentation and insensitive analytical methods; failure to analytically confirm the concentrations of H_2S to which the test animals or human subjects were exposed; failure to maintain uniform concentrations of H_2S in the exposure chamber; inadequacies with respect to the number of test animals/subjects employed; general lack of detail concerning test animals (*i.e*., source, strain, age, sex, pre-study health status) and animal husbandry; and, inattention to detail leading to "accidental" exposures because of equipment malfunction or technician error.
- Approximately 40% of the studies received a "moderate" or higher grade, signifying that the findings and conclusions are reasonably technically robust, and that the data add to understanding of the concentration-time-response characteristics of H_2S vis-à-vis lethality. These data were judged to be

suitable for use in "toxic load" calculations.

- The remaining 20% of the studies were deemed to be of no practical use in providing an understanding of the concentration-time-response characteristics of H_2S vis-à-vis lethality. In most instances, these studies either lacked fundamental information or provided information that could not be substantiated. In some cases, the information was irrelevant.
- With one exception, the subset of studies specifically selected by the AEUB for the calculation of the EPZ endpoints for H_2S received a grade of "moderate" ... signifying that the dataset selected was fit-for-purpose and scientifically defensible. In the consultant's opinion, the findings and conclusions from these studies can be accepted with a reasonable degree of confidence. Despite some weaknesses, the results from the studies add to understanding of the concentration-time-response characteristics of H_2S vis-à-vis lethality, and were judged to be suitable for use in "toxic load" calculations. The exception was a review article (Back *et al.* (1972), which the consultant concluded was simply a summary of data originally collected by MacEwen and Vernot (1972). The former study was deemed to be of no practical use, whereas the latter study received a "moderate" grade.

The principal conclusions and recommendations arising from the work are:

- The outcomes and conclusions reached in the Discussion Paper relating to the proposed EPZ endpoints for H_2S are based on moderate quality studies. The lethality data upon which the endpoints are based are reasonably technically robust and defensible. They originate from studies that achieved "moderate" scores when reviewed against very strict standards for proper design, execution and reporting.
- For added refinement, the EPZ endpoints should be re-calculated with the data from the paper by Back *et al*. (1972) removed. The paper was deemed to be of "no practical use" since, according to the consultant, it is simply a review article summarizing original data collected by others (MacEwan and Vernot, 1972). Use of the summary data in the calculations is redundant and misleading since it assigns extra weight to the original findings, possibly skewing the outcome.
- The EPZ endpoints might benefit from a broader literature search to identify other health effects studies that might contribute to added understanding of the concentration-time-response characteristics of H_2S vis-à-vis lethality following short-term exposure. The subset of studies that formed the basis of the "toxic load" calculations on which the endpoints were based was somewhat narrow in breadth, consisting of three studies only. Other reliable studies may exist to complement the subset.
- The EPZ endpoints also might benefit from examination of exposure concentration-exposure time combinations beyond those corresponding to LC_{50} values.[5] It might be equally useful to examine combinations associated with no lethality … or alternatively, combinations at

[5] The proposed EPZ endpoints were based strictly on exposure concentration-exposure time combinations corresponding to LC_{50} values, which were then adjusted through the use of uncertainty factors to afford the level of protection demanded (*i.e.*, protection against serious irreversible health effects, including fatalities, with a conservative margin of safety).

which deaths are first reported or combinations corresponding to LC_{10} values or some other lower lethality index. The results of "toxic load" calculations using these alternate combinations could be used to expand and/or validate the outcomes and conclusions reached in the Discussion Paper.

- Some attempt should be made to explore the impact of differences in physiology, anatomy and metabolism between humans and laboratory rodents on the outcome of the "toxic load" calculations used to determine the EPZ endpoints. These differences will influence the total "dose" of H_2S received, which, in turn, will govern the nature and severity of any response, including lethality. Since the proposed endpoints are based entirely on lethality data from studies with mice and rats, their relevance to the human condition should be carefully examined, taking the above differences into consideration.

1.0 BACKGROUND AND INTRODUCTION

In December 2003, the Alberta Energy and Utilities Board (AEUB) published a series of proposed requirements for calculating Emergency Planning Zones (EPZs) for "sour" gas wells, pipelines and production facilities.[6] The proposed requirements were developed in response to recommendations concerning emergency planning and preparedness made by the Provincial Advisory Committee on Public Safety and Sour Gas in its final report entitled *Findings and Recommendations – Final Report.*[7] In order to facilitate meaningful stakeholder consultation with respect to the proposed requirements, the AEUB subsequently issued a Discussion Paper outlining the basis and substance of the proposed methodology to be used for determining EPZs.[8] Comments were invited from a number of different stakeholders, and a workshop was held to gather input concerning the proposed approach.[9] The comments are now under review by the AEUB.

The main features of the proposed methodology as outlined in the Discussion Paper are as follows:

- The EPZ defines the priority area in which immediate action must be taken to protect people in the event of a "sour" gas release.
- The immediate action is intended to protect people from serious, irreversible health effects, including fatalities.
- The EPZ will be defined on the basis of a series of calculations supported by the EUBMODELS® Emergency Planning Tool.
- A key input to the EUBMODELS is the EPZ "endpoint" for hydrogen sulphide (H_2S). The endpoint is a combination of H_2S concentration and exposure time at which serious, irreversible health effects, including death, will be avoided through prompt action.[10]
- The development of the EPZ endpoint(s) relies on the "toxic load" approach, which embodies the mathematics relating exposure concentration and exposure time, coupled with the use of uncertainty factors to ensure the necessary level of protection.
- The "toxic load" approach, in turn, relies on the review and interpretation of health effects data on H_2S specific to the endpoint of concern (*e.g.*, lethality), with consideration given to the influence of both concentration and exposure time on the outcome.
- The EUBMODELS then uses dispersion modeling of an uncontrolled release of "sour" gas to estimate the distance to the pre-determined emergency planning criterion as the basis for defining the EPZ.

As part of the development of the proposed approach (… and, more specifically, the determination of the proposed EPZ endpoints for H_2S), the AEUB relied largely on health effects data for H_2S referenced by other regulatory authorities and used as the basis of emergency response planning guidelines in their respective jurisdictions. A complete listing of the health effects literature considered by the Board can be found in Appendix 2 of the Discussion Paper. A summary listing of the various jurisdictions and guidelines considered

[6] See www.eub.gov.ab.ca/BBS/new/Projects/sgr.htm.
[7] Provincial Advisory Committee of Public Safety and Sour Gas. 2000. Findings and Recommendations – Final Report, December 2000.
[8] Alberta Energy and Utilities Board. 2004. Proposed Hydrogen Sulphide Endpoints for Emergency Response Planning – A Discussion Paper for the November 26 Stakeholder Meeting, October 2004.
[9] A multi-stakeholder workshop was convened by the AEUB on November 26, 2004 in Calgary, AB.

[10] For the purposes of the Discussion Paper, the AEUB proposed an endpoint "range" of $1.2x10^{10}$ to $1.2x10^{11}$ ppm^4minute. The range reflects the use of 100-fold and 1000-fold uncertainty factors.

is shown in Table 1-1. The health effects data were assembled, reviewed and arranged in the context of the "toxic load" approach (*i.e.*, segregated by exposure concentration and exposure time) in order to ultimately derive the proposed EPZ endpoints. Some key items concerning the retrieval and review of the health effects data by the AEUB are listed below:

- The collection of health effects data was not based on an exhaustive search. As indicated above, the search was limited largely to data cited by other regulatory authorities as part of the development of emergency planning guidelines.
- The original scientific papers supporting the health effects information were not retrieved and reviewed. The full extent of data available in the original papers was not necessarily captured in the documentation prepared by the other authorities … and accordingly, was not available to the AEUB as part of its review.
- Only lethality data were reviewed and incorporated into the "toxic load" calculations.
- Reliance was placed largely on health effects data sourced from non-clinical laboratory studies (*i.e.*, animal testing) involving exposure of rats and mice. Few studies with humans exposed to the concentrations of H_2S under consideration were identified.
- Only studies that provided LC_{50} data were used to calculate the proposed EPZ endpoints.[11]
- Lethality data involving exposures to H_2S for greater than three (3) hours were excluded from the calculations.[12]
- The technical quality of the health effects information vis-à-vis the scientific integrity of the design, conduct and reporting of the studies was not assessed.
- A total of 23 studies were included in the original dataset examined by the AEUB. The list was subsequently narrowed to four (4) studies on the basis of the 3-hour exposure time restriction as well as the dependency on LC_{50} data (see above). The "toxic load" calculations were performed on these four studies.[13]

[11] The LC_{50} refers to the "lethal concentration" causing death in 50% of a test population.

[12] According to the Discussion Paper, the upper-bound exposure duration of three hours was selected based on statistical evidence indicating that meteorological conditions (*i.e.*, wind speed, wind direction, stability class) affecting the dispersion of a "sour" gas plume would not remain constant for more than three hours.

[13] For a listing of the 23 studies originally considered, see Table 1 of Appendix 2 of the Discussion Paper. The four studies selected for use in the "toxic load" calculations and ultimately used in the derivation of the proposed EPZ endpoints are listed in Table 2 of Appendix 2.

TABLE 1-1
SUMMARY OF EMERGENCY PLANNING GUIDELINES CONSIDERED BY THE AEUB AS PART OF THE DEVELOPMENT OF THE PROPOSED EPZ ENDPOINTS FOR H_2S[1]

Guideline	Authority	Target Group	Description
Acute Exposure Guideline Level (AEGL)	U.S. Environmental Protection Agency (US EPA)	Public	Three-tier guideline for emergency response
Emergency Response Planning Guideline (ERPG)	American Industrial Hygiene Association (AIHA)	Public	Three-tier guideline for emergency response planning
Immediately Dangerous to Life and Health (IDLH)	U.S. National Institute for Occupational Safety and Health (NIOSH)	Worker	Highest concentration from which escape is possible without permanent damage
Specified Level of Toxicity (SLOT)	U.K. Health and Safety Executive (UK HSE)	Public	Dangerous "toxic load" used in context of land use planning

[1] Re-produced, in part, from Table 1 of Appendix 2 of the Discussion Paper (October 2004). A full description of the various guidelines is contained in the Discussion Paper.

Subsequent to the release of the Discussion Paper and in response to comments received, the AEUB elected to commission a review of the technical quality of the health effects information used as the basis of the proposed EPZ endpoints for H_2S. The intent was to ensure that:

- The health effects data that were considered in the development of the proposed endpoints were both representative and technically robust.
- The technical quality of the health effects data was such that the information could be used with confidence to develop a scientifically defensible EPZ endpoint.
- The health effects data considered were appropriate for the determination of the concentration-time-response characteristics of H_2S in terms of serious, irreversible outcomes, most notably lethality.

The review was performed by CANTOX ENVIRONMENTAL INC ("the consultant"). This report summarizes the work that was performed. It includes a description of the methodology that was followed, the findings and conclusions that were reached, and recommendations for future work aimed at refining and/or validating the proposed EPZ endpoints as well as advancing the state-of-the-art of the toxicology used in EUBMODELS.

2.0 OBJECTIVE AND TERMS OF REFERENCE

The objective of the work was to gauge the technical quality of each of the health effects studies considered by the AEUB in setting the proposed EPZ endpoints for H_2S, as outlined in the Discussion Paper. In order to meet the objective, the following Terms of Reference were developed by the AEUB and the consultant:

- The entire set of 23 studies would be subject to review.[14]
- Each study would be rated in terms its technical quality based on consideration of experimental design, conduct and reporting.
- The rating would specifically reflect the adequacy and usefulness of the study for establishing the concentration-time-response characteristics of H_2S vis-à-vis lethality. Other health endpoints were to be considered (*i.e.*, signs and symptoms, necropsy/autopsy findings), but were to be given less emphasis as part of the ratings.
- The ratings would be based, in part, on comparison of the experimental design, conduct and reporting features of each study against "benchmarks" for proper design, conduct and reporting established by leading scientific and regulatory authorities. Reliance would be placed on the guidelines for the testing of chemicals developed by the Organization for Economic Cooperation and Development (OECD), the U.S. Environmental Protection Agency (US EPA), the European Union (EU), and others.[15]
- The rating scheme would include a series of "grades" to permit distinction between studies of low *vs*. moderate *vs*. high quality and those studies having no practical use.
- The rating scheme would allow for objectivity, consistency and fairness in grading.
- Each study would be subject to detailed review, with the main design, conduct and reporting features to be documented and summarized, and a rationale to be provided for the grade assigned.
- The work would be strictly limited to a review of the technical quality of each study. Interpretation of the significance of the study findings did not form part of the work. Likewise, the scope of work did not include collating, "fitting" or incorporating the lethality data into "toxic load" calculations.

[14] Note that several of the studies were dated and/or published in foreign language journals. Certain of these studies proved to be difficult to retrieve. After careful consideration of the difficulties and costs involved with obtaining these papers as well as the likelihood that such studies would provide meaningful and defensible data, a decision was taken to omit these studies from the review. This decision applied to two foreign-language papers published prior to 1900.

[15] The overall approach, rating scheme and quality criteria used to assess the 23 papers were similar to those described in the Alberta Health and Wellness report entitled: "Health Effects Associated with Short-term Exposure to Low Levels of Hydrogen Sulphide (H2S) – A Technical Review" (October 2002). The latter review was performed under the auspices of an Expert Scientific Panel.

3.0 METHODS

The work proceeded in stages, beginning with the retrieval of the original 23 scientific papers referenced in the Discussion Paper. Upon retrieval, the papers were segregated by study type (*i.e*., non-clinical *vs*. clinical *vs*. case report *vs*. review article) and then assigned a unique study "code" for cataloguing and review purposes. Each study was subsequently subjected to a detailed review, which encompassed an evaluation of the technical quality of the investigation based on consideration of experimental design, conduct and reporting features. A set of "quality benchmarks" was developed for each study type to permit consistent and objective assessment of technical strengths and weaknesses. The benchmarks were based on the recommendations of leading scientific and regulatory authorities for the proper design, execution and reporting of health effects studies, with particular emphasis on "acute" or short-term inhalation tests. A grading system was established and, on the basis of the evaluation of technical quality, a grade or score was assigned to each study, reflecting the level of confidence that could be assigned to the findings and conclusions.

It is important to note that the grade principally reflected the adequacy and usefulness of the study for establishing the concentration-time-response characteristics of H_2S vis-à-vis lethality following short-term exposure. Other health endpoints were examined as part of the review, but did not significantly influence the grading.

The detailed review included documenting and summarizing the main design, conduct and reporting features of each study as well as the principal observations, especially with respect to lethality. Attention also was given to any clinical signs or symptoms as well as gross pathological (*i.e*., necropsy/autopsy) findings. In addition, the degree to which these latter health endpoints correlated with the lethality data was noted.[16] The above information was captured in a 'Document Review Form', with separate forms developed for each of the study types.

A more detailed description of the various stages comprising the work follows.

A. Document Retrieval

Full citations for each of the studies listed in Tables 1 and 2 of Appendix 2 of the Discussion Paper were compiled and first compared against an historical in-house database of studies assembled by CANTOX ENVIRONMENTAL INC. Any available papers held by the consultant were immediately retrieved. The citations for the remaining studies were submitted to the AEUB library for retrieval. The outstanding papers were either sourced directly from the library's collection or ordered through local, national or international sources. Once sourced, the original papers or copies were supplied to CANTOX ENVIRONMENTAL INC. Upon retrieval and/or receipt, each paper was catalogued and assigned a unique alpha-numeric code to permit tracking during the course of the work.[17] The "alpha" designation was used to distinguish between study types as follows:

[16] In instances in which deaths were recorded, any clinical signs and/or gross pathological findings recorded were examined to determine the degree to which the outcomes matched (*i.e*., the degree of consistency between the observed clinical signs, the gross pathological findings and the deaths formed part of the review). The intent was to confirm that the deaths were exposure-related and not due to other causes (*e.g*., pre-existing health conditions, poor animal husbandry, experimental error, *etc*.).

[17] Note that the alpha-numeric codes were not always assigned in sequence. The in-house database of studies assembled by the consultant included papers with pre-assigned codes that were not included as part of the scope of the present work (*i.e*., the cataloguing system of the consultant was already in place and included a number of studies other than those listed in Tables 1 and 2 of Appendix 2 of the Discussion Paper).

- NC – assigned to non-clinical studies involving exposure of test animals to H_2S under controlled laboratory conditions.
- CL – reserved for clinical studies involving controlled exposures of human subjects to H_2S.
- CR – assigned to case studies involving accidental and uncontrolled exposure of humans to H_2S
- RE – assigned to review articles describing the acute toxicity of H_2S, but without presentation of any original research findings.

In a few cases, the papers described a combination of different study types (*e.g.*, findings from separate clinical testing with humans and non-clinical investigations with animals were combined in a single paper). In these instances, a set or series of alpha-numeric codes were assigned to the paper, capturing each of the study types involved.

Some challenges were presented in terms of document retrieval since certain of the papers were dated (*i.e.*, published before 1900) and/or published in foreign-language journals. Special efforts were made to retrieve English translations of the foreign-language papers through the services of U.S.-based authorities, which had cited the studies as part of earlier reviews of the health effects of H_2S and/or the development of emergency response planning guidelines (*e.g.*, NIOSH). These efforts proved to be successful in some cases; however, certain of the papers remained elusive. After careful consideration of the difficulty and cost involved in retrieving these papers, a decision was taken to omit them from the review. Only two papers fit this category, both of which were dated and likely of suspect technical quality and little practical use.[18] Accordingly, the work comprised review of the technical quality of 21 of the 23 papers referenced in the Discussion Paper.

A summary of the number of studies for which the original papers were retrieved, catalogued and reviewed, arranged by study type, is shown in Table 3-1.

TABLE 3-1
NUMBER OF STUDIES FROM ORIGINAL PAPERS SUBJECTED TO REVIEW

Study Type	Number of Studies[1]
Non-clinical	15
Clinical	2
Case report	3
Review	5
Total	25

[1] The number of studies reviewed does not match the number of original papers retrieved since certain of the papers included a combination of different study types.

B. Quality Benchmarks

The assessment of the technical quality of each study involved comparison of the experimental design, conduct and reporting features of the investigation against "benchmarks" recommended by leading scientific and regulatory authorities. The benchmarks were based on testing protocols outlining the proper design, execution and reporting features of health effects studies, with an aim toward harmonization as well as the objective analysis and interpretation of findings. Because of the nature of the studies involved, emphasis was given to protocols specific to acute inhalation

[18] The following two papers were not retrieved and subjected to review:
Biefel, R. and Polek, T.H. 1880. Uber kohlendunst und leuchtgasvergiftung. Zeitschr. F. Biologie 16, 279-366.
Eulenberg, H. 1865. Die lehre von den schadlichen gasen und dampfen. Braunschweig.

toxicity testing. Reliance was placed on the following testing guidelines and/or guidance documents:

- Clarke, M. and Oxman, A.D. (eds). 2001. Cochrane Reviewers Handbook 4.1.4 (updated October 2001). In: Cochrane Library, Issue 4, 2001. Oxford: Update Software.
- Diener, W., *et al*. 1997. The inhalation acute toxic class method: test procedures and biometric evaluations. Arch. Toxicol. 71, 537-549.
- EU Guideline 92/69/EEC. 1992. B.2. Acute toxicity – inhalation. Official Journal of the European Community L383A, December 29, 1992.
- Holzhutter, H.G., *et al*. 2003. Dermal and inhalation acute toxic class methods: test procedures and biometric evaluations for the Globally Harmonized Classification System. Arch. Toxicol. 77, 243-254.
- OECD. 1981. Test Guideline 403. Acute inhalation toxicity. OECD, Paris.
- OECD. 2004. Draft Guidance Document on Acute inhalation Toxicity Testing. OECD Environment Directorate, Publication Series on Testing and Assessment No. 39B, December 8, 2004.
- Society of Toxicology (SOT). 1992. Commentary: Recommendations for the conduct of acute inhalation limit tests. Fundam. Appl. Toxicol. 18, 321-327.
- US EPA. 1998. Health Effects Test Guidelines. OPPTS 870.1300. Acute Inhalation Toxicity. U.S. Environmental Protection Agency. August, 1998.

The benchmarks used for comparison covered a number of different aspects of study design, conduct and reporting, including:

- Test material (*i.e*., source, purity, *etc*. of the H_2S).
- Test animals and/or human subjects (*i.e*., species/strain, source, number, sex, age, health status, husbandry, acclimation, *etc*.).
- Test equipment (*i.e*., exposure chamber, gas delivery system, metering devices, *etc*.).
- Exposure conditions (*i.e*., chamber equilibration, gas flow rates, exposure concentrations, exposure times, whole body *vs*. head only exposure, *etc*.).
- Procedural (*i.e*., randomization and assignment of test animals/subjects to groups, placement of animals/subjects into exposure chamber, monitoring of exposure conditions, length of post-exposure recovery period, *etc*.).
- Observations (*i.e*., nature and frequency of observations, including body weights, signs and symptoms, deaths, time to death, necropsy/autopsy findings, *etc*.).
- Test report (*i.e*., description of methods, documentation and tabulation of findings, statistical treatment of data, interpretation of data, study conclusions, *etc*.).

To permit ease of comparison, the various benchmarks were compiled into "check-lists", covering each of the above aspects and organized in a Q&A format. The check-lists formed part of the Document Review Form. Examples of the completed forms and check-lists can be found in Appendix A. The primary purpose of the check-lists was to facilitate the review process by providing a convenient mechanism whereby the descriptions contained in the original papers of the various study aspects could be compared to the recommended protocols. A grade could then be assigned to the study based, in part, on how well the descriptions matched the recommendations.

It is important to note the comparisons focused principally on each of the study aspects as it related to the determination, description and documentation of lethality. Other health endpoints were examined as part of the review,

but were given less emphasis. This approach was consistent with the Terms of Reference developed for the work and the AEUB's reliance on the use of lethality data to establish the proposed EPZ endpoints for H_2S.

The rating or grading of the studies necessarily involved weighing the strengths and weaknesses of each investigation, using the appropriate check-list for guidance. The rating scheme included a full spectrum of grades, ranging from high to low. The grading criteria are described in Table 3-2. Again, it is important to note that the grade assigned to any given study principally reflected the adequacy and usefulness of the study for establishing the concentration-time-response characteristics of H_2S vis-à-vis lethality following short-term exposure. Other health endpoints were examined as part of the review, but did not significantly influence the grading.

TABLE 3-2
GRADING CRITERIA USED IN THE RATING OF THE TECHNICAL QUALITY OF THE STUDIES

Grade[1]	Criteria
High	The study meets or exceeds the recommended guidelines, with no serious weaknesses in experimental design, conduct or reporting. All aspects of the study satisfy the "quality benchmarks". Procedures are well described and the results are properly disclosed to permit meaningful interpretation. Study validity is obvious. Confidence in the findings and conclusions is high.
Moderate	The study approaches the recommended guidelines, but minor deficiencies in experimental design, conduct and/or reporting detract from its usefulness. One or more aspects of the study are somewhat deficient relative to the "quality benchmarks". Study validity is evident, but not entirely obvious. Careful attention to detail in describing procedures and/or documenting results may be somewhat lacking.
Low	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.
No practical use	The study fails to meet the recommended guidelines, with obvious and fundamental weaknesses in experimental design, conduct and/or reporting. Virtually all aspects of the study are deficient when measured against the "quality benchmarks". Critical information is lacking to permit meaningful interpretation of the findings. Procedures are poorly described. (Note that this grade was typically assigned by default to review articles in which the original research was not described in sufficient detail to allow comparison against the recommended guidelines and "quality benchmarks").

[1] Note that, in some cases, intermediate grades (*i.e.*, low-to-moderate or moderate-to-high) were assigned for added refinement. In these instances, the study generally failed to meet the upper grade because of some uncertainty or doubt surrounding one or more aspects.

As noted above, certain of the papers cited by the AEUB proved to be review articles (see Table 3-1) and did not contain original research findings. It was deemed impractical to complete a detailed comparison of the information presented in the review articles against the quality benchmarks since, as would be expected, most details concerning experimental design, conduct and reporting were missing. Comparison was further hindered by the fact that, in some cases, the original source of the lethality data shown in Table 1 of Appendix 2 of the Discussion Paper was either not indicated or not obvious from the review article (*i.e.*, the original findings could not be identified, sourced and reviewed). As a result, the detailed checklists were not completed for the review articles. Instead, a condensed version of the Document Review Form was used.

C. Review Process

Each study was subjected to independent review by at least two members of the consultant's team. In the event of a disagreement between the two reviewers over the assignment of a grade, a third reviewer was engaged and any differences were resolved through discussion among all three individuals. Disagreement was very rare. In virtually all cases, the grade was obvious.

The review process began with a detailed assessment of the technical quality of each study and the assignment of grades (see above). Upon completion, a further review was performed to ensure that the grading was objective and fair across all studies, with the ratings assessed on a comparative basis. In other words, the initial detailed review was directed at grading each study on a stand alone basis after comparison of the experimental design, conduct and reporting features of the investigation against the recommended "quality benchmarks", whereas the subsequent review was aimed at examining each of the grades assigned to ensure consistency in the approach followed and objectivity, continuity and fairness in the scoring across all studies. In keeping with the Terms of Reference (see Section II), the subsequent review was concerned only with the consistency of grade assignment, and not the consistency of findings across studies (*i.e.*, the significance of the findings was not assessed and the lethality data *per se* were not compared across individual studies).

For ease of review of the grades assigned as well as the sorting of studies by grade, a colour and symbol scheme was devised, as shown opposite. The scheme was applied to the text and tables that follow.

Grading Scheme

High	▲
Moderate or Moderate-to-High	◆
Low-to-Moderate	■
Low	●
No Practical Use	★

4.0 RESULTS

4.1 General Comments

The review of the technical quality of the studies considered by the AEUB in the development of the proposed EPZ endpoints for H_2S revealed considerable diversity in the adequacy and usefulness of the investigations. Some studies were deemed to be of no practical use, whereas other investigations achieved a moderate-to-high rating. A summary of the grades assigned, arranged by study type, is captured in Table 4-1. A detailed listing of the studies and the assigned grades can be found in Table 4-2. Some general comments concerning the outcome of the review follow:

- None of the studies followed a conventional, standardized protocol using the methods recommended by a number of leading authorities for acute inhalation toxicity testing. A variety of different experimental approaches were employed, with the differences related largely to differences in the specific objective(s) of each study. Some of the approaches resembled those recommended, whereas other studies showed significant departures from conventional methods. In addition, there was no indication that any of the studies was performed with attention to Good Laboratory Practice (GLP) or Good Clinical Practice (GCP) guidelines.
- None of the studies received a "high" grade (▲). In other words, none of the studies fully met the "quality benchmarks" for design, conduct and reporting recommended by leading scientific and regulatory authorities. In all cases, one or more deficiencies were identified which detracted from the quality of the study and undermined confidence in the findings and conclusions. In some instances, the deficiencies were modest, which allowed a "moderate-to-high" grade to be assigned.
- One factor contributing to the absence of "high" grades was the fact that the majority of studies were performed before publication of the recommended testing protocols. More than 80% of the studies were completed prior to 1980 (*i.e.*, the time at which the testing guidelines first appeared). This invariably deflated the grades that were assigned. Certain of the studies were very dated (*i.e.*, completed before 1900) and featured archaic design, conduct and/or reporting elements that have since been replaced with more reliable, informative and meaningful measures.
- A second factor that may have contributed to the lack of "high" scores was the fact that the quality benchmarks used for comparison represented exacting standards that demand careful attention to detail and full disclosure of all study features. Word restrictions and other limits placed on the authors (*i.e.*, study investigators) by the journal editors may have prevented the reporting of all details needed to satisfy the benchmarks. Given this possibility, the consultant was left with two choices: i) relax the benchmarks and presume the missing details formed part of the study but were not reported; or, ii) maintain the benchmarks and grade the study accordingly. The first choice was deemed to be too arbitrary. The second choice … although stricter … was needed to meet the requirement for objectivity and consistency in grading demanded by the Terms of Reference for the work (see Section II).
- Approximately 40% of the studies received a "moderate" or "moderate-to-high" rating (◆), signifying that the findings can be used with a reasonable degree of confidence to understand the concentration-time-response characteristics of H_2S vis-à-vis lethality. A number of these studies documented the deaths (… as well as clinical signs and necropsy findings) witnessed among test animals exposed to different exposure concentration-exposure time "combinations".

These data were judged by the consultant to be suitable for performing "toxic load" calculations.

- Approximately 40% of the studies were assigned a grade of "low" (●) or "low-to-moderate" (■), indicating serious weaknesses in experimental design, conduct and/or reporting. Common deficiencies among these studies included inadequate description of the exposure chamber and gas delivery system, use of makeshift and/or "dated" instrumentation, failure to analytically confirm the concentrations of H_2S to which the test animals/subjects were exposed and/or inadequacies with respect to the number of test animals/subjects employed, the health status of the animals and/or animal husbandry. In some cases, the study investigators reported difficulties in maintaining stable, uniform concentrations of H_2S in the exposure chamber. In one instance, the investigator openly admitted to having little confidence in the actual exposure concentrations tested (Lehmann, 1892 - NC070●).
- The remaining 20% of the studies were deemed to be of no practical use (★) in providing an understanding of the concentration-time-response characteristics of H_2S vis-à-vis lethality. In most instances, these studies either lacked fundamental information or provided information that could not be substantiated. In some cases, the information was irrelevant (see below).
- With one exception, the studies specifically selected by the AEUB for the calculation of the EPZ endpoints for H_2S (*i.e.*, the studies listed in Table 2 of the Discussion Paper, in which the 3-hour exposure time restriction was applied) received a grade of "moderate" ... signifying that the dataset selected was fit-for-purpose and technically robust. The exception was a review article by Back *et al.* (1972 – RE003★), which simply summarized the data collected by MacEwen and Vernot (1972 – NC072◆). The former study was deemed to be of no practical use, whereas the latter study received a "moderate" grade.
- The relevance of certain of the studies was highly questionable. Two of the studies referenced in the Discussion Paper (Table 1 of Appendix 2) were classified by the consultant as review articles, one of which consisted only of a table of physical-chemical properties of a series of alcohols, aldehydes and ketones, without mention of H_2S (Tabulae Biologicae Periodicae, 1933 - RE004★). The other paper (Lefaux, 1968 - RE001★) was dedicated to a discussion of the toxicology of plastics and included a single summary table outlining the "toxic effects" of different concentrations of H_2S (… presumably as a combustion by-product of certain types of plastics), without reference to the source(s) and basis of the information (*i.e.*, the data could not be substantiated). Neither study represented original scientific research. Both studies were deemed to be of no practical use.[19]
- The care and attention to detail exercised in certain of the studies was dubious. In one case, so-called "accidents" involving the inadvertent exposure of test animals to H_2S because of technician error occurred on two separate occasions (O'Donoghue, 1961 – NC034●). In both instances, the animals died. In another case, an equipment malfunction occurred, allowing H_2S to penetrate an entire animal room, contaminating all surfaces, including the control chamber … yet, the study investigator proceeded undeterred and chose to include the accidental exposure as part of the experimental dataset (Hays, 1972 – NC057■). Each incident conveyed a certain disregard for proper training, equipment maintenance and safety precautions as well as a departure from conventional testing

[19] Both of these studies were cited by NIOSH as part of the development of the IDLH guidelines.

protocols. The incidents seriously undermined confidence in the studies, and the grades were adjusted downwards accordingly.

- The more "dated" studies suffered from a number of common deficiencies, including the use of makeshift chambers and gas delivery systems, difficulty in maintaining uniform concentrations of H_2S in the exposure chamber, use of test animals from undisclosed sources and of unknown health status, use of unconventional designs, lack of ethics committee oversight, and inadequate reporting (Haggard, 1925 – NC067●; Lehmann, 1892 – NC070●/CL011●; Mitchell and Yant, 1925 – NC032●/CL010★; Weedon *et al*., 1940 – NC054■). In all cases, the experimental designs were not only very exploratory, with an emphasis on comparatively high exposure concentrations, often approaching several hundred parts-per-million ... but also somewhat remarkable given that certain of the studies involved the use of human subjects (*i.e*., the subjects were deliberately exposed to relatively high concentrations of H_2S, with little apparent regard for safety). The quality of these dated studies is perhaps aptly illustrated by the first-person account of Lehmann (1892) in which he speaks of the circumstances surrounding the necropsy (… or lack thereof) of a rabbit exposed to H_2S for several hours in a chamber also occupied by a cat. He laments that "the rabbit was obviously killed the following day by the cat and was found half-eaten, such that no dissection was carried out"(!!!). He also offers that, in retrospect, he "would no longer choose this method" when describing the analytical means by which he attempted to confirm the concentrations of H_2S in the exposure chamber. Finally, when introducing his human test subjects (including his servant and one of his students) he admits that he "prevailed upon them to undertake a large series of experiments on themselves", with no mention of voluntary consent or medical ethics oversight. In virtually all cases, the dated studies were assigned a "low" grade.
- The review articles were consistently deemed to be of no practical use in the context of the present exercise. The information provided in the review articles was either: i) judged to be irrelevant (Lefaux, 1968 - RE001★ and Tabulae Biologicae Periodicae, 1933 - RE004★ – see above); or, ii) could not be substantiated in the absence of the original scientific findings (Haggard, 1925 - RE002★; Back *et al*., 1972 - RE003★; and NIOSH, 1977 - RE005★). It was beyond the scope of the current work to retrieve all of the various original scientific papers relating to lethality and other health effects cited in the latter articles (*e.g*., the dataset referenced by NIOSH). In some cases, the amount of original literature involved was shown to be considerable, partly because of the "cascading citation effect" (*i.e*., a review article citing an earlier review article, leading to a compounding of the number of original scientific papers involved). In other instances, the source(s) of the original findings was either not indicated or not obvious from the review article.

4.2 Technical Quality and Grades Assigned

Table 4-1 provides a summary of the grades that were assigned as part of the review of the technical quality of the studies, with the scores arranged by study type. As already indicated, the grades reflect the adequacy and usefulness of the study in providing an understanding of the concentration-time-response characteristics of H_2S vis-à-vis *lethality* following short-term exposure. The grades assigned to the specific studies that were used by the AEUB in the calculation of the proposed EPZ endpoints for H_2S (*i.e*., the four studies which served as the basis of the "toxic load' calculations shown in

Table 2 of the Discussion Paper) are highlighted.

Table 4-2 provides more detailed listing of the grades, arranged by individual study, with the specific studies cited by the AEUB and used as the basis of the proposed EPZ endpoints, again highlighted. An expanded listing showing the major strengths and weaknesses of each study can be found in Table 4-3.

For a more complete description of each study, including details respecting design, execution and reporting as well as study outcomes (e.g., the number of deaths recorded, the time to death, etc.), the reader is referred to the Document Review Forms found in Appendix A. The forms also provide complete details opposite the strengths and weaknesses of each study that formed the basis of the grading.

TABLE 4-1
SUMMARY OF GRADES ASSIGNED (ARRANGED BY STUDY TYPE)

Study Type	Total Number of Studies	Number of Studies Achieving Grade					
		High	Moderate-to-High	Moderate	Moderate-to-Low	Low	No Practical Use
Non-clinical	15	-	2	6	2	5	-
Clinical	2	-	-	-	-	2	-
Case Report	3	-	-	-	-	2	1
Review Article	5	-	-	-	-	-	5
Studies Selected by AEUB[1]	4	-	-	3	-	-	1

[1] Refers to the set of four studies that served as the basis of the "toxic load" calculations used in the development of the proposed EPZ endpoints for H_2S. Each of the four studies was a non-clinical investigation. See Table 2 of the Discussion Paper.

TABLE 4-2
SUMMARY OF GRADES ASSIGNED (ARRANGED BY INDIVIDUAL STUDY)[1]

Author(s)	Study Code	Grade[2]
Non-clinical studies		
Clanachan (1979)	NC002◆	Moderate
Haggard (1925)	NC067●	Low
Hays (1972)	NC057■	Low-to-Moderate[3]
Lehmann (1892)	NC070●	Low
Lopez *et al*. (1986)	NC069◆	Moderate-to-High
Lopez *et al*. (1987)	NC027◆	Moderate
Lopez *et al*. (1989)	NC031◆	Moderate-to-High
Lund and Wieland (1966)	NC073●	Low
MacEwen and Vernot (1972)	NC072◆	Moderate
Mitchell and Yant (1925)	NC032●	Low

Author(s)	Study Code	Grade[2]
O'Donoghue (1961)	NC034●	Low
Prior *et al*. (1988)	NC035◆	Moderate
Tansy *et al*. (1981)	NC047◆	Moderate
Weedon *et al.* (1940)	NC054■	Low-to-Moderate
Zwart et al. (1990)	NC056◆	Moderate
Clinical studies		
Lehmann (1892)	CL011●	Low
Mitchell and Yant (1925)	CL010●	Low
Case reports		
Mitchell and Yant (1925)	CR066★	No Practical Use
Prouza (1972)	CR067●	Low
Winek (1968)	CR002●	Low
Review articles		
Back *et al.* (1972)[4]	RE003★	No Practical Use
Haggard (1925)	RE002★	No Practical Use
Lefaux (1968)	RE001★	No Practical Use
NIOSH (1977)	RE005★	No Practical Use
Tabulae Biologicae Periodicae (1933)	RE004★	No Practical Use

[1] Highlighted studies are those selected by the AEUB which served as the basis of the "toxic load" calculations used to derive the proposed EPZ endpoints for H_2S.
[2] The grade principally reflects the adequacy and usefulness of the study for establishing the concentration-time-response characteristics of H_2S vis-à-vis lethality following short-term exposure. Assignment of grades was based, in part, on comparison of study design, conduct and reporting features against "benchmarks" recommended by leading scientific and regulatory authorities. The strengths and weaknesses listed are among those considered in the grading. For complete details, the reader is referred to the Document Review Forms found in Appendix A.
[3] Note that the "low" rating applies to a specific portion of the study involving an "accidental" exposure of mice to 30 ppm of H_2S resulting from an equipment malfunction.
[4] Note that it was concluded that the paper by Back *et al*. (1972 – RE003★) is a review article summarizing the original data collected by MacEwan and Vernot (1972 – NC072◆).

TABLE 4-3
SUMMARY OF PRINCIPAL STRENGTHS AND WEAKNESSES (ARRANGED BY INDIVIDUAL STUDY)[1]

Author(s)	Study Code	Overall Technical Quality		Grade[2]
		Key Strengths	Major Weaknesses	
Non-clinical studies				
Clanachan (1979)	NC002	• Use of gradient of exposure concentrations (500 to 1300 ppm) and exposure times (1 to 30 minutes) to permit assessment of comparative influence of each parameter on lethality and other health endpoints. • Use of adequate numbers of test animals of both sexes (at least 20 mice per exposure concentration-exposure time combination). • Adequate description of exposure chamber and gas delivery system. • Customized exposure chamber design which allowed for careful control of entry and exit of test mice from the exposure chamber (*i.e.*, exposure times were well controlled). • Complete documentation of deaths recorded for each exposure concentration-exposure time combination. • Selected clinical signs (*i.e.*, loss of righting reflex, unconsciousness) monitored and documented. • Time course of effects, including lethality, well described.	• Although testing was performed in both sexes of mice, the findings were not segregated by sex. • No indication that actual exposure concentrations were analytically confirmed. • Exact time to death was not specified. • Post-exposure observation period was limited to 5 days only. • No indication that test animals were necropsied. • No control group.	Moderate (◆)
Haggard (1925)	NC067	• Use of gradient of exposure concentrations (100-1800 ppm) and exposure times (up to several hours) to permit assessment of the influence of each parameter on lethality and other health endpoints. • Test animals monitored for lethality and clinical signs.	• Very limited description of exposure chamber. • No description of gas delivery system. • No indication of source of H_2S. • No details provided concerning sampling and analytical methodology used to evidently confirm exposure concentrations. • Inadequate number of test animals (1 dog per	Low (●)

Author(s)	Study Code	Overall Technical Quality		Grade[2]
		Key Strengths	Major Weaknesses	
			exposure regimen – sex not specified). • No control group. • Complete lack of details concerning source, age, sex, health status, husbandry, *etc.* of test animals. • Only general description of clinical signs (*i.e.*, signs were simplified classified as "systemic" or "irritant"). No details concerning exact nature, severity, *etc*. • No indication that test animals were necropsied. • Lack of detail to allow critical assessment of concentration and time-responsiveness since exposure levels and exposure times most often were reported as ranges only.	
Hays (1972)	NC057	• Use of multiple test species (mice, goats, cows). • Use of control groups. • Use of gradient of exposure concentrations for studies with mice and goats (0 to 100 ppm). • Detailed description of gas delivery system and exposure chamber/ exposure "hood". • Analytical confirmation of exposure concentrations (… albeit methodology relied on colorimetric analysis of limited sensitivity). • Measurement of some indicators of clinical toxicity (*e.g.*, feed intake, water intake, body weight, heart rate, respiration rate, and/or blood pressure, *etc*).	• "Accidental" exposure resulting in contamination of animal room, including control chambers, suggests lack of care and attention to detail. • Reliability of findings from "accidental" exposure portion of study highly questionable. • Lack of monitoring of conventional clinical signs. • No necropsy records. • Time course of deaths witnessed among certain groups of mice (30 ppm) judged to be questionable because of unusual pattern (*i.e.*, sudden collapse and death within minutes after 18 hours of continuous exposure).	Low-to-Moderate[3] (■)
Lehmann (1892)	NC070	• Use of gradient of exposure concentrations and exposure times to permit assessment of comparative influence of each parameter on lethality and other health endpoints.	• Use of limited number of test animals (*i.e.*, only 1-2 test animals for each exposure concentration/exposure time combination). • Repeated use of the same test animals in different	Low (●)

Author(s)	Study Code	Overall Technical Quality		Grade[2]
		Key Strengths	**Major Weaknesses**	
		• Use of multiple animal species (guinea pig, rabbit, cat, dog). • Detailed observations of clinical signs. • Regular attempts to measure H_2S concentrations in the chamber during exposure (albeit methods were suspect in terms of reliability). • Necropsy findings reported and summarized for animals which died on test.	experiments (*i.e.*, animals which survived exposures were often subsequently exposed to a different exposure concentration/exposure time combination). • Inadequate description of test animals (*e.g.*, source, age, sex, strain, pre-study health status). • Failure to include control animals • Limited description of gas delivery system and exposure chamber. • Uncertainty with respect to actual exposure concentrations used (*i.e.*, study investigator admitted lack of confidence in several of the analytical methods employed). • Complete lack of detail concerning animal housing and husbandry • Failure to observe surviving animals for 14 days post-exposure	
Lopez *et al*. (1986)	NC069	• Use of two exposure levels (40 ppm and 300 ppm) as well as two separate control groups (0 ppm). • Use of relatively large numbers of test animals (rats) per exposure level for mortality assessment (n=12). • Use of three different time intervals post-exposure for sacrifice of surviving rats to assess potential recovery from exposure-related effects. • Regular observation of test animals during exposure, allowing approximate time of death to be determined. • Good description of exposure chamber and gas delivery system. • Direct and regular monitoring of H_2S	• Use of male rats only. • Insufficient post-observation period in surviving rats with respect to mortality (<42 hours *versus* recommended 14 days). • Exact times of death not specified. • No examination of different exposure concentration-exposure time combinations to permit assessment of comparative influence of concentration and time on lethality outcomes. Only a single exposure time (6 hours) was used. (Although the use of concentration-time combinations is not a guideline requirement, it can broaden understanding of acute lethality of gases vis-à-vis Haber's law).	Moderate-to-High (◆)

Author(s)	Study Code	Overall Technical Quality		Grade[2]
		Key Strengths	Major Weaknesses	
		concentration during exposure in both test and control atmospheres. • Detailed reporting of gross and histopathologic findings. • Monitoring of clinical signs during exposure, including weight loss.		
Lopez *et al*. (1987)	NC027	• Use of gradient of exposure concentrations (0, 10, 200 or 400 ppm), including control exposure(s). • Use of adequate number of test animals (n=12) per exposure concentration. • Good description of exposure chamber and gas delivery system. • Exposure concentrations were analytically confirmed. • Regular monitoring of test animals for clinical signs.	• Use of male rats only. • Failure to follow animals for recommended 14-day observation period (*i.e.*, animals were sacrificed with 1 to 44 hours post-exposure). • No examination of different exposure concentration-exposure time combinations to permit assessment of comparative influence of concentration and time on lethality outcomes. Only a single exposure time (4 hours) was used. (Although the use of concentration-time combinations is not a guideline requirement, it can broaden understanding of acute lethality of gases vis-à-vis Haber's law). • Lack of necropsy of animals at study termination.	Moderate (◆)
Lopez *et al*. (1989)	NC031	• Good description of exposure chamber and gas delivery system. • Analytical confirmation of exposure concentration. • Time to death known within 3 minutes. • Good descriptions of clinical signs and pathological findings. • Control group (air only) included. • Concentration-time-response well defined for specific test conditions used.	• Use of male sex only. (… which, in turn, limited number of test animals to 5 per treatment). • Use of a single exposure concentration only. • No examination of different exposure concentration-exposure time combinations to permit assessment of comparative influence of concentration and time on lethality outcomes. Only a single exposure concentration was used.	Moderate-to-High (◆)

Author(s)	Study Code	Overall Technical Quality		Grade[2]
		Key Strengths	Major Weaknesses	
Lund and Wieland (1966)	NC073	• Use of a higher order test species (*i.e.*, monkey), bearing a comparatively close resemblance to man. • Use of different acute exposure regimens (*i.e.*, 500 ppm exposure delivered one or twice for periods ranging from 17 to 35 minutes). • Study design included monitoring and recording of clinical signs both during and following exposure. • Study design included detailed pathological examination of selected tissues, including the brain and heart.	• Number of test animals (n=3) was somewhat limited. • Use of single exposure concentration. • Complete lack of detail concerning test animals and animal husbandry (*i.e.*, information respecting source, age, sex, body weight, pre-study health status, caging, feed supply, *etc*. was lacking). • Lack of detail concerning source and purity of H_2S, as well as lack of information respecting the gas delivery system and exposure chamber. • No indication that exposure concentration (*i.e.*, 500 ppm nominal) was analytically confirmed. • Post-exposure monitoring period was somewhat limited (*i.e*. confined to 5-10 days for surviving monkeys). • Pathological assessment did not include examination of the lungs (*i.e.*, one of the primary target tissues).	Low (●)
MacEwen and Vernot (1972)	NC072	• Use of gradient of exposure concentrations (400, 504, 635 and 800 ppm) • Use of adequate numbers of animals (10 per exposure concentration). • Use of two test species (rats and mice). • Animals monitored for recommended 14-day post-exposure observation period. • Adequate description of exposure chamber and gas delivery system. • Direct monitoring of H_2S during exposure to confirm nominal concentrations. • Monitoring of clinical signs during and after exposure, including weight loss.	• Use of male sex only. • Use of a single exposure time only (one-hour). • No control group. • No examination of different exposure concentration-exposure time combinations to permit assessment of comparative influence of concentration and time on lethality outcomes. Only a single exposure time (1 hour) was used. • Limited reporting of clinical signs (*e.g.*, number of animals exhibiting signs was not indicated, nor were signs segregated by exposure concentration). • No reporting of necropsy findings in animals that died on test. • Exact times to death were not reported.	Moderate (◆)

Author(s)	Study Code	Overall Technical Quality		Grade[2]
		Key Strengths	Major Weaknesses	
Mitchell and Yant (1925)	NC032	• Use of a wide range of test animal species (*i.e.*, canary birds, rats, guinea pigs, dogs and goats). • Use of gradient of exposure concentrations and exposure times. • Good description of clinical signs. • Approximate time to death recorded.	• Use of limited numbers of test animals for certain exposure conditions (... numbers ranged from 1 to 40 per treatment) • Failure to distinguish between sexes of test animals. • Limited description only of gas delivery system and exposure chamber (… details evidently available in companion report). • Purity of H_2S gas not provided (… the H_2S was generated *in situ* by combining FeS and HCl using a "Kipp generator"). • Lack of detail concerning confirmation of exposure concentrations (… test concentrations evidently were measured using the "calcium chloride method", but no details were supplied). • Lack of detail to allow critical assessment of concentration and time-responsiveness since exposure levels and exposure times most often were reported as ranges only. • Failure in many instances to report actual numbers of test animals that either died or were afflicted with clinical signs. • Exact time to death not specified. • Complete lack of data with respect to control animals. • Limited necropsy data (… findings were reported for dogs only and only for dogs exposed to selected concentrations).	Low (●)
O'Donoghue (1961)	NC034	• Unique experimental design involving exposure to gradually increasing concentrations of H_2S over varying time periods, allowing for assessment of onset and/or recovery from clinical signs.	• Lack of description of exposure chamber and gas delivery system. • Lack of detail surrounding analytical confirmation of exposure concentrations.	Low (●)

Author(s)	Study Code	Overall Technical Quality		Grade[2]
		Key Strengths	**Major Weaknesses**	
		• Use of different exposure concentration-exposure time combinations, permitting assessment of the influence of each parameter on lethality and other health endpoints. • Use of two test animal species (pig and rabbit). • Time to death documented. • Clinical signs well documented (*i.e.*, nature, onset, duration and severity). • Necropsy findings documented.	• Use of restricted numbers of test animals (1 to 3 per treatment). • Reference to "accidental" exposures leading to death of animals signifies general lack of attention and carelessness, and seriously detracts from the level of confidence that can be assigned to the study. • Lack of detail concerning post-exposure observation period. • Complete lack of detail concerning control animals. • Inconsistencies in the reporting of exposure times.	
Prior *et al*. (1988)	NC035	• Use of adequate numbers of test animals (12 rats per sex per exposure level). • Use of both sexes as well as multiple strains of rats. • Use of a gradient of exposure concentrations (approx. 300 to 800 ppm) and exposure times (2, 4, or 6 hours). • Full description of exposure chamber and gas delivery system. • Analytical confirmation of exposure concentrations. • Summary descriptions of weight loss and necropsy findings.	• Failure to specify actual exposure concentrations tested. (A probit distribution of concentration-response was shown graphically, but the resolution was not adequate to discern the exact exposure levels tested). • Evident failure to include control group(s) of animals. • Reliance on summary data. Individual animal/individual group data were not provided for any of the outcomes reported (*i.e.*, lethality, weight loss, necropsy). • Failure to report clinical signs other than weight loss.	Moderate (◆)
Tansy *et al*. (1981)	NC047	• Use of adequate numbers of test animals (rats) of both sexes (10 per exposure level). • Use of gradient of exposures concentrations, albeit range was somewhat narrow (*i.e.*, 400 to 600 ppm). • Animals monitored for recommended 14-day post-exposure observation period.	• Failure to analytically confirm exposure concentrations. • No examination of different exposure concentration-exposure time combinations to permit assessment of comparative influence of concentration and time on lethality outcomes. Only a single exposure time (4 hour) was used.	Moderate (◆)

Author(s)	Study Code	Overall Technical Quality		Grade[2]
		Key Strengths	**Major Weaknesses**	
		• Adequate description of exposure chamber and gas delivery system. • Use of control group of animals.	• Lack of mention of presence or absence of clinical signs despite the fact that such signs evidently were monitored as part of the study. • Limited reporting of necropsy findings. • Failure to report actual time of death of rats that died on test.	
Weedon *et al.* (1940)	NC054	• Use of gradient of exposure concentrations of H_2S (16 to 1,000 ppm). • Use of two species of test animals (*i.e.*, rats and mice). • Use of limited, but adequate numbers of test animals. • Use of both sexes. • Adequate description of gas delivery system and exposure chamber (… provided in companion paper). • Good description of concentration-time response for mortalities, clinical signs and necropsy findings.	• Lack of detail concerning control animals. • Questionable health status of some animals at start of study (… based on necropsy findings). • Failure to specifically report on confirmation of nominal test concentrations (… reference only to the use of "autometers" in the companion paper … no confirmation that test concentrations were actually measured as part of the studies). • Failure to distinguish between the sexes in terms of the reporting of results. • Use of relatively antiquated equipment for generating test concentrations, with use of manometers, chart recorders, and "warning bells".	Low-to-Moderate (■)
Zwart *et al.* (1990)	NC056	• Use of two species (rat and mouse) and use of both sexes. • Use of adequate numbers of test animals (5 per sex per exposure concentration). • Use of gradient of exposure concentrations covering a fairly broad range (≈300 to 1300 ppm). • Use of multiple exposure times (5, 10, 30, and 60 minutes). • Use of varied concentration-time combinations to permit assessment of comparative effects of exposure concentration	• Lack of reporting of clinical signs and body weights, despite the fact that these parameters evidently were monitored as part of the study. • Lack of reporting of gross pathological findings despite the fact that the animals evidently were necropsied at the end of the observation period. • Lack of control group(s). • Lack of in-depth description of exposure chamber and gas delivery system, as well as failure to describe sampling and analytical methodology used to confirm the exposure concentrations.	Moderate (◆)

Author(s)	Study Code	Overall Technical Quality		Grade[2]
		Key Strengths	Major Weaknesses	
		and exposure time on lethality and other health endpoints.		
Clinical studies				
Lehmann (1892)	CL011	• Use of gradient of exposure concentrations (20 to 575 ppm) and exposure times (30 minutes to 4 hours) to permit assessment of comparative influence of each parameter on acute toxicity. • Detailed observations of clinical symptoms, including duration and/or reversibility of symptoms in most instances. • Use of human subjects (… thereby avoiding uncertainties associated with extrapolating findings from test animals to humans).	• Use of limited numbers of subjects (1-3 test subjects for each exposure concentration/exposure time combination) • Use of test subjects repeatedly exposed to H_2S as part of separate experiments. • Inadequate description of test subjects (*e.g.*, occupation, exact age, prior exposures to H_2S). • Failure to include control subjects. • Unusual chamber selection. Chamber was described as a "washhouse" in which the H_2S was produced by combining ferrous sulphide with acid. • Significant uncertainty surrounding the actual exposure concentrations that were tested. Analytical methodology used to measure H_2S concentrations was suspect. Author admitted that maintenance of uniform concentrations of H_2S was "difficult".	Low (●)
Mitchell and Yant (1925)	CL010	• Use of human subjects (… thereby avoiding uncertainties associated with extrapolating findings from test animals to humans). • Use of gradient of exposure concentrations (100 to 450 ppm). • Regular monitoring and recording of clinical symptoms during exposure.	• Study was "preliminary" in nature only (… by authors' admission). • Lack of detail concerning test subjects (*i.e.*, age, weight, health status, occupation, smoking history, *etc*.) • Use of male subjects only. • Lack of detail concerning number of test subjects used. • Limited description only of gas delivery system and exposure chamber. • Inadequate detail concerning analytical confirmation of exposure concentrations.	Low (●)

Author(s)	Study Code	Overall Technical Quality		Grade[2]
		Key Strengths	Major Weaknesses	
			• Lack of detail concerning exact exposure concentrations and times examined (… concentrations and times were reported as ranges only). • No records with respect to post-exposure observations. • Lack of control group of subjects.	
Case reports				
Mitchell and Yant (1925)	CR066	• Paper encompasses description of "real world" incidents involving over-exposure of humans to H_2S. • Some attempt made to correlate findings with observations from non-clinical and clinical investigations described as part of same paper.	• No information respecting concentrations of H_2S to which workers may have been exposed. • Limited reporting of clinical symptoms. • No indication of medical intervention, treatment or follow-up.	No Practical Use (★)
Prouza (1972)	CR067	• Case report describing circumstances surrounding "real world" incident involving the death of a worker over-exposed to H_2S. • Some indication of approximate exposure concentration (*i.e.*, greater than 2,850 ppm) and exposure time (*i.e.*, "a few minutes") resulting in death. • Good correlation between clinical symptoms, death and autopsy findings.	• Actual exposure concentration and exposure time leading to death not known. • Actual exposures received by rescue workers who survived the incident not known. • Measurement of H_2S concentrations within vicinity of incident involved use of detector tubes with limited sensitivity.	Low (●)
Winek (1968)	CR002	• Description of "real world" incident involving over-exposure to H_2S leading to death. • Some indication of potential exposure concentration(s) that might have been encountered as well as indication of exposure time. • Good description of autopsy findings,	• Actual concentration of H_2S to which subject may have been exposed unknown. Evidence points to possibly higher concentration than that measured and reported. • Details concerning measurements of H_2S taken in relation to the incident were limited. The time interval between the incident and the measurements	Low (●)

Author(s)	Study Code	Overall Technical Quality		Grade[2]
		Key Strengths	Major Weaknesses	
		including results from analysis of tissues for the presence of H_2S. • Good correlation between symptoms (unconsciousness), eventual outcome (death) and autopsy findings.	was not indicated, nor were details given concerning the sampling and analytical methodology employed.	
Review articles				
Back *et al.* (1972)[4]	RE003	• No obvious strengths	• The paper contains only a brief summary of work seemingly performed by others, and limited to a listing of one-hour LC_{50} values for rats and mice from a single study. • Technical quality of the data could not be determined directly, but only through retrieval and review of the original study conducted by MacEwan and Vernot (1972 – NC072).	No Practical Use (★)
Haggard (1925)		• Provides a general overview of the toxicology of H_2S, with reference to systemic poisoning.	• Information is "dated". • Extent of literature review was very limited. • Reliability of the information could not be readily established (*i.e.*, information from other sources was simply summarized, with very little detail provided). • No information provided specific to concentration-time-response characteristics of H2S vis-à-vis lethality or any other health endpoint.	No Practical Use
Lefaux (1968)	RE001	• The paper provides a listing of health effects according to both exposure concentration and exposure time, with concentration-time combinations associated with lethality indicated.	• Source of health effects information was not provided (*i.e.*, the information could not be substantiated). • Descriptions of health effects were very brief. • Technical quality of the information could not be determined.	No Practical Use

Author(s)	Study Code	Overall Technical Quality		Grade[2]
		Key Strengths	Major Weaknesses	
NIOSH (1977)	RE005	• Comprehensive review of the toxicology of H_2S, including summary of findings from case reports involving systemic poisonings, epidemiological studies and animal toxicity tests.	• Reliability and technical quality of original studies were not readily apparent and were not determined.	No Practical Use
Tabulae Biologicae Periodicae (1933)		• None	• The paper contains no information relating to H_2S.	No Practical Use

[1] Highlighted studies are those selected by the AEUB which served as the basis of the "toxic load" calculations used to derive the proposed EPZ endpoints for H_2S.

[2] The grade principally reflects the adequacy and usefulness of the study for establishing the concentration-time-response characteristics of H_2S vis-à-vis lethality following short-term exposure. Assignment of grades was based, in part, on comparison of study design, conduct and reporting features against "benchmarks" recommended by leading scientific and regulatory authorities. The strengths and weaknesses listed are among those considered in the grading. For complete details, the reader is referred to the Document Review Forms found in Appendix A.

[3] Note that the "low" rating applies in most part to the portion of the study involving the "accidental" exposure of mice due to equipment malfunction. The remainder of the study was deemed to be of moderate quality.

[4] The paper by Back *et al*. (1972) is a listing of one-hour LC_{50} values and confidence limits for H_2S for rats and mice. The values closely match those reported by MacEwan and Vernot (1972). Given the similarity in the reported LC_{50} values, the date of issue of both papers (August 1972), and the fact that both papers indicated that the testing was commissioned by the same sponsors (U.S. Department of Transportation and the U.S. Air Force Toxic Hazards Research Unit at Wright-Patterson Air Force Base), it was concluded that the two sets of data originated from the same source.

5.0 DISCUSSION

The present work was concerned with assessing the technical quality of the health effects data cited by the AEUB as part of the development of the proposed EPZ endpoints for H_2S outlined in the Discussion Paper entitled *"Proposed Hydrogen Sulphide Endpoints for Emergency Response Planning"* (October 2004). A primary objective of the work was to determine whether or not the data were representative and scientifically defensible. Beyond this objective, the work also was meant to provide some indication of the level of confidence that could be assigned to the proposed endpoints in terms of defining EPZs that afford protection against serious, irreversible health outcomes in the event of an emergency involving the release of "sour" gas.

In keeping with the Terms of Reference developed for the work, the assessment consisted largely of evaluating the strengths and weaknesses of each of the various health effects studies cited by the AEUB based on comparison of the design, conduct and reporting features of the study against "benchmarks" developed by a number of leading scientific and regulatory authorities. As part of the assessment, a grade or score was assigned to each study, with the grade not only signalling the outcome of the comparison, but also signifying the adequacy and usefulness of the investigation in providing an understanding of the concentration-time-response characteristics of H_2S vis-à-vis lethality. The emphasis on lethality was based on the proposed use of this health endpoint in the Discussion Paper as the leading measure of serious, irreversible health effects, coupled with the AEUB's reliance on the use of LC_{50} data in the "toxic load" calculations that formed the basis of the proposed EPZ endpoints.

The assessment revealed a diversity of grades, with some studies receiving a moderate-to-high rating, whereas other studies were deemed to be of no practical use. Deficiencies which seriously undermined the adequacy and usefulness of the lower-grade studies included:

- Lack of detail concerning the actual concentration(s) of H_2S to which the test animals or human subjects were exposed. This lack of detail was a common weakness among the case reports of accidental poisonings in the workplace (Winek, 1968 - CR002●; Mitchell and Yant - CR066★; and Prouza, 1972 - CR067●). Although, in some instances, attempts were made to determine the concentrations(s) of H_2S involved, the measurements invariably were taken after-the-fact (*i.e.*, several hours after the occurrence of the incident) and/or at locations that did not exactly match the whereabouts of the stricken workers. In other cases, no measurements were taken. In each case, the lack of detail effectively precluded determination of the "toxic load" (*i.e.*, the combination of exposure concentration and exposure time) leading to the deaths. The lack of detail also applied to certain of the controlled clinical and non-clinical investigations, largely as a result of the failure to analytically confirm the test concentrations of H_2S to which the animals or subjects were exposed while in the exposure chamber. In some instances, there was no record of any attempt to confirm the concentration(s), while in other cases, the measurements were deemed to be suspect because of the use of dated and/or insensitive metering devices and analytical methods. In some cases, even the study investigators admitted to difficulties in maintaining uniform concentrations of H_2S within the exposure chambers. In other instances, the concentrations were listed only as ranges, often having a fairly wide spread (*i.e.*, ranging over tens of parts-per-million). Again, this detracted from the usefulness of the studies for the specific purpose of

defining an EPZ endpoint that requires a high degree of confidence in the exposure concentration-time response.

- Lack of detail respecting basic design features, including specifics concerning the source and purity of the H_2S, the gas delivery system, the exposure chamber and/or the monitoring equipment used to sample and test the chamber atmospheres. This lack of detail detracted from the technical quality of a number of the clinical and non-clinical investigations since it effectively breached the benchmark requirements for the proper design and reporting of health effects studies. It also confounded interpretation of the findings and conclusions from the investigations since the lack of detail precluded or confused the determination of chamber equilibration times, actual exposure times, actual exposure concentrations, *etc*.

- Use of limited numbers of test animals or human subjects of unspecified source, age, sex, weight, and/or pre-study health status. Both the limited numbers and the lack of detail detracted from the technical quality of the studies and undermined confidence in the study findings and conclusions. In one instance, even the study investigators admitted that the data should only be considered "preliminary" in nature owing to the use of restricted numbers of subjects (Michell and Yant, 1925 - CL010●).

- Lack of detail concerning actual time to death. In many instances, the exact time to death of the test animals or stricken workers following exposure to H_2S could not be discerned from the information supplied. In some cases, the time period over which deaths were reported to occur was sizeable, stretching over several hours. This lack of detail detracted from the usefulness of the studies in terms of defining the concentration-time-response characteristics of H_2S vis-à-vis lethality, and would necessarily hinder and/or confuse use of the data in the calculation of "toxic load".[20] This hindrance is especially relevant since much of the data that surfaced during the course of the review demonstrated a steep dose-response for lethality, with modest shifts in exposure concentration and/or exposure time having a marked influence on the outcome.

- Use of data from supposedly controlled tests involving "accidental" exposures to H_2S. Some of the findings from certain of the non-clinical studies were judged to be highly suspect since they were based on responses witnessed following accidental exposures of the test animals to H_2S as a result of technician error or equipment malfunction (O'Donoghue, 1961 - NC034●; Hays, 1972 - NC057■). The actual exposure concentrations to which the animals were exposed in these experiments could not be confirmed. The reported concentrations were simply estimates based on measurements taken after-the-fact. The circumstances surrounding these findings (including the reported deaths) effectively precludes use of the data in any "toxic load" calculations.

Interestingly, none of the studies received a "high" grade. Each study showed at least one departure from the recommended "benchmarks" that was judged to detract from its adequacy and usefulness. In some cases, the departures were minor, allowing the studies to achieve a "moderate-to-high" rating. As indicated earlier (see Results), the lack of "high" grades was ascribed, in part, to the age of most of the studies (*i.e*., pre-dating the testing guidelines), together with the use of stringent "benchmarks"

[20] Since the "toxic load" is calculated on a "ppm^n x minute" basis, the actual time to death becomes an important consideration. In some cases, the reported times to death stretched over a 4- to 6-hour period or greater, covering a spread of 240 minutes or more.

that were strictly applied as part of the rating system. The demand for high quality features and the consistent use of stringent grading criteria across all studies constituted a reasonable and necessary approach given the need for objectivity as well as the sheer importance of the data as an integral part of the development of the EPZ endpoints for H_2S. Relaxing the grading criteria simply to inflate the scores and create an impression of overall high quality was not an option. It would misrepresent the adequacy and usefulness of the data, and confuse the entire approach.

With respect to the specific set of studies selected by the AEUB and used in the "toxic load" calculations that formed the basis of the proposed EPZ endpoints for H_2S, a few comments are in order:

- The dataset did not include any low-grade studies. Accordingly, the "toxic load" calculations and the corresponding EPZ endpoints were not compromised by the use of highly suspect data.

- Apart from the review article by Back *et al.* (RE003★), each of the studies received a "moderate" grade, signifying that the data are reasonably technically robust and can be used with a reasonable degree of confidence as part of any "toxic load" calculations, despite showing some weaknesses in experimental design, conduct and/or reporting. In all cases, the deficiencies were judged not to detract significantly from the overall weight-of-evidence that signalled the usefulness of the study for advancing understanding of the concentration-time-response characteristics of H_2S vis-à-vis lethality.

- The paper by Back *et al.* (RE003★) is a review article summarizing the work performed by MacEwen and Vernot (NC072◆).[21] It was deemed to be of "no practical use" since it consisted only of a table listing the LC_{50} values for rats and mice previously reported by the original study investigators, with nothing further added. It should be removed from consideration since its inclusion in the "toxic load" calculations is redundant and misleading since it assigns extra weight to the original findings, possibly skewing the mean outcome of the calculations.

- The "toxic load" calculations (… as well as the determination of the EPZ endpoints …) could possibly benefit from inclusion of the lethality data gathered as part of the other non-clinical studies which achieved a "moderate" or higher grade. Although the 3-hour upper-bound exposure duration restriction would eliminate some of these studies, certain of the investigations either involved exposure times lasting less than 3 hours or recorded deaths within 3 hours. In particular, the study by Clanachan (NC002◆) might be useful since it involved exposure of mice to a series of exposure concentration-exposure time combinations, with the exposures lasting 2.5 to 30 minutes.

The present work did reveal some errors and omissions in certain of the "records" contained in Table 1 of Appendix 2 of the Discussion Paper (*i.e.*, the complete summary of lethality data). These errors are highlighted in Table 5-1. The source of the errors and omissions is unknown. Since in developing the Discussion

[21] The paper by Back *et al.* (1972) simply contains a listing of one-hour LC_{50} values and confidence limits for H_2S for rats and mice. The values closely match those reported by MacEwan and Vernot (1972). Given the similarity in the reported LC_{50} values, the date of issue of both papers (August 1972), and the fact that both papers indicated that the testing was commissioned by the same sponsors (U.S. Department of Transportation and the U.S. Air Force Toxic Hazards Research Unit at Wright-Patterson Air Force Base), it was assumed that the two sets of data originated from the same source.

Paper, reliance was placed on health effects data compiled by other authorities, without referral to the original scientific papers, it is possible that the errors originated from the other jurisdictions and were simply perpetuated. Regardless of the source, the discovery of the errors points out the need for careful scrutiny of the information, particularly since errors in the exposure concentrations and/or exposure times can have a direct and considerable bearing on the reliability of any "toxic load" calculations. Fortunately, none of the errors applied to the any of the studies selected by the AEUB and used as the basis of the proposed EPZ endpoints for H_2S. Accordingly, confidence remains in the outcomes and conclusions reached in the Discussion Paper. Nevertheless, the records should be corrected, otherwise the information could easily be misconstrued.

TABLE 5-1
ERRORS AND OMISSIONS DISCOVERED IN THE SUMMARY TABLE OF H2S LETHALITY DATA[1]

Record No.	Original Reference	Study Code	Description of Error
4	Lopez *et al.* (1987)	NC027◆	This single record devoted to the study indicates that 4 rats were exposed 400 ppm of H_2S for 240 minutes. In actuality, the study involved the exposure of groups of 12 rats to 0, 10, 200 or 400 ppm of H_2S for 4 hours.
7	Lefaux (1968)	RE001★	The record suggests that a single human subject was exposed to 600 ppm of H_2S for 30 minutes and survived. In fact, the paper by Lefaux is simply a review article which contains a table showing the toxic effects associated with different concentrations of H_2S, including an entry that 600 ppm is fatal in ½ hour. The basis of the entry is unknown and the information could not be substantiated.
16	Prouza (1970)	CR067●	The record suggests that 10 humans were exposed to 1,000 ppm of H_2S for one minute and that one of the subjects died. In actuality, the study consists of a case report of an industrial accident in which a worker was overcome by H_2S fumes after entering a tank and died. The exact details surrounding the exposure were unknown; however, according to the author, the worker was exposed to H_2S at a concentration greater than 2,850 ppm for "a few minutes". Nine other workers were involved in the attempt to rescue and resuscitate the stricken worker. The exposures received by these other workers were unknown.
18	Weedon *et al.* (1940)	NC054■	The record shows 1 of 8 rats dying after exposure to 250 ppm of H_2S for 1074 minutes. The record fails to indicate that an additional 3 rats were found dead at the end of 1374 minutes, at which time exposure was discontinued.
26	Hays (1972)	NC057■	The record indicates that exposure to 30 ppm of H_2S for 1110 minutes will kill 50% of a test population of mice. In fact, the findings from the study show that 3 of 8 mice were found dead within 1110 minutes following an "accidental" exposure to H_2S in which it was "estimated" that the gas level in the chamber was 30 ppm.
28	Weedon *et al.* (1940)	NC054■	The record indicates that 2 of 4 mice died following exposure to 63 ppm of H_2S for 804 minutes. In actuality, the results from the study show one mouse dying after 57 minutes, two of the mice dying within 960 minutes (… after which exposure was discontinued), and the remaining mouse dying 23 hours later.
30	Weedon *et al.* (1940)	NC054■	The record lists 2 of 4 mice dying after exposure to 250 ppm of H_2S for 410 minutes. In fact, the results from the study reveal that all 4 mice died within 6.9 to 7 hours of exposure (*i.e.*, within 414 to 420 minutes).
47	Weedon *et al.* (1940)	NC054■	The record indicates that 4 of 8 rats died after exposure to 1000 ppm of H_2S for 14 minutes. In fact, the paper reports only that all of the rats (n=8) were found dead with 29 to 37 minutes.
48	Weedon *et al.* (1940)	NC054■	The record lists 2 of 4 mice dying within 18 minutes following exposure to 1000 ppm of H_2S, whereas the actual study results show all 4 mice dying within 18 to 20

Record No.	Original Reference	Study Code	Description of Error
			minutes.
68	Mitchell and Yant (1925)	NC032●	The record suggests that 2 of 2 dogs exposed to 103 ppm of H_2S died after 960 minutes of exposure. In fact, it is not clear from the study that both of the dogs died, and the report only indicates that death occurred within 8 to 16 hours (*i.e.*, within 480 to 960 minutes).
74	Mitchell and Yant (1925)	NC032●	The record shows 2 of 2 dogs exposed to 350 ppm of H_2S dying after 480 minutes. In actuality, the results from the study show one of the dogs dying within 240 to 480 minutes, and the remaining dog dying within 480 to 960 minutes.
79	Lund and Wieland (1966)	NC073●	The record shows 100% mortality among monkeys following exposure to 500 ppm of H_2S for 35 minutes, based on the use of a single test animal. The record fails to indicate that, as part of the same study, two additional monkeys survived separate exposures to 500 ppm for 22 to 25 minutes.
108	Winek *et al.* (1968)	CR002●	The record indicates that exposure to 6100 ppm of H_2S for 5 minutes proved fatal to a human. In actuality, the paper describes a case involving the death of a worker overcome by fumes while working in a tank containing coal-tar resins. Evidence collected at the scene following the incident suggests that H_2S was implicated; however, the actual exposure received by the worker was unknown. Concentrations of H_2S measured near the top and mid-way point of the 15-foot high tank were reported to be 1900 to 6100 ppm, respectively, suggesting stratification of the gas in the tank.

[1] Refers specifically to the lethality data summarized in Table 1 of Appendix 2 of the Discussion Paper.

6.0 OTHER CONSIDERATIONS

Although the Terms of Reference developed for the work were aimed exclusively at the assessment of the technical quality of the health effects data used by the AEUB as part of the determination of the proposed EPZ endpoints for H_2S, the review of the studies did reveal a number of other findings that deserve consideration in expanding understanding of the concentration-time-response characteristics of H_2S vis-à-vis lethality. Some of these findings are meant to promote and/or complement the outcomes and conclusions reached in the Discussion Paper, whereas others serve as recommendations to improve and strengthen the approach followed by the Board. These findings are discussed below.

- The Discussion Paper relies heavily on health effects data cited by other authorities and used to establish emergency planning guidelines in other jurisdictions. This approach limits the number of studies considered. In fact, after discounting the review articles (… all of which were deemed to be of no practical use since the information either was of questionable relevance or could not be substantiated …), only 17 papers comprising 20 different studies remained. It is very likely that other studies exist that could further advance understanding of the concentration-time-response characteristics of H_2S. Efforts might be taken to identify these other studies as a means to validate and/or strengthen the outcomes and conclusions reached in the Discussion Paper. This finding assumes added significance since the initial list of 17 papers was eventually narrowed to only three original papers (*i.e.*, Prior *et al.*, 1988 - NC035◆; Zwart *et al.*, 1990 - NC056◆; and MacEwan and Vernot, 1972 - NC072◆), which ultimately served as the basis of the "toxic load" calculations that were used to develop the proposed EPZ endpoints. The use of only three studies may be too restrictive, and serves to emphasize the need for an expanded search. Any additional studies identified should necessarily be subjected to detailed review in order to establish the adequacy and reliability of the results. The present work clearly demonstrated that studies may be of little or no practical use in developing emergency planning guidelines because of serious weaknesses in experimental design, conduct and/or reporting.

- The Discussion Paper relies strictly on the use exposure concentration-exposure time combinations corresponding to LC_{50} values as inputs to the "toxic load" calculations that served as the basis of the proposed EPZ endpoints.[22] The LC_{50} "combinations" chosen as inputs were those reported directly by the study investigators. No apparent attempt was made to calculate additional LC_{50} values based on the lethality data reported as part of other studies included in the complete dataset (... admittedly, however, the number of additional reliable studies from which LC_{50} estimates might be derived was determined to be rather limited)[23]. Two

[22] The "toxic load" calculations are shown in Table 2 of the Discussion Paper. The proposed EPZ endpoints were based strictly on exposure concentration-exposure time combinations corresponding to LC_{50} values, which were then adjusted through the use of uncertainty factors to afford the level of protection demanded (*i.e.*, protection against serious irreversible health effects, including fatalities, with a conservative margin of safety).

[23] Of the additional studies included in the complete dataset that were not chosen for use in the "toxic load" calculations, only 5 received a grade of "moderate" or higher, possibly qualifying them as sources of reliable LC_{50} estimates . However, the findings from the majority of these studies proved unsuitable for calculating LC_{50} values. Specifically, the study by Lopez *et al.* (1986 – NC069◆) produced either no deaths or 100% mortality among rats exposed to 40 or 300 ppm of H_2S,

items arise from this finding. First, the LC_{50} estimates reported by Clanachan (1979 – NC002◆ – see footnote below) should be incorporated into the "toxic load" calculations. The addition of these estimates will act to augment and strengthen the approach. Second, the use of lethality data only from exposure concentration-exposure time combinations corresponding to LC_{50} values in the "toxic load" calculations may be overly restrictive. It fails to consider the wealth of lethality data that exist for other combinations. These data could be used to expand and/or validate the outcomes and conclusions reached in the Discussion Paper. In addition to examining the combinations corresponding to 50% mortality, it might be equally useful to consider the combinations associated with no deaths … or alternatively, the combinations at which deaths were first reported. Use of these latter combinations might allow for less reliance on the use of "uncertainty factors" in the determination of the EPZ endpoints since the response is much more tempered (*i.e.*, little or no mortality *vs*. 50% mortality) and closer to the outcome demanded (*i.e*., avoidance of serious, irreversible health effects, including fatalities). To facilitate the possible use of these other combinations in any refinement of the Discussion Paper, the relevant lethality data have been extracted from the original papers and are summarized in Table 6-1. The table lists the exposure concentration-exposure time combination(s) from each of the studies reviewed that correspond with no observed mortality, minimal observed mortality, and 50% mortality (based on LC_{50} values calculated by the study investigators). Additional details are available in the Document Review Forms found in Appendix A. For completeness, the entire complement of papers is included, regardless of grade. However, emphasis should necessarily be given to the higher quality papers when using the data. For added perspective, Table 6-2 is included, which lists the more significant clinical signs and symptoms, if any, that were observed for each of the exposure concentration-exposure time combinations that produced no deaths. Many of the observations shown would reasonably qualify as "serious" health effects (*e.g*., loss of consciousness, severe dyspnea, unsteady gait, clonic and/or tonic convulsions). The listing also indicates whether or not the effects were reversible. These observations should be considered when assessing the level of protection afforded by the proposed EPZ endpoints for H_2S. The level of protection should be sufficient to guard against the occurrence of these types of serious health outcomes in order to meet the criterion proposed in the Discussion Paper.

- Not only does the Discussion Paper rely exclusively on exposure concentration-exposure time combinations corresponding to LC_{50} values as inputs to the "toxic load" calculations (see above), it relies only on LC_{50} values derived from studies with laboratory rodents (*i.e*., rats and mice). Use of these LC_{50} values is understandable given that the rodent data were generally of comparatively higher quality than the data

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respectively, for 6 hours. Similarly, no deaths were recorded among rats exposed to 10, 200 or 400 ppm of H_2S for 4 hours in a subsequent study by Lopez *et al.* (1987 – NC027◆). In the remaining study by Lopez *et al.* (1989 – NC031◆), all rats died within 5 minutes following exposure to 1655±391 ppm of H_2S. The data from these studies are not amenable to the calculation of LC_{50} values since only 0% or 100% mortality was observed. Clanachan (1979 – NC002◆) did report a series of LC_{50} values for mice based on different exposure concentration-exposure time combinations. The reason for excluding these values from the "toxic load" calculations is not known. The study by Tansy *et al.* (1981 –NC047◆) produced graded mortality among rats exposed to H_2S at concentrations ranging from 400 to 600 ppm; however, the exposure time was 4 hours, and therefore, outside the 3-hour exposure duration limit specified for the "toxic load" calculations in the Discussion Paper.

obtained from other species. The lack of reliable mortality data from the human case reports and the absence of deaths (… as expected …) in the human clinical studies also support the use of the rodent findings. However, although use of the rodent data may be justifiable, the "toxic load" calculations might benefit from consideration of the physiological, anatomical and metabolic differences that exist between humans and rodents that can have a direct bearing on the "dose" of H_2S received. Although the available data do not suggest remarkable distinctions in sensitivity to H_2S between mammalian species, acknowledgement and incorporation of these differences as part of the calculations could lead to refinement of the "toxic load" estimates. This, in turn, could contribute to more selective use of "uncertainty factors" in the development of the EPZ endpoints for H_2S as opposed to the somewhat arbitrary choice of factors outlined in the Discussion Paper. Work aimed at examining the species differences and determining how they might be incorporated into the calculations should be explored.

TABLE 6-1

SUMMARY OF LETHALITY DATA EMPHASIZING EXPOSURE CONCENTRATION-EXPOSURE TIME COMBINATIONS RESULTING IN LITTLE OR NO MORTALITY[1]

Author(s)	Study Code	Grade	Species	Upper-End Exposure Concentration-Exposure Time Combination(s) Resulting in No Mortality		Lower-End Exposure Concentration-Exposure Time Combination(s) Resulting in Mortality[2]			Exposure Concentration-Exposure Time Combination(s) Corresponding to LC_{50} Values (calculated)[3]		
				Concentration (ppm)	Time	Concentration (ppm)	Time	% Mortality	Concentration (ppm)	Time	% Mortality
Non-clinical studies											
Clanachan (1979)	NC002	Moderate	Mouse	500	30 minutes	800	30 minutes	5	700	>30 minutes	50
				600	30 minutes	900	15 minutes	10	800	>30 minutes	50
				700	30 minutes	1100	2.5 minutes	5	900	>30 minutes	50
				800	15 minutes	1200	2.5 minutes	10	961	30 minutes	50
									1,000	18.6 minutes	50
									1,003	15 minutes	50
									1,059	12.5 minutes	50
									1,097	10 minutes	50
									1,100	10.3 minutes	50
									1,132	7.5 minutes	50
									1,200	5.2 minutes	50
									1,207	5 minutes	50
									1,300	4.3 minutes	50
									1,734	2.5 minutes	50
Haggard (1925)	NC067	Low	Dog	100 to 150	Several hours	500 to 700	Several hours	Not specified	No calculated LC_{50} value(s) were provided by the study investigator(s).		
				200 to 300	Several hours	900	< 60 minutes	Not specified			
Hays (1972)	NC057	Low-to-Moderate	Mouse	10	120 hours	50	16 hours	50	30	18.5 hours	50
				20	48 hours	100	8 hours	40	50	15 hours	50
									100	7.5 hours	50
			Goat	10	96 hours	No goats died on test					
				50	96 hours						
				100	96 hours						
			Cow	20	21 days	No cows died on test					
Lehmann (1892)	NC070	Low	Cat	130	480 minutes	720	330 minutes	100 (single cat)	No calculated LC_{50} value(s) were provided by the study investigator(s).		
				140	600 minutes	710	490 minutes	100 (single cat)			
				220	480 minutes	3250	10 minutes	100 (single cat)			

Author(s)	Study Code	Grade	Species	Upper-End Exposure Concentration-Exposure Time Combination(s) Resulting in No Mortality		Lower-End Exposure Concentration-Exposure Time Combination(s) Resulting in Mortality[2]			Exposure Concentration-Exposure Time Combination(s) Corresponding to LC_{50} Values (calculated)[3]		
				Concentration (ppm)	Time	Concentration (ppm)	Time	% Mortality	Concentration (ppm)	Time	% Mortality
				360	210 minutes	3400	2 minutes	100 (single cat)			
				490	160 minutes	5200	4 minutes	100 (single cat)			
				700	255 minutes						
				760	109 minutes						
			Dog	380	65 minutes	1880	1.5 minutes	100 (single dog)			
				560	41 minutes	5200	4 minutes	100 (single dog)			
				3400	2 minutes	140	600 minutes	100 (single rabbit)			
			Rabbit	130	480 minutes	470	495 minutes	100 (single rabbit)			
				220	480 minutes	750	265 minutes	100 (single rabbit)			
				360	210 minutes	710	230 minutes	100 (single rabbit)			
				490	160 minutes	5200	4 minutes	100 (single rabbit)			
				760	10 minutes						
				1300	3 minutes						
				3250	2.5 minutes						
			Guinea pig	No combinations tested were without mortality		470	530 minutes	100 (single g. pig)			
						1300	90 minutes	100 (single g. pig)			
Lopez *et al.* (1986)	NC069	Moderate-to-High	Rat	40	360 minutes	300	360 minutes	100	No calculated LC_{50} value(s) were provided by the study investigator(s).		
Lopez *et al.* (1987)	NC027	Moderate	Rat	10	240	No combinations tested produced mortality			No calculated LC_{50} value(s) were provided by the study investigator(s).		
				200	240						
				400	240						
Lopez *et al.* (1989)	NC031	Moderate-to-High	Rat	Only a single combination was tested and it resulted in mortalities		1655±391	< 3 minutes	100	No calculated LC_{50} value(s) were provided by the study investigator(s).		

Author(s)	Study Code	Grade	Species	Upper-End Exposure Concentration-Exposure Time Combination(s) Resulting in No Mortality		Lower-End Exposure Concentration-Exposure Time Combination(s) Resulting in Mortality[2]			Exposure Concentration-Exposure Time Combination(s) Corresponding to LC_{50} Values (calculated)[3]		
				Concentration (ppm)	Time	Concentration (ppm)	Time	% Mortality	Concentration (ppm)	Time	% Mortality
Lund and Wieland (1966)	NC073	Low	Monkey	500	Up to 25 minutes	500	35 minutes	100 (single monkey)	No calculated LC_{50} value(s) were provided by the study investigator(s).		
MacEwen and Vernot (1972)	NC072	Moderate	Rat	400	60	635	60 minutes	10	712	60 minutes	50
				504	60						
			Mouse	504	60	400	60 minutes	20	634	60 minutes	50
Mitchell and Yant (1925)	NC032	Low	Canary	440 to 620	2 minutes	35 to 65	Up to 18 hours	100	No calculated LC_{50} value(s) were provided by the study investigator(s).		
						97 to 100	Up to 8 hours	100			
						140	Up to 8 hours	100			
						190 to 210	Up to 60 minutes	Not specified			
						280 to 310	Up to 30 minutes	Not specified			
						730	20 seconds	100			
			Rats	36 to 65	48 hours	A number of different combinations were tested that resulted in mortalities; however, presentation of the findings is hindered because of lack of definition of exposure concentrations, exposure times and/or percent mortality.					
			Guinea pig	35 to 65	48 hours	103	Up to 48 hours	50			
				820	30 minutes	1500	Up to 30 minutes	50			
			Dog	No combinations tested were without mortality		760 to 800	Up to 60 minutes	50			
						All other combinations produced 100% mortality					
			Goat	820	30 minutes	Other combinations were tested that resulted in mortalities; however, presentation of the findings is hindered because of lack of definition of exposure concentrations, exposure times and/or percent mortality.					
O'Donoghue (1961)	NC034	Low	Pig	50 to 100 (progressively increasing)	120 minutes	250 to 1000 (progressively increasing)	130 minutes	100 (single pig)	No calculated LC_{50} value(s) were provided by the study investigator(s).		
				250 to 970 (progressively increasing)	230 minutes	350 to 1200 (progressively increasing)	44 minutes	100 (single pig)			

Author(s)	Study Code	Grade	Species	Upper-End Exposure Concentration-Exposure Time Combination(s) Resulting in No Mortality		Lower-End Exposure Concentration-Exposure Time Combination(s) Resulting in Mortality[2]			Exposure Concentration-Exposure Time Combination(s) Corresponding to LC_{50} Values (calculated)[3]		
				Concentration (ppm)	Time	Concentration (ppm)	Time	% Mortality	Concentration (ppm)	Time	% Mortality
			Rabbit	50	16 hours	1000 "accidental"	Momentary	30			
Prior *et al*. (1988)	NC035	Moderate	Rat	≈340	360 minutes	299 (calculated)	360 minutes	10	335	360 minutes	50
				≈460	240 minutes	422 (calculated)	240 minutes	10	501	240 minutes	50
				≈635	120 minutes	549 (calculated)	120 minutes	10	587	120 minutes	50
Tansy *et al*. (1981)	NC047	Moderate	Rat	No combinations tested were without mortality		400	240 minutes	30	444	240 minutes	50
						440	240 minutes	30			
Weedon *et al*. (1940)	NC054	Low-to-Moderate	Rat	16	16 hours	63	Up to 16 hours	12.5	16	>960 minutes	50
									63	>960 minutes	50
									250	>960 minutes	50
									1,000	14 minutes	50
			Mouse	16	16 hours	63	Up to 16 hours	100	16	>960 minutes	50
									63	804 minutes	50
									250	410 minutes	50
									1,000	18 minutes	50
Zwart *et al*. (1990)	NC056	Moderate	Rat	665	5 minutes	854	5 minutes	20	679	50 minutes	50
				665	10 minutes	668	30 minutes	10	721	30 minutes	50
				321	30 minutes	694	30 minutes	20	829	10 minutes	50
				504	30 minutes	737	30 minutes	30			
				581	30 minutes						
				595	30 minutes						
				320	60 minutes						
				502	60 minutes						
				553	60 minutes						
				576	60 minutes						
				590	60 minutes						
			Mouse	665	5 minutes	1308	5 minutes	30	671	50 minutes	50
				854	5 minutes	629	30 minutes	20	793	30 minutes	50
				665	10 minutes	668	30 minutes	10	1,150	10 minutes	50
				856	10 minutes	694	30 minutes	30			
				321	30 minutes	553	60 minutes	20			

Author(s)	Study Code	Grade	Species	Upper-End Exposure Concentration-Exposure Time Combination(s) Resulting in No Mortality		Lower-End Exposure Concentration-Exposure Time Combination(s) Resulting in Mortality[2]			Exposure Concentration-Exposure Time Combination(s) Corresponding to LC_{50} Values (calculated)[3]		
				Concentration (ppm)	Time	Concentration (ppm)	Time	% Mortality	Concentration (ppm)	Time	% Mortality
				504	30 minutes	576	60 minutes	30			
				581	30 minutes						
				737	30 minutes						
				320	60 minutes						
Clinical studies											
Lehmann (1892)	CL011	Low	Human	100 to 150	60 minutes	No combinations tested produced mortality			No calculated LC_{50} value(s) were provided by the study investigator(s).		
				145	236 minutes						
				210	60 minutes						
				250	184 minutes						
				210	158 minutes						
				261	46 minutes						
				326	145 minutes						
				331	53 minutes						
				531	40 minutes						
				575	199 minutes						
				20 to 40	60 minutes						
				70 to 90	60 minutes						
				140 to 150	60 minutes						
				210 to 280	30 minutes						
				210 to 230	52 minutes						
				370 to 490	95 minutes						
				250 to 410	110 minutes						
				530	30 minutes						
				120 to 200	180 minutes						
				100 to 140	181 minutes						
				100 to 130	83 minutes						
Mitchell and Yant (1925)	CL010	Low	Human	100 to 150	240 minutes	No combinations tested produced mortality			No calculated LC_{50} value(s) were provided by the study investigator(s).		
				150 to 200	240 minutes						
				250 to 350	240 minutes						
				350 to 450	60 minutes						

Author(s)	Study Code	Grade	Species	Upper-End Exposure Concentration-Exposure Time Combination(s) Resulting in No Mortality		Lower-End Exposure Concentration-Exposure Time Combination(s) Resulting in Mortality[2]			Exposure Concentration-Exposure Time Combination(s) Corresponding to LC_{50} Values (calculated)[3]		
				Concentration (ppm)	Time	Concentration (ppm)	Time	% Mortality	Concentration (ppm)	Time	% Mortality
Case Reports											
Mitchell and Yant (1925)	CR066	No practical use	Human	Unknown Unknown	Approx. 1 minute <1 minute	Unknown	Approx. 5 minutes	67 (2 of 3 workers died)	No calculated LC_{50} value(s) were provided by the study investigator(s).		
Prouza (1970)	CR067	Low	Human	100 to greater than 2850	Unknown	>2850	"A few minutes"	33 (1 of 3 workers died)	No calculated LC_{50} value(s) were provided by the study investigator(s).		
Winek *et al.* (1968)	CR002	Low	Human	No cases without mortality were described		1900 to 6100	5 minutes	100 (single worker)	No calculated LC_{50} value(s) were provided by the study investigator(s).		

[1] The information listed is meant to emphasize the exposure concentration-exposure time combinations that were tested in each study and resulted in little or no mortality. Not all combinations are listed. Complete details concerning the various combinations tested in each study are contained in the Document Review Forms found in Appendix A.

[2] In some cases, the % mortality is seemingly very high owing largely to experimental designs involving separate exposures of single test animals or accidental exposure of single workers (*i.e.*, the use of single test animals or exposure of single workers necessarily meant that the % mortality would register 100 if the animal or worker died).

[3] The exposure concentration-exposure time combinations listed are those reported by the study investigator(s) to cause death in 50% of the test population based on calculations or use of concentration-time-mortality plots.

TABLE 6-2
SUMMARY OF LETHALITY DATA EMPHASIZING EXPOSURE CONCENTRATION-EXPOSURE TIME COMBINATIONS RESULTING IN NO MORTALITY WITH OR WITHOUT OTHER SERIOUS IRREVERSIBLE HEALTH EFFECTS [1]

Author(s)	Study Code	Grade	Species	Upper-End Exposure Concentration-Exposure Time Combination(s) Resulting in No Mortality		
				Concentration (ppm)	Time	Other Indications of Serious, Irreversible Health Effects[2]
Non clinical Studies						
Clanachan (1979)	NC002	Moderate	Mouse	500	30 minutes	None reported
				600	30 minutes	Loss of righting reflex (*i.e.*, unconsciousness)
				700	30 minutes	Loss of consciousness within 12.5 minutes
				800	15 minutes	Loss of consciousness within 7.5 minutes
Haggard (1925)	NC067	Low	Dog	100 to 150	Several hours	No effects other than local irritation
				200 to 300	Several hours	"Slight general symptoms", together with local irritation.
Hays (1972)	NC057	Low-to-Moderate	Mouse	10	120 hours	Reduced feed and water intake (… possibly consistent with generalized systemic toxicity and/or secondary to local irritation caused by exposure or discomfort experienced in exposure chamber). No recovery period included.
				20	48 hours	Loss of body weight, reduced feed and water intake, and evidence of hypothermia (*i.e.*, reduced rectal temperatures) consistent with generalized systemic toxicity. Some indication of recovery within 14 days (based on "accidental" exposure to 30 ppm for 24 hours).
			Goat	10	96 hours	Modest, temporary reduction in feed and water intake only (… possibly due to discomfort from confinement in exposure chamber).
				50	96 hours	Reduced feed and water intake … with some evidence of recovery toward end of exposure period.
				100	96 hours	Some reduction in respiration rate, as well as reduced feed and water intake. Evidence of transient effect on thermoregulation (*i.e.*, increased rectal temperature)
			Cow	20	21 days	No effects other than local irritation.
Lehmann (1892)	NC070	Low	Cat	130	480 minutes	No effects other than "varying respiration" and minimal salivation. Full recovery upon cessation of exposure.
				140	600 minutes	None reported.

Author(s)	Study Code	Grade	Species	Upper-End Exposure Concentration-Exposure Time Combination(s) Resulting in No Mortality		
				Concentration (ppm)	Time	Other Indications of Serious, Irreversible Health Effects[2]
				220	480 minutes	Difficulty walking and generally unresponsive … animal was reported to be "half narcotized".
				360	210 minutes	Gradual reduction in respiration rate, sleepiness, lack of responsiveness.
				380	65 minutes	None reported.
				490	160 minutes	Unsteady gait, sleepiness, with or without vomiting … complete recovery.
				560	41 minutes	None reported.
				700	255 minutes	Unsteady gait, difficulty standing, generally unresponsive … complete recovery within 24 hours.
				760	109 minutes	Laboured respiration, staggered gait, general unsteadiness, tonic contractions … recovery within a few hours.
			Dog	380	65 minutes	Restlessness, slight convulsions in limbs, laboured respiration, numbness, retching … complete recovery.
				560	41 minutes	Epilepsy-like attacks, unsteady gait, retching, "stretching" convulsions … recovered.
				3400	2 minutes	Restlessness, stretching convulsions, alternating clonic and tonic convulsions, moribund … moderate recovery after 4.5 hours.
			Rabbit	130	480 minutes	Laboured respiration … complete recovery upon cessation of exposure.
				220	480 minutes	Few symptoms other than laboured respiration … full recovery upon cessation of exposure.
				360	210 minutes	None reported.
				490	160 minutes	Laboured respiration, unsteady gait, stretching convulsions, generalized weakness … still unsteady 30 hours post-exposure.
				760	10 minutes	Clonic and tonic convulsions, loss of righting ability, "rolling" movements … recovery within a few hours.
				1300	3 minutes	Staggering movements, unsteady gait, laboured respiration, loss of righting ability … recovery within 15 minutes.
				3250	2.5 minutes	Collapse, laboured respiration, convulsive movements … recovery within 10 minutes.

Author(s)	Study Code	Grade	Species	Upper-End Exposure Concentration-Exposure Time Combination(s) Resulting in No Mortality		
				Concentration (ppm)	Time	Other Indications of Serious, Irreversible Health Effects[2]
			Guinea pig	No combinations tested were without mortality		
Lopez *et al.* (1986)	NC069	Moderate-to-High	Rat	40	360 minutes	Agitated movements, laboured respiration and loss of body weight possibly due to general malaise … full recovery with no noticeable residual signs of toxicity … no macroscopic lesions … mild necrosis of nasal epithelium noted histologically.
Lopez *et al.* (1987)	NC027	Moderate	Rat	10	240	None reported.
				200	240	None reported.
				400	240	Moderate lethargy … rapid recovery.
Lopez *et al.* (1989)	NC031	Moderate-to-High	Rat	Only a single combination was tested and it resulted in mortalities		
Lund and Wieland (1966)	NC073	Low	Monkey	500	Up to 25 minutes	Laboured respiration, loss of consciousness within 15 minutes … ataxic movements, loss of appetite, and somnolescence for 10 days post-exposure.
MacEwen and Vernot (1972)	NC072	Moderate	Rat	400	60	Laboured respiration (?)
				504	60	Laboured respiration (?)
			Mouse	504	60	Laboured respiration and convulsions (?)
Mitchell and Yant (1925)	NC032	Low	Canary	440 to 620	2 minutes	Laboured respiration, loss of consciousness
			Rats	35 to 65	48 hours	No signs other than local irritation.
			Guinea pig	35 to 65	48 hours	Cough as well as local irritation.
				820	30 minutes	Increased respiration.
			Dog	No combinations tested were without mortality		
			Goat	820	30 minutes	Increased respiration.
O'Donoghue (1961)	NC034	Low	Pig	50 to 100 (progressively increasing)	120 minutes	None reported.

Author(s)	Study Code	Grade	Species	Upper-End Exposure Concentration-Exposure Time Combination(s) Resulting in No Mortality		
				Concentration (ppm)	Time	Other Indications of Serious, Irreversible Health Effects[2]
				250 to 970 (progressively increasing)	230 minutes	Laboured respiration, unsteady gait, loss of consciousness … full recovery, with no after effects.
			Rabbit	50	16 hours	None reported
Prior *et al.* (1988)	NC035	Moderate	Rat	≈340	360 minutes	Weight loss (?)
				≈460	240 minutes	Weight loss (?)
				≈635	120 minutes	Weight loss (?)
Tansy *et al.* (1981)	NC047	Moderate	Rat	No combinations tested were without mortality		
Weedon *et al.* (1940)	NC054	Low-to-Moderate	Rat	16	16 hours	Transient and slight restlessness only.
			Mouse	16	16 hours	Transient and slight restlessness only.
Zwart *et al.* (1990)	NC056	Moderate	Rat	665	5 minutes	None reported[3]
				665	10 minutes	None reported
				321	30 minutes	None reported
				504	30 minutes	None reported
				581	30 minutes	None reported
				595	30 minutes	None reported
				320	60 minutes	None reported
				502	60 minutes	None reported
				553	60 minutes	None reported
				576	60 minutes	None reported
				590	60 minutes	None reported
			Mouse	665	5 minutes	None reported
				854	5 minutes	None reported
				665	10 minutes	None reported
				856	10 minutes	None reported
				321	30 minutes	None reported
				504	30 minutes	None reported
				581	30 minutes	None reported
				737	30 minutes	None reported
				320	60 minutes	None reported
Clinical Studies						
Lehmann (1982)	CL011	Low	Human	100 to 150	60 minutes	No symptoms other than local irritation.
				145	236 minutes	Persistent headache, pain in eyes

Author(s)	Study Code	Grade	Species	Upper-End Exposure Concentration-Exposure Time Combination(s) Resulting in No Mortality		
				Concentration (ppm)	Time	Other Indications of Serious, Irreversible Health Effects[2]
				210	60 minutes	Headache and eye irritation … continuing for several hours post-exposure.
				250	184 minutes	Light headache, inflammation of eyelids … recovery within 2.5 hours post-exposure
				210	158 minutes	Headache, pain in eyes … symptoms persisted for 24 hours.
				261	46 minutes	No symptoms other than local irritation of eyes and trachea … rapid recovery.
				326	145 minutes	Pain in head and eyes … rapid recovery.
				331	53 minutes	Local irritation and latent headache.
				531	40 minutes	Persistent headache and local irritation of eyes and trachea.
				575	199 minutes	Headache and persistent pain in eyes.
				20 to 40	60 minutes	None reported.
				70 to 90	60 minutes	No symptoms other than slight local irritation.
				140 to 150	60 minutes	No symptoms other than slight to unpleasant local irritation.
				210 to 280	30 minutes	No symptoms other than local irritation.
				210 to 230	52 minutes	Progressive local irritation, otherwise no symptoms … latent diarrhea.
				370 to 490	95 minutes	Cough, pain in eyes, swelling of eyelids, light intolerance … latent diarrhea.
				250 to 410	110 minutes	Difficult respiration, pain in eyes, light intolerance … latent diarrhea, slight bladder pain.
				530	30 minutes	Headache, unsteadiness, giddiness, trembling of the extremities, accompanied by local irritation… latent diarrhea, headache, pain in bladder.
				120 to 200	180 minutes	Transient difficulty in breathing, slight irritation of eyes and throat … latent headache, slight bronchitis.
				100 to 140	181 minutes	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.
				100 to 130	83 minutes	No symptoms other than slight nasal irritation.
Mitchell and Yant (1925)	CL010	Low	Human	100 to 150	240 minutes	Cough, disturbed respiration, accompanied by pain in eyes and throat irritation.

Author(s)	Study Code	Grade	Species	Upper-End Exposure Concentration-Exposure Time Combination(s) Resulting in No Mortality		
				Concentration (ppm)	Time	Other Indications of Serious, Irreversible Health Effects[2]
				150 to 200	240 minutes	Cough, difficult respiration, irritation of eyes and throat, light intolerance.
				250 to 350	240 minutes	Headache, difficult respiration, weariness, irritation of eyes and nasal passages, light intolerance.
				350 to 450	60 minutes	Headache, cough, difficult respiration, irritation of eyes and nasal passages.
Case Reports						
Mitchell and Yant (1925)	CR066	No practical use	Human	Unknown Unknown	Approx. 1 minute <1 minute	Loss of consciousness … recovery within a few days. Loss of consciousness, headache, nausea, stomach pain … recovery within 24 hours.
Prouza (1970)	CR067	Low	Human	100 to greater than 2850	Unknown	Loss of consciousness, nausea, general weakness, pain in chest … recovery with 14 days.
Winek *et al.* (1968)	CR002	Low	Human	No cases without mortality were described		

[1] The information listed is limited to the exposure concentration-exposure time combinations that were tested in each study and resulted in no mortality. Complete details concerning the various combinations tested in each study are contained in the Document Review Forms found in Appendix A.
[2] 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. In some instances (… designated by (?) …), reporting was such that it was not clear whether the signs and symptoms were observed as part of the specific exposure concentration-exposure time combination shown (*i.e.*, the signs and symptoms were reported as generic entries without indication of the exposure concentration-exposure time combination(s) involved).
[3] No clinical signs were reported by Zwart *et al.* (1990) regardless of exposure concentration-exposure time combination, despite reference in the paper that during the 14-day observation period, clinical signs were monitored at least once per day.

7.0 SUMMARY AND CONCLUSIONS

A principal objective of this work was to determine the adequacy and technical quality of the health effects data used by the AEUB in deriving the proposed EPZ endpoints for H_2S. The assessment was completed in accordance with specific Terms of Reference developed for the work, with full respect for the need for objectivity, consistency and scientific rigour.

A total of 21 papers comprising 25 original health effects studies and/or summaries of health effects data on H_2S were reviewed. Each of the papers was cited in the Discussion Paper (October 2004) prepared by the AEUB, in which the approach taken to develop the proposed EPZ endpoints was described. The approach relied, in part, on a series of "toxic load" calculations that considered exposure concentration-exposure time combinations corresponding to LC_{50} values sourced from a smaller subset of four (4) studies. The entire dataset reviewed by the consultant included non-clinical studies involving controlled exposures of test animals to H_2S, clinical investigations involving controlled exposures of human subjects, case reports describing accidental exposures in the workplace, and review articles summarizing health effects data gathered by others. The smaller subset of studies used by the AEUB consisted entirely of tests using rats and/or mice. Much of the information reviewed concerned the health effects associated with short-term inhalation exposures to H_2S, with an emphasis on exposures causing death.

The review consisted largely of comparison of the design, conduct and reporting features of each study against a series of "quality benchmarks". The benchmarks were based on the recommendations of a number of leading scientific and regulatory authorities for the proper design, execution and reporting of health effects studies. Each study was graded in terms of how well the design, conduct and reporting features matched the recommendations. A grading system was developed to distinguish between low *vs*. moderate *vs*. high quality studies as well as to identify any studies having no practical value. The grading system was intended principally to gauge the adequacy and usefulness of each study in terms of advancing understanding of the concentration-time-response characteristics of H_2S vis-à-vis lethality following short-term exposure.

The principal findings that emerged from the work were:

- None of the studies received a "high" rating, signifying that each of the studies suffered from one or more weaknesses that detracted from its usefulness and limited the level of confidence that could be assigned to its findings and conclusions. The lack of high grades was due, in part, to the age of the most of the studies, with many pre-dating the testing guidelines (circa 1980) and some performed in the late 1800's using archaic designs, make-shift equipment, and poor reporting standards. The absence of high grades also may have resulted from the strict application of the "quality benchmarks" throughout the review, which demanded that each study meet very stringent and exacting standards. In some cases, the weaknesses were modest, allowing a "moderate-to-high" grade to be assigned.

- A number of the studies (≈40%) received a "low" grade, signalling significant deficiencies in experimental design, conduct and reporting that seriously detracted from their usefulness. Weaknesses common to these studies included: inadequate description of equipment (*i.e*., exposure chamber, gas delivery system, metering devices); use of make-shift and "dated" instrumentation and insensitive analytical

methods; failure to analytically confirm the concentrations of H_2S to which the test animals or human subjects were exposed; failure to maintain uniform concentrations of H_2S in the exposure chamber; inadequacies with respect to the number of test animals/subjects employed; general lack of detail concerning test animals (*i.e*., source, strain, age, sex, pre-study health status) and animal husbandry; and, inattention to detail leading to "accidental" exposures because of equipment malfunction or technician error. The findings from these studies were judged to be unsuitable for use in "toxic load" calculations.

- Approximately 40% of the studies received a "moderate" or higher grade, signifying that the findings and conclusions can be accepted with a reasonable degree of confidence, and that the data add to understanding of the concentration-time-response characteristics of H_2S vis-à-vis lethality. These data were judged to be suitable for use in "toxic load" calculations.

- The remaining 20% of the studies were deemed to be of no practical use in providing an understanding of the concentration-time-response characteristics of H_2S vis-à-vis lethality. In most instances, these studies either lacked fundamental information or provided information that could not be substantiated. In some cases, the information was irrelevant.

- With one exception, the subset of studies specifically selected by the AEUB for the calculation of the EPZ endpoints for H_2S received a grade of "moderate" ... signifying that the dataset selected was fit-for-purpose, and scientifically defensible. The findings and conclusions from these studies can be accepted with a reasonable degree of confidence. Despite some weaknesses, the results from these studies add to understanding of the concentration-time-response characteristics of H2S vis-à-vis lethality, and were judged to be suitable for use in "toxic load" calculations. The exception was a review article (Back *et al.* (1972), which simply summarized the original data collected by MacEwen and Vernot (1972). The former study was deemed to be of no practical use, whereas the latter study received a "moderate" grade.

The principal conclusions and recommendations arising from the work are:

- The outcomes and conclusions reached in the Discussion Paper relating to the proposed EPZ endpoints for H_2S are based on studies that achieved "moderate" scores when reviewed against very strict standards for proper design, execution and reporting. The findings and conclusions of these studies can be accepted with a reasonable degree of confidence. The lethality data upon which the endpoints are based are reasonably technically robust and defensible.

- For added refinement, the EPZ endpoints should be re-calculated with the data from the paper by Back *et al*. (1972) removed. The paper was deemed to be of "no practical use" since the consultant concluded that it is review article summarizing original data collected by others (MacEwan and Vernot, 1972). Use of the summary data in the calculations is redundant and misleading since it assigns extra weight to the original findings, possibly skewing the overall outcome.

- The EPZ endpoints for H_2S might benefit from a broader literature search to identify other health effects studies that might contribute to added understanding of the concentration-time-response characteristics

of H_2S vis-à-vis lethality following short-term exposure. The subset of studies that formed the basis of the "toxic load" calculations on which the endpoints were based was narrow in breadth, consisting of three studies only. Other reliable studies may exist to complement the subset.

- The EPZ endpoints for H_2S also might benefit from examination of exposure concentration-exposure time combinations beyond those corresponding to LC_{50} values. It might be equally useful to examine combinations associated with no lethality … or alternatively, combinations at which deaths are first reported or combinations corresponding to LC_{10} values or some other lower lethality index. The results of "toxic load" calculations using these alternate combinations could be used to expand and/or validate the outcomes and conclusions reached in the Discussion Paper.

- Some attempt should be made to explore the impact of differences in physiology, anatomy and metabolism between humans and laboratory rodents on the outcome of the "toxic load" calculations used to determine the EPZ endpoints. These differences will certainly influence the total "dose" of H_2S received, which, in turn, will govern the nature and severity of any response, including lethality. Since the proposed endpoints are based entirely on lethality data from studies with mice and rats, their relevance to the human condition should be carefully examined, taking the above differences into consideration.

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Appendix A

Document Review Forms

Rating Legend:	**High**	▲
	Moderate, Moderate to High	◆
	Low to Moderate	■
	Low	●
	No Practical Use	★

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NON-CLINICAL STUDIES

Document Review - Non-Clinical Studies

Author:	Clanachan, A.S.	**Study Code:**	NC002
Title:	H_2S Toxicity Analysis		
Year:	1979		

Paper Description:	Full length paper: ☒ Peer-reviewed ☐ Non-peer reviewed ☒	Abstract: ☐	Review article: ☐	Cited in-review article[1] ☐ Details:

Abstract:	*The potential interaction between the concentration and the duration of exposure on the acute toxicity of hydrogen sulphide (H_2S) was investigated. Groups of mice (BALB/CCR strain) were exposed to various H_2S concentrations (prepared by dynamic dilution) for seven different time intervals (2.5 – 30 min.). Exposure times and concentrations were randomized. An LC50 value (the concentration of gas which kills 50% of test subjects) was calculated for each exposure duration. The LC50 (±SD; n = 20) at 2.5 min. exposure was 1734 ppm (±110) whereas following 30 min. exposure the LC50 was 961 (±19). Death appeared to result from respiratory arrest. Surviving animals recovered rapidly (~ 2 min.), and were retained for a further 5 days. There were no additional deaths. These results indicate that the LC50 is indeed time-dependent – higher concentrations of gas were required to cause death at the shorter exposure durations.* *LC50 values, although time-dependent, were confided to a narrow concentration range (961-1734 ppm). However, in the general population, where many factors can influence sensitivity, lethality may extend over greater concentration ranges.* *Supported by Alberta Environment.*
Objective:	To investigate different combinations of exposure duration and exposure concentration in relation to the acute toxicity of H_2S. Of particular interest was the examination of combinations involving short exposure times (*i.e.*, 1 to 15 minutes) as the author suspected exposures of such duration might occur following a "sour" gas pipeline failure.
Primary focus of the study:	Lethality/fatality: ☒ — Other: Loss of righting reflex (unconsciousness)

Overall study design:

Exposure level(s)	Exposure frequency/duration	Species	Strain/ Breed	Age at initiation	Sex	Number of test animals	Pre-study health status
600-1300 ppm	Single exposure lasting 1 to 30 minutes	Mice	BALB/CCR	5-6 weeks	Both	Each exposure concentration-exposure time combination was examined twice using 10 mice, for a total of 20 mice per combination. (Note that certain combinations were examined in triplicate and involved the use of larger numbers of mice)	Not specified

[1] Refers to a paper describing the original paper that was either unattainable or in a foreign language.

Observations:

Lethality/Fatality

Were deaths observed? Yes ☒ No ☐

If so, were deaths exposure-related? Yes ☒ No ☐

If not, provide an explanation (e.g., trauma, concurrent disease, improper and/or inadequate husbandry, etc.).

If so, were the exposure-related deaths observed within 14 days of the initial exposure? Yes ☒ No ☐

Details:

Exposure Level (ppm)	Exposure Time (min)	Number of Deaths / Number of Animals Tested	Time to Death (min)
500	30	0/20	N/A
600	12.5	0/20	"
600	15	0/20	"
600	30	0/20	"
700	7.5	0/20	"
700	10	0/20	"
700	12.5	0/20	"
700	15	0/20	"
700	30	0/20	"
800	2.5	0/20	"
800	5	0/20	"
800	7.5	0/20	"
800	10	0/46	"
800	12.5	0/20	"
800	15	0/20	"
800	30	1/20	Not specified
900	15	2/20	"
900	30	7/20	"
1000	10	9/46	"
1000	12.5	6/20	"
1000	15	14/20	"
1000	30	12/20	"
1100	2.5	1/20	"
1100	5	4/20	"
1100	7.5	8/20	"
1100	10	25/46	"
1100	12.5	13/20	"
1100	15	13/20	"
1100	30	17/20	"
1200	2.5	2/20	"
1200	5	13/20	"

1200	7.5	14/20	“
1200	10	34/46	“
1200	12.5	17/20	“
1200	15	19/20	“
1200	30	20/20	“
1300	2.5	3/20	“
1300	5	12/20	“
1300	7.5	17/20	“
1300	10	44/46	“
1300	12.5	20/20	“
1300	15	20/20	“
1300	30	20/20	“

Were any exposure-related deaths observed more than 14 days after the initial exposure? Yes ☐ No ☒

Details:

Exposure Level (ppm)	Exposure Time (min)	Number of Deaths / Number of Animals Tested	Time to Death (min)

Were animals that died subjected to gross pathological examination (*i.e.*, necropsy)? Yes ☐ No ☒

If so, were necropsy findings consistent with exposure-related cause of death? Yes ☐ No ☐

List major necropsy findings:

Were lethal concentrations (LCs) reported? Yes ☒ No ☐

If so, describe:LC50s for 2.5-,5-, 7.5-, 10-,12.5-, 15-, and 30-minute exposure times were 1734, 1207, 1132, 1097, 1059, 1003 and 961 ppm, respectively

Were time concentrations (TCs) reported? Yes ☒ No ☐

If so, describe: LT50s for 700, 800, 900, 1000, 1100, 1200 and 1300 ppm exposure concentrations were >30, >30,>30, 18.6, 10.3, 5.2 and 4.3 minutes, respectively

Signs & Symptoms

Were clinical signs monitored as part of the study? Yes ☒ No ☐

Were any clinical signs consistent with life-threatening, serious and/or irreversible health outcomes reported as a part of the study (*e.g.*, convulsions, coma, unconsciousness, laboured breathing, abnormal gait, etc.)? Yes ☒ No ☐

If so, were the clinical signs exposure-related? Yes ☒ No ☐

If not, provide an explanation:

If so, were these exposure-related clinical signs observed within 14 days of the initial exposure? Yes ☒ No ☐

Details:

Nature of Symptom	Exposure Level	Exposure Time	Number of	Time to Onset	Duration

	(ppm)	(min)	Animals Affected	(min)	
Loss of righting reflex (*i.e.*, unconsciousness)	500	30	0/20	Not applicable	
	600	12.5 or 15	0/20	Not applicable	
	600	30	5/20	15-30 min.	Until death or end of exposure*
	700	7.5 or 10	0/20	Not applicable	
	700	12.5	2/20	10-12.5 min	Until death or end of exposure*
	700	15	3/20	10-15 min	"
	700	30	9/20	10-30 min	"
	800	1, 2.5 or 5	0/20	Not applicable	
	800	7.5	3/20	5-7.5 min	Until death or end of exposure*
	800	10	5/20	5-10 min	"
	800	12.5	9/20	5-12.5 min	"
	800	15	14/20	5-15 min	"
	800	30	18/20	5-30 min	"
	900	1 or 2.5	0/20	Not applicable	
	900	5	2/20	2.5-5 min	Until death or end of exposure*
	900	7.5	4/20	2.5-7.5 min	"
	900	10	6/20	2.5-10 min	"
	900	12.5	11/20	2.5-12.5 min	"
	900	15	15/20	2.5-15 min	"
	900	30	16/20	2.5-30 min	"
	1000	1	0/5	Not applicable	
	1000	2.5	2/20	1-2.5 min.	Until death or end of exposure*
	1000	5	6/20	1-5 min	"
	1000	7.5	11/20	1-7.5 min	"
	1000	10	16/20	1-10 min	"
	1000	12.5	16/20	1-12.5 min	"
	1000	15	20/20	1-15 min	"
	1000	30	20/20	1-30 min	"
	1100	1	1/20	<1 min	"
	1100	2.5	12/20	<1-2.5 min.	"
	1100	5	17/20	<1-5 min	"
	1100	7.5	20/20	<1-7.5 min	"
	1100	10	20/20	<1-10 min	"
	1100	12.5	20/20	<1-12.5 min	"
	1100	15	19/20	<1-15 min	"
	1100	30	20/20	<1-30 min	"
	1200	1	9/20	<1 min	"
	1200	2.5	17/20	<1-2.5 min.	"

	1200	5	18/20	<1-5 min	“
	1200	7.5	19/20	<1-7.5 min	“
	1200	10	20/20	<1-10 min	“
	1200	12.5	20/20	<1-12.5 min	“
	1200	15	20/20	<1-15 min	“
	1200	30	20/20	<1-30 min	“
	1300	1	11/20	<1 min	
	1300	2.5	18/20	<1-2.5 min.	
	1300	5	18/20	<1-5 min	
	1300	7.5	19/20	<1-7.5 min	
	1300	10	20/20	<1-10 min	
	1300	12.5	20/20	<1-12.5 min	
	1300	15	20/20	<1-15 min	
	1300	30	20/20	<1-30 min	

Did any of these exposure-related clinical signs first appear more than 14 days after the initial exposure? Yes ☐ No ☒

Details:

Nature of Symptom					Duration

Were any other exposure-related clinical signs observed? Yes ☒ No ☐

If yes, list other clinical signs: Animals appeared “stressed” for 1-2 days following exposure and showed marked piloerection

Review & Assessment: Study Design, Conduct & Reporting:

A. Test Animals:	+ Adequate numbers of test animals (*i.e.*, at least 20 per concentration-time combination). +/- Both sexes employed (… however findings were not segregated by sex). +/- Body weights of animals at initiation was provided, but weight variation was greater than recommended in OECD test guidelines (25% versus less than 20%). - Source of test animals was not provided. - The age of test animals was lower than recommended in the OECD guidelines (5-6 weeks versus 8-12 weeks). - It was not reported whether a pre-test health assessment was conducted. - No indication of whether or not test animals were acclimated to the laboratory environment prior to exposure.
B. Exposure conditions:	+ Durations of exposure were clearly defined and were appropriate to the investigative objective; *i.e.*, potential immediate acute toxicity following a “sour” gas pipeline failure. + Exposure chamber design consisted of two separate chambers … a larger chamber in which the exposures occurred, and a

	smaller chamber that served as an entry-exit portal for the mice. The design allowed for very good control of exposure times. + Exposure chamber design allowed for clear observation of test animals. + Larger exposure chamber was allowed to equilibrate for "at least" 45 minutes for each exposure concentration-exposure time combination before introduction of the test animals (… this equilibration period was judged to be adequate given that the volume of the larger chamber was 120 liters and the air flow rate through the chamber was 20 liters per minute). - Test concentrations of H_2S were not analytically confirmed. - No evidence that temperature and humidity within the exposure chamber were monitored. + Rapid transfer of animals in and out of the exposure chamber increased the accuracy of exposure durations, especially for the shorter exposure periods (1-15 minutes). + Source and purity of H_2S were provided.
C. Housing/Feeding	- No details supplied concerning animal housing or husbandry (*i.e.*, no information provided concerning caging, feed or water supply, bedding or temperature, humidity and photoperiod within the animal room).
D. Exposure equipment:	+ Details concerning the exposure chamber and gas delivery system were adequate. +/- Exposure concentrations prepared by dynamic dilution involving mixing controlled flows of H_2S and air. + Air flow rates were monitored continuously with flow meters and checked intermittently before and during each experiment. High line pressure and fine control valves were used throughout the system to result in steady accurate flows. - Exposure concentrations were not analytically confirmed.
E. Procedural:	- No evidence that a control group was employed +/- No random assignment of test animals to groups, but exposure time-concentrations were randomized. (Unclear how this was achieved). + Each experimental test run (concentration-time test) was performed in duplicate. Some combinations were performed in triplicate. - Survivors were observed for 24 h to 5 days following exposure for additional deaths. OECD test guideline recommends a post-exposure observation period of 14 days or longer
F. Data collection:	+ Individual group data were supplied.
G. Data analysis:	+ The statistical method employed (computer-assisted probit analysis) was appropriate. - Confidence intervals were not reported.
H. Interpretations:	+ Explored potential interaction between duration of exposure and concentration on LC50 values and EC50 for loss of righting reflex for exposure durations from 1 minute to 30 minutes. Study was designed, in part, to test the hypothesis that the toxicity of H_2S is more closely related to concentration than to duration of exposure. + Large number of animals studied per concentration-time exposure yields statistically meaningful results and lends confidence to findings. +/- Authors suggested future experiments to further elucidate time-concentration curves for LC50 and EC50 values.

Review & Assessment - Summary:

Discussion of findings: LC50 and EC50 values in BALB/CCR mice were determined for various exposure times. LC50s ranged from 961 ppm for a 30-minute exposure to 1734 ppm for a 2.5-minute exposure. EC50s for loss of righting reflex (indicates unconsciousness) ranged from 693 ppm for a 30-minute exposure to 1101 ppm for a 2.5-minute exposure. The authors concluded that the death rate and the rate at which unconsciousness occurred were dependent upon both exposure duration and exposure concentration, with higher concentrations required at the shorter exposure durations to produce a standard effect. A greater dependence upon exposure duration was observed at the lower (2.5-10 minutes) than at the higher (>10 min) exposure durations. LT50 and ET50 (Loss RR)

were also reported and determined to be dependent upon the exposure concentration. Surviving mice were noted to make a rapid recovery, although they appeared "stressed" for 2 days post-exposure.

Review & Assessment - Scoring[2] and Rational:

No practical use	☐
Low	☐
Low – Moderate	☐
Moderate	☒
Moderate – High	☐
High	☐

Rationale: This study is useful for development of emergency planning endpoints (based on the use of lethality as the endpoint of interest) in that it reported acute lethality data in mice for durations of exposure up to 30 minutes and at concentrations ranging from 700-1300 ppm The overall study design and conduct were adequate for the purposes of the investigation. Confidence in the results could have been improved through the employment of a control group and confirmation of test concentrations in the exposure chamber.

Strengths:

- Use of adequate numbers of test mice of both sexes (at least 20 mice per exposure concentration-exposure time combination).
- Use of gradient of exposure concentrations (500 to 1300 ppm) and exposure times (1 to 30 minutes) to permit assessment of comparative influence of each parameter on acute toxicity.
- Customized exposure chamber design allowed for careful control of entry and exit of test mice from the exposure chamber (*i.e.*, exposure times were well controlled).
- Observation of test animals for both mortality and loss of righting reflex (*i.e.*, unconsciousness) as indicators of acute toxicity.

Weaknesses:

- Although testing was performed in both sexes of mice, the findings were not segregated by sex.
- Exposure concentrations were not analytically confirmed.
- Post-exposure observation period was limited to 5 days (… whereas guidelines generally recommend a 14-day post-exposure observation period).
- Monitoring of clinical signs was limited to loss of righting reflex only.
- Exact time to death or loss of unconsciousness was not specified.
- No indication that test animals were necropsied.
- No indication that study was subjected to independent peer review.

[2] Score reflects usefulness of study for development of emergency planning criteria vis-à-vis acute lethality.

Reviewers:

DD	☒
RT	☒
CM	☐

Document Review - Non-Clinical Studies

Author:	Lopez, A. Prior, M., Yong, S., Albassam, M. and Lillie, L.E.	**Study Code:**	NC027
Title:	Biochemical and cytologic alterations in the respiratory tract of rats exposed for 4 hours to hydrogen sulfide.		
Year:	1987		

Paper Description:	Full length paper: ☒ Peer-reviewed ☒ Non-peer reviewed ☐	Abstract: ☐	Review article: ☐	Cited in-review article[3] ☐ Details:

Abstract:

"Fischer-344 rats were killed by exsanguination 1, 20, and 44 hr after a single 4-hr exposure to an atmosphere of 0, 10, 200, and 400 ppm of hydrogen sulfide (H_2S). Alterations in the activities of lactate dehydrogenase and alkaline phosphatase, and cytomorphology of epithelial cells in fluids obtained by nasal and broncoalveolar lavage were used as indicators of cell injury. Changes in the number of leukocytes were used as indicators of inflammatory response, and changes in the concentration of protein were used as indicators of altered vascular permeability. Inhalation of H_2S resulted in 139, 483, and 817% increased cellularity in the nasal cavity of rats exposed to 10, 200, and 400 ppm, respectively. This was due to marked exfoliation of degenerated epithelial cells and exudation of neutrophils. The high dose of H_2S resulted in a moderate increase in lactate dehydrogenase and protein in nasal passages; values returned to baseline levels 20 hr later. Bronchoalveolar cell counts were decreased in rats exposed to 400 ppm and unchanged in those exposed to 10 and 200 ppm. Enzymatic activities in lung lavage fluid were moderately elevated (up to 90%), yet protein concentrations were increased by more that 3000 % and remained significantly elevated up to 44 hr after exposure to 400 ppm. It was concluded that inhalation of H_2S has a severe cytotoxic effect on the nasal epithelium and a severe edematogenic effect on lung parenchyma. These results are in agreement with autopsy findings of individuals killed by accidental exposure to H_2S-containing sour gas."

Objective:	To evaluate the early injury and inflammatory response occurring in the respiratory tract of rats after a single 4-hr exposure to 0,10, 200 or 400 ppm of H_2S.	
Primary focus of the study:	Lethality/fatality: ☒	Other: Biochemical and cytological alterations in the respiratory tract of rats following single acute exposure to H_2S.

Overall study design:

Exposure level(s)	Exposure frequency/duration	Species	Strain/ Breed	Age at initiation	Sex	Number of test animals	Pre-study health status
0,10,200, or 400 ppm (nominal) 0, 9.6 ± 1.0, 197.8 ± 1.6, and 387.7 ± 11.1 ppm (actual)	Single exposure lasting 4 hrs. (Note that exposures were performed on two different days, with the control (0 ppm), 10 ppm and 400 ppm groups exposed on the first day, and another control and 200 ppm groups exposed two days later).	Rat	Fischer-344	12 weeks at time of exposure	Male	12 rats per exposure concentration. (Note that following exposure, each group of rats was sub-divided into 3 groups of 4 rats each, and followed for 1, 20 or 44 hours post-treatment, after which the animals were sacrificed and the respiratory tracts examined).	Not specified. (Rats were sourced from a reputable supplier and presumed to be healthy)

[3] Refers to a paper describing the original paper that was either unattainable or in a foreign language.

Observations:

General

Did the study follow a standardized test protocol? Yes ☐ No ☒

If yes, which test protocol did the study follow? OECD ☐
USEPA ☐
Other:

Was the study conducted under Good Laboratory Practice (GLP)? Yes ☐ No ☒

Lethality/Fatality

Were deaths observed? Yes ☐ No ☒

If so, were deaths exposure-related? Yes ☐ No ☐

If not, provide an explanation (e.g., trauma, concurrent disease, improper and/or inadequate husbandry, etc.).

If so, were the exposure-related deaths observed within 14 days of the initial exposure? Yes ☐ No ☐

Details:

Exposure Level (ppm)	Exposure Time (min)	Number of Deaths / Number of Animals Tested	Time to Death (min)

Were any exposure-related deaths observed more than 14 days after the initial exposure? Yes ☐ No ☐

Details:

Exposure Level (ppm)	Exposure Time (min)	Number of Deaths / Number of Animals Tested	Time to Death (min)

Were animals that died subjected to gross pathological examination (*i.e.*, necropsy)? Yes ☐ No ☒

If so, were necropsy findings consistent with exposure-related cause of death? Yes ☐ No ☐

List major necropsy findings:

Were lethal concentrations (LCs) reported? Yes ☐ No ☒

If so, describe:

Were time concentrations (TCs) reported? Yes ☐ No ☒

If so, describe:

Signs & Symptoms

Were clinical signs monitored as part of the study? Yes ☒ No ☐

Were any clinical signs consistent with life-threatening, serious and/or irreversible health outcomes reported as a part of the study (e.g., convulsions, coma, unconsciousness, laboured breathing, abnormal gait, etc.)? Yes ☐ No ☒

If so, were the clinical signs exposure-related? Yes ☐ No ☐

If not, provide an explanation:

If so, were these exposure-related clinical signs observed within 14 days of the initial exposure? Yes ☐ No ☐

Details:

Nature of Symptom	Exposure Level (ppm)	Exposure Time (min)	Number of Animals Affected	Time to Onset (min)	Duration

Did any of these exposure-related clinical signs first appear more than 14 days after the initial exposure? Yes ☐ No ☐

Details:

Nature of Symptom	Exposure Level (ppm)	Exposure Time (min)	Number of Animals Affected	Time to Onset (min)	Duration

Were any other exposure-related clinical signs observed? Yes ☒ No ☐

If yes, list other clinical signs: By the end of the 4-hour exposure, moderate transient lethargy was observed in the 400 ppm group. The authors reported that no obvious effects on respiratory movements were observed among any of the rats throughout the exposure period.

Review & Assessment: Study Design, Conduct & Reporting:

A. Test Animals:	+ Adequate number of test animals per exposure concentration (12) - Only male rats employed + Details concerning source, age and acclimation of test animals were provided
B. Exposure conditions:	+/- A whole body exposure chamber was used. - It was not stated whether exposure chambers were equilibrated before the test animals were placed inside. +/- Since the exposure system could only expose three groups in a single trial, two separate trials were performed within two days, each with a separate control group. + The exposure chamber and gas delivery system were adequately described. + The actual gas concentrations were determined and recorded. Gas concentrations were monitored 3 times an hour and analyzed by gas chromatography + The exposure chamber was maintained at negative pressure in compliance with guideline recommendations.

C. Housing/Feeding	+/- The material from which cages were constructed was noted (*i.e.,* stainless steel, wire mesh bottomed). However, the dimensions of these cages were not included in the description. + Test animals were individually-housed, therefore, clear observation of each animal was possible. - Feeding restrictions imposed during exposure were not noted. + Animals were housed in an environmentally-controlled room (*e.g.* 19 to 24 °C; 30 to 70% humidity; monitored photoperiod)
D. Exposure equipment:	+ All exposure, monitoring and analysis equipment used was adequately described. + Gas flow rate in the chamber was recorded and complied with OECD guidelines (*i.e.,* 12 to 15 changes per hour)
E. Procedural:	+ Rats were randomly allotted to exposure groups and to cage location within the exposure chamber. The method of randomization (*e.g.,* table of random numbers, computer generated) was not noted. + Controls were also placed within chambers to account for physiological responses associated with this stress. - Unclear whether technicians and handlers were blinded to exposure conditions. - No indication that the study was conducted under Good Laboratory Practice (GLP) conditions. - Animals were only followed for up to 44 hours post-exposure (… assessment of exposure-related clinical signs and mortality were limited to this period). Guidelines for acute toxicity testing generally recommend that animals be followed for 14 days post-exposure.
F. Data collection:	- Individual data were not provided for each test animal - No pre-determined scales were used to assess clinical responses.
G. Data analysis:	+/- Description of statistical methods was judged to be adequate
H. Interpretations:	+ Use of 12 animals per exposure concentration adds confidence to the findings - Effects on the nasal epithelium and lung parenchyma were confined largely to animals exposed to 400 ppm. Accordingly, the conclusion that *"inhalation of H_2S has a severe cytotoxic effect on the nasal epithelium and a severe edematogenic effect on lung parenchyma"* must be interpreted with caution.

Review & Assessment - Summary:

Discussion of findings: No deaths or symptoms consistent with life-threatening, serious and/or irreversible health outcomes were observed in Fischer-344 rats after a single 4-hour exposure to H_2S concentrations as high as 400 ppm. Apart from transient lethargy at 400 ppm, clinical signs of toxicity were not evident either during or following exposure. Test animals were followed for only 44 hours post-exposure, consistent with the primary objective of the study (*i.e.*, to examine the effects of acute exposure to H_2S on the structural, cytological and biochemical integrity of the respiratory tract).

Review & Assessment - Scoring[4] and Rational:

No practical use	☐
Low	☐
Low – Moderate	☐
Moderate	☒
Moderate – High	☐
High	☐

[4] Score reflects usefulness of study for development of emergency planning criteria vis-à-vis acute lethality.

Rational: The study design, conduct and reporting were judged to be adequate for the stated purposes of the investigation. Descriptions of test systems and exposure conditions were clear. Nominal concentrations of H_2S in the exposure chamber were analytically confirmed. Added confidence in the findings and conclusions might have been achieved through the use of both sexes of test animals and a 14-day post-exposure observation period to assess survival and/or clinical signs. This study is judged to be of moderate usefulness for the development of emergency planning endpoints (based on the use of lethality as the endpoint of interest) in that it is an acute exposure study examining moderately high concentrations of H_2S (*i.e.*, 10 to 400 ppm), with monitoring for clinical signs and mortality during and after exposure.

Strengths:

- Use of adequate number of test animals (12) per exposure concentration.
- Use of gradient of exposure concentrations (0, 10, 200 or 400 ppm), including control exposure(s).
- Clear description of exposure chamber and gas delivery system.
- Nominal exposure concentrations analytically confirmed.

Weaknesses:

- Use of male sex only.
- Failure to follow animals for recommended 14-day observation period (*i.e.*, animals were sacrificed with 1 to 44 hours post-exposure).
- No examination of different exposure concentration-exposure time combinations to permit assessment of influence of concentration and time on lethality outcomes. (Although the use of concentration-time combinations is not a guideline requirement, it can broaden understanding of acute lethality).
- Lack of general necropsy of animals at study termination (*i.e.*, description of necropsy findings was limited to respiratory tissues).

Reviewers:

DD	☒
RT	☒
CM	☐

Document Review - Non-Clinical Studies

Author:	Lopez, A., Prior, M.G., Reiffenstien, R.J. and Goodwin, L.R.	**Study Code:**	NC031
Title:	Peracute toxic effects of inhaled hydrogen sulfide and injected sodium hydrosulfide on the lungs of rats		
Year:	1989		

Paper Description:	Full length paper: ☒ Peer-reviewed ☒ Non-peer reviewed ☐	Abstract: ☐	Review article: ☐	Cited in-review article[5] ☐ Details:

Abstract:

This study was designed to test whether intraperitoneally injected sodium hydrosulfide (NaHS) would mimic the pulmonary alterations induced by lethal peracute exposure to an atmosphere containing hydrogen sulfide. Groups of five Sprague-Dawley rats were exposed to an atmosphere of either 2317.6 ∀ 547.3 mg m^{-3} H_2S (H_2S group) or no H_2S (air group), or were injected intraperitoneally with a solution containing 30 mg kg^{-1} sodium hydrosulfide (NaHS group or saline solution (vehicle control). Rats of the air and saline groups were killed by cervical dislocation. All rats exposed to H_2S or injected with NaHS died within 3 min; however, only rats exposed to H_2S showed severe respiratory distress in the agonic phase preceding death. In addition, rats in the H_2S group had a notable discharge of serous fluid from the mouth and nostrils. At necropsy, all rats in the H_2S group had gross and Histologic evidence of pulmonary edema characterized by massive extravasation of eosinophilic fluid into the bronchoalveolar space. In contract, the lungs of rats injected with NaHS or saline or exposed to air were unaffected. It was concluded that the edematogenic effect of H_2S in the lungs cannot be reproduced by injection of NaHS. The severity of lung edema induced by a peracute exposure to H_2S was extensive enough to account for death.

Objective:	To investigate: i) whether pulmonary edema would develop in rats after a rapidly lethal, peracute (5-min) exposure to H_2S; and, ii) to compare whether pulmonary lesions in rats killed by a lethal injection of NaHS are similar to those found in the lungs of rats killed by inhalation of H_2S.	
Primary focus of the study:	Lethality/fatality: ☒	Other: pulmonary lesions

Overall study design:

Exposure level(s)	Exposure frequency/duration	Species	Strain/ Breed	Age at initiation	Sex	Number of test animals	Pre-study health status
1655 ± 391 ppm	Single exposure/until death	Rat	Sprague-Dawley	6-months	Male	5 per exposure group	Not specified

Observations:

General

Did the study follow a standardized test protocol? Yes ☐ No ☒

If yes, which test protocol did the study follow? OECD ☐ USEPA ☐ Other:

Was the study conducted under Good Laboratory Practice (GLP)? Yes ☐ No ☒

[5] Refers to a paper describing the original paper that was either unattainable or in a foreign language.

Lethality/Fatality

Were deaths observed? Yes ☒ No ☐

If so, were deaths exposure-related? Yes ☒ No ☐

If not, provide an explanation (e.g., trauma, concurrent disease, improper and/or inadequate husbandry, etc.).

If so, were the exposure-related deaths observed within 14 days of the initial exposure? Yes ☒ No ☐

Details:

Exposure Level (ppm)	Exposure Time (min)	Number of Deaths / Number of Animals Tested	Time to Death (min)
1655 ±391 ppm	Until death	5/5	<3 minutes

Were any exposure-related deaths observed more than 14 days after the initial exposure? Yes ☐ No ☒

Details:

Exposure Level (ppm)	Exposure Time (min)	Number of Deaths / Number of Animals Tested	Time to Death (min)

Were animals that died subjected to gross pathological examination (*i.e*., necropsy)? Yes ☒ No ☐

If so, were necropsy findings consistent with exposure-related cause of death? Yes ☒ No ☐

List major necropsy findings: severe gross and microscopic pulmonary edema including foamy fluids in the trachea and severe congestion of the lungs

Were lethal concentrations (LCs) reported? Yes ☐ No ☒

If so, describe:

Were time concentrations (TCs) reported? Yes ☐ No ☒

If so, describe:

Signs & Symptoms

Were clinical signs monitored as part of the study? Yes ☒ No ☐

Were any clinical signs consistent with life-threatening, serious and/or irreversible health outcomes reported as a part of the study (*e.g*., convulsions, coma, unconsciousness, laboured breathing, abnormal gait, *etc*.)? Yes ☒ No ☐

If so, were the clinical signs exposure-related? Yes ☒ No ☐

If not, provide an explanation:

If so, were these exposure-related clinical signs observed within 14 days of the initial exposure? Yes ☒ No ☐

Details:

Nature of Symptom	Exposure Level (ppm)	Exposure Time (minutes)	Number of Animals Affected	Time to Onset (min)	Duration
Severe respiratory distress	1655	3	5/5	Immediately	Until death
Large frothy fluid pouring from nose and mouth	1655	3	5/5	Immediately	Until death
Unconsciousness	1655	3	Not specified	Within 3 minutes	Until death

Did any of these exposure-related clinical signs first appear more than 14 days after the initial exposure? Yes ☐ No ☒

Details:

Nature of Symptom	Exposure Level (ppm)	Exposure Time (min)	Number of Animals Affected	Time to Onset (min)	Duration

Were any other exposure-related clinical signs observed? Yes ☐ No ☒

If yes, list other clinical signs:

Review & Assessment: Study Design, Conduct & Reporting:

A. Test Animals:	+ Adequate number of test animals per exposure concentration (5), in compliance with OECD test guidelines - Only male rats employed + Details concerning source, age and weight of test animals were provided - No indication that test animals were acclimated to the laboratory environment prior to exposure.
B. Exposure conditions:	+ The exposure chambers were equilibrated before test animals were placed inside and the use of an access chamber with slides allowed for rapid transfer of animals in and out of the exposure chamber. + The actual gas concentrations in the exposure chamber were determined and recorded. - Only one concentration of H_2S was tested.
C. Housing/Feeding	+/- The access and exposure chambers were adequately described and permitted clear observation of each animal. + Animals were housed in an environmentally-controlled room (*e.g.* 22 °C; 30 to 70% humidity; monitored photoperiod). + Source and type of feed and water were described.
D. Exposure equipment:	+ The exposure chamber and gas delivery system were adequately described. + The exposure chamber consisted of two parts: a 110-liter inhalation chamber, and a smaller access chamber which allowed the test animals to be placed into and removed from the inhalation chamber quickly. + The nominal exposure concentration was analytically confirmed. The single test concentration of H_2S was reported to be 1655 ± 391 ppm.

	+/- Flow rates of H_2S and air into the inhalation chamber were reportedly controlled and monitored; however, the flow rates were not given. Equilibration times could not be calculated in the absence of the flow rate data.
E. Procedural:	+/- Rats were randomly allotted to exposure groups, but the method of randomization was not stated + A control group exposed to air only was employed.
F. Data collection:	+ Clinical, gross and histological pathology data were collected
G. Data analysis:	+/- Findings were simple statistics and were well presented.
H. Interpretations:	+ Study published in a peer-reviewed journal

Review & Assessment - Summary:

Discussion of findings: All rats exposed to 1655 ± 391 ppm H_2S died within 3 minutes and displayed severe respiratory distress and/or unconsciousness prior to death. Pathological examination revealed gross and histologic evidence of pulmonary edema, which was reportedly severe and extensive enough to account for death.

Review & Assessment - Scoring[6] and Rational:

No practical use ☐

Low ☐

Low – Moderate ☐

Moderate ☐

Moderate – High ☒

High ☐

Rational:

This study is useful for development of emergency planning endpoints in that it is a well-conducted acute exposure study examining clinical signs, lethality and pathology in rats exposed to a high concentration of H_2S. The study would have benefited from the use of both male and female rats.

Strengths:

- Good description of exposure chamber and gas delivery system.
- Analytical confirmation of nominal test concentration.
- Good descriptions of clinical signs and pathological findings.
- Adequate descriptions of test animals and husbandry.
- Adequate description of concentration-time response relationship.

[6] Score reflects usefulness of study for development of emergency planning criteria vis-à-vis acute lethality.

Weaknesses:

- Use of male sex only (… which, in turn, limited number of test animals to 5 per treatment).
- Use of only a single test concentration of H_2S.

Reviewers:

DD	☒
RT	☒
CM	☐

Document Review - Non-Clinical Studies

Author:	Mitchell, C.W. and Yant, W.P.	**Study Code:**	NC032 (see also NC010)
Title:	Correlation of the data obtained from refinery accidents with a laboratory study of H_2S and its treatment.		
Year:	1925		

Paper Description:	Full length paper: ☒ Peer-reviewed ☐ Non-peer reviewed ☒	Abstract: ☐	Review article: ☐	Cited in-review article[7] ☐ Details:
Abstract:	*"In the laboratory study, the symptoms of hydrogen sulphide (H2S) poisoning in animals and men were found to be almost identical with those caused by gases in the refineries. The need for a definite method of treating H2S poisoning was evident. The medical findings, the study on toxicity of H2S, and the treatment for H2S poisoning will be discussed in turn."*			
Objective:	To investigate the toxicity of hydrogen sulphide in various laboratory animal species as a possible means to further understanding of the onset, progress and treatment of H2S poisoning among refinery workers. The animal species tested were canary birds, rats, guinea pigs, dogs and goats. Canaries were chosen because of their susceptibility to poisonous gases, and goats for their resistance. Preliminary studies involving exposure of human subjects to H2S under controlled conditions were also performed (…see CL010 for complete review and ranking of the clinical portion of the study).			
Primary focus of the study:	Lethality/fatality: ☒	Other: Clinical signs		

Overall study design:

Exposure level(s)	Exposure frequency/duration	Species	Strain/ Breed	Age at initiation	Sex	Number of test animals	Pre-study health status
35 to 1600 ppm (animals) 100-350 ppm (humans)	Single exposures lasting up to 100 hours, depending on species 1-4 hours (humans)	(a) Canary (b) Rat (c) Guinea pig (d) Dog (e) Goat (f) Human	Not specified	Not specified	Not specified for animal species. Male subjects were used in the human study	Total numbers of animals exposed were as follows: (a) 27 exposed (b) 101 exposed (c) 27 exposed (d) 32 exposed (e) 9 exposed (f) unknown Number of animals exposed at each exposure concentration varied within and between species, and ranged from 1 to 40.	Stated to be "healthy"

[7] Refers to a paper describing the original paper that was either unattainable or in a foreign language.

Observations:

General

Did the study follow a standardized test protocol? Yes ☐ No ☒

If yes, which test protocol did the study follow? OECD ☐ USEPA ☐ Other:

Was the study conducted under Good Laboratory Practice (GLP)? Yes ☐ No ☒

Lethality/Fatality

Were deaths observed? Yes ☒ No ☐

If so, were deaths exposure-related? Yes ☒ No ☐

If not, provide an explanation (e.g., trauma, concurrent disease, improper and/or inadequate husbandry, etc.)

If so, were the exposure-related deaths observed within 14 days of the initial exposure? Yes ☒ No ☐

Details:

Exposure Level (ppm)	Exposure Time (min)	Number of Deaths / Number of Animals Tested	Time to Death (min)
Canaries			
35-65 ppm	Up to 18 hours	2/2	Between 8 to 18 hours
97-100 ppm	Up to 8 hours	6/6	At least one death occurred between 4 to 8 hours. Birds that did not die during the experiment died 12 to 36 hours post-exposure.
140 ppm	Up to 8 hours	4/4	At least one death occurred between 4 to 8 hours. Birds that did not die during the experiment died 12 to 36 hours post-exposure.
190-210 ppm	Up to 1 hour	Not specified; 4 animals tested: death during exposure or unconsciousness and subsequent recovery reported	During exposure period.
280-310 ppm	Up to 30 minutes	Not specified; 3 animals tested: death during exposure or unconsciousness and subsequent recovery reported	During exposure period.
440-620 ppm	Up to 2 minutes	0/7	Not applicable
730 ppm	Up to 20 seconds	1/1	18-20 seconds
Rats			
36-65 ppm	Up to 48 hours	0/4	Not applicable

100 - 140 ppm	Up to 48 hours	Not specified; 19 animals tested: death reported, but slow recovery also reported for surviving animals	Between 18 to 48 hours
190-240 ppm	Up to 18 hours	Not specified; 17 animals tested: death reported but slow recovery also reported for surviving animals	Between 18 to 48 hours
310-350 ppm	Up to 8 hours	Not specified; 13 animals tested: death reported, but slow recovery also reported for surviving animals	Between 4 to 8 hours
450 ppm	Up to 4 hours	1/2	Between 1 to 4 hours
520-530ppm	Up to 4 hours	Not specified; 3 animals tested: death reported, but slow recovery also reported for surviving animals	Between 1 to 4 hours.
620 ppm	Up to 1 hour	Not specified; 3 animals tested: death reported, but slow recovery also reported for surviving animals	Between 30 minutes to 1 hour.
790-900 ppm	Up to I hour	Not specified; 40 animals tested: death reported, but slow recovery also reported for surviving animals	Between 2 minutes and 1 hour..
Guinea Pig			
35-65 ppm	Up to 48 hours	0/2	Not applicable
103 ppm	Up to 48 hours	1/2	Between 18 to 48 hours.
240 ppm	Up to 18 hours	2/3	Between 8 to 18 hours.
350 ppm	Up to 18 hours	3/3	2 within 8 to 18 hours; 1 four days later
820 ppm	Up to 30 minutes	0/5	Not applicable
1000-1100 ppm	Up to 30 minutes	10/10 (?) … not clearly specified	Between 2 to 30 minutes
1500 ppm	Up to 30 minutes	1/2	Between 2 to 30 minutes.
Dog			
103 ppm	Up to 16 hours	2/2 (?) … not clearly specified	Between 8 to 16 hours
240 ppm	Up to 16 hours	2/2	Between 8 to 16 hours
350 ppm	Up to 16 hours	2/2	Between 4 to 16 hours
760-800 ppm	Up to 1 hour	1/2	Between 30 minutes to 1 hour
850-890 ppm	Up to 30 minutes	3/3 (?) … not clearly specified	Between 2 to 30 minutes
1000-1140 ppm	Up to 30 minutes	8/8 (?) … not clearly specified	Between 2 to 30 minutes
1280 ppm	Up to 30 minutes	4/4 (?) … not clearly specified	Between 2 to 30 minutes
1500-1600 ppm	Up to 30 minutes	9/9 (?) … not clearly specified	Between 2 to 30 minutes
Goat			
820 ppm	Up to 30 minutes	0/1	Not applicable
1000 - 1100 ppm	Up to 30 minutes	4/4 (?) … not clearly specified	Between 2 to 30 minutes

1280-1330 ppm	Up to 30 minutes	4/4(?) … not clearly specified	Between 2 to 30 minutes
Humans			
100-150 ppm	Up to 4 hours	No deaths reported (number exposed unknown)	Not applicable
150–200 ppm	Up to 8 hours	No deaths reported (number exposed unknown)	Not applicable
250-350 ppm	Up to 4 hours	No deaths reported (number exposed unknown)	Not applicable
350-450 ppm	Up to 1 hour	No deaths reported (number exposed unknown)	Not applicable

Were any exposure-related deaths observed more than 14 days after the initial exposure? Yes ☐ No ☒

Details:

Exposure Level (ppm)	Exposure Time (min)	Number of Deaths / Number of Animals Tested	Time to Death (min)

Were animals that died subjected to gross pathological examination (*i.e.*, necropsy)? Yes ☒ No ☐

If so, were necropsy findings consistent with exposure-related cause of death? Yes ☒ No ☐

List major necropsy findings: collapsed lungs, congestion in lungs, hemorrhage in nose, mouth or lungs, dilated heart, distended liver, congestion in the abdomen and kidneys. Necropsy findings were listed only for dogs, but authors implied that the findings were "typical".

Were lethal concentrations (LCs) reported? Yes ☐ No ☒

If so, describe:

Were time concentrations (TCs) reported? Yes ☐ No ☒

If so, describe:

Signs & Symptoms

Were clinical signs monitored as part of the study? Yes ☒ No ☐

Were any clinical signs consistent with life-threatening, serious and/or irreversible health outcomes reported as a part of the study (*e.g.*, convulsions, coma, unconsciousness, laboured breathing, abnormal gait, *etc*.)? Yes ☒ No ☐

If so, were the clinical signs exposure-related? Yes ☒ No ☐

If not, provide an explanation:

If so, were these exposure-related clinical signs observed within 14 days of the initial exposure? Yes ☐ No ☐

Details:

Nature of Symptom	Exposure Level (ppm)	Exposure Time (min)	Number of Animals Affected	Time to Onset (min)	Duration

Canary birds					
Labored breathing	35-65 ppm	Until death or unconsciousness	2/2	4-8 hours	Not specified
	97-140 ppm	"	10/10	1-4 hours	"
	190-310 ppm	"	7/7	2-30 minutes	"
	440 ppm	"	4/4	0-2 min	"
Dizziness; general stupidity	35-65 ppm	"	2/2	4-8 hours	"
	97-140 ppm	"	10/10	1-4 hours	"
	190-210 ppm	"	4/4	2-30 min	"
	440	"	4/4	0-2 min	"
Unconsciousness	97-140 ppm	"	10/10	4-8 hours	"
	190-210 ppm	"	4/4	30 min-1 hour	"
	280-310 ppm	"	3/3	2-30 min	"
	440-730 ppm	"	8/8	0-2 min	"
Rats					
Labored breathing	100-140 ppm	"	19/19	8-18 hours	"
	190-240 ppm	"	17/17	1-4 hours (panting)	"
	190-240 ppm	"	17/17	4-8 hours (forced respiration)	"
	310-350 ppm	"	13/13	30 min-1 hour	"
	450 ppm	"	2/2	30 min-1 hour	"
	520-530 ppm	"	3/3	2-30 min	"
Excitement/distress	310-350 ppm	"	13/13	1-4 hours (great distress)	"
	450-620 ppm	"	8/8	0-2 min	"
Unconsciousness	310-350 ppm	"	13/13	4-8 hours	"
	450 ppm	"	2/2	1-4 hours	"
	620 ppm	"	3/3	2-60 min	"
	790-900 ppm	"	40/40	Few seconds	"
Guinea Pigs					
Labored breathing	103 ppm	"	2/2	8-18 hours	"
	240 ppm	"	1/3 (2 died)	8-18 hours	"
	350 ppm	"	3/3	4-8 hours	"
	820 ppm	"	5/5	2-30 min	"
Unconsciousness	1000-1500 ppm	"	12/12	0-2 min	"
Dogs					
Depression	103 ppm	"	2/2	4-8 hours	"

	240 ppm	"	2/2	1-4 hours	"
	350 ppm	"	2/2	30 min-1 hour	"
Labored breathing	240-350 ppm	"	4/4	4-8 hours	"
Unconsciousness/spasms	760-1600 ppm	"	26/26	0-2 min	"
Goats					
Excitement/distress	1000 ppm	"	4/4	0-2 min	
Unconsciousness, spasms, convulsions	1000 ppm	"	4/4	2-30 min	
	1280-1330	"	4/4	0-2 min	
Humans					
Disturbed respiration	100-150 ppm	1-4 hours	Not specified	15-30 min	
Difficulty breathing	150-200 ppm	1-4 hours	Not specified	1-4 hours	
	350-450 ppm	1-4 hours	Not specified	15-30 min	

Did any of these exposure-related clinical signs first appear more than 14 days after the initial exposure? Yes ☐ No ☒

Details:

Nature of Symptom	Exposure Level (ppm)	Exposure Time (min)	Number of Animals Affected	Time to Onset (min)	Duration

Were any other exposure-related clinical signs observed? Yes ☒ No ☐

If yes, list other clinical signs: slight edema, eye and nose irritation, continual face washing, "quiet" disposition, pus in eyes and nose, lachrymation, increased respiration, coughing. In men, coughing, eye, throat and trachea irritation, loss of sense of smell, sleepiness, pain in eyes, painful secretion of tears, weariness, light shy, pain in head, infection of conjunctiva, nasal catarrh

Review & Assessment: Study Design, Conduct & Reporting:

A. Test Animals:	- The number of treated/control animals was limited in many cases and the number of treated/control human subjects was not stated. - Principle characteristics of exposed animals and subjects were not defined beyond the statement that they *"all were healthy and representative of their kind."* - The source of the test animals was not indicated and the manner by which human subjects were recruited was not stated. - It is unknown whether human subjects provided informed consent.

B. Exposure conditions:	- The manufacturer and purity of the FeS and HCl used to generate the H_2S was not reported. - The precise exposure concentration(s) was not stated. Only a range was quoted. +/- The duration of exposure was defined; however, only time intervals were listed for the reporting of signs and symptoms. + A whole body exposure chamber was used. + Animals/subjects were placed in the exposure chamber after the equilibration period. - Whether oxygen content, pressure, humidity, and photoperiod were monitored throughout exposure was not reported. + The distribution of the gas within the chamber was maintained through the use of a fan to ensure homogeneous mixing. +/- At intervals during the exposure period, the H_2S concentration in the chamber was determined by the cadmium chloride method. How sampling was performed without altering the concentration within the chamber was not specified. +/- Exposures were stated to be "continuous"
C. Housing/Feeding	- Details concerning animal housing (*i.e.*, temperature, humidity, and photoperiod) were lacking. - Information pertaining to animal caging (*i.e.*, type and dimensions) was not provided. - Bedding material was not specified. - The type and source of feed was not reported. The feeding schedule was also omitted. - Details concerning the water supply were lacking.
D. Exposure equipment:	- Exposure was completed in a 1000-cubic foot gas chamber. +/- Description of the exposure chamber was limited. Further description is provided in US Public Health Reports, vol. 37(19), May 12, 1922 pp.1127-1142, which was not readily available. +/- A Kipp generator was employed to generate the H_2S gas. - The cadmium chloride method was used to measure the gas concentration within the chamber. The method was judged to provide limited sensitivity.
E. Procedural:	- No acclimatization period was specified. - No description of pre-test conditions was provided. - Detail pertaining to the randomization of test animals and assignment to test groups was lacking. +/- Control experiments were conducted in pure air. +/- Following death, a pathological examination was made for gross changes, and specimens of the lungs, heart, liver, and kidneys were microscopically examined. +/- Study pre-dated Good Laboratory Practice (GLP) guidelines. - Unclear whether technicians and handler were blinded to exposure conditions.
F. Data collection:	+/- General comments on reversibility of symptoms were made; however, they were not specific to each symptom observed. +/- All symptoms were noted, as well as the time of occurrence. However, only a time range was provided. - Individual data were not provided for each test subject/animal, thereby limiting the independent assessment of the findings. - Other than the statement that control results were *"negative,"* no further data were provided. - All observational data were generalized in tables. +/- Necropsy and histology data were provided only for the dogs and only for exposure levels of 350ppm and above. - In most cases, the number of animals in which the symptoms were noted per exposure group was not recorded.
G. Data analysis:	- Data were not statistically analyzed.

H. Interpretations:	- The authors believed that based on the results of human exposure trials up to 350 ppm for 4 hours and data from canine studies, it is possible to predict the reaction of men to higher concentrations. The validity of this statement is questionable, as it is unclear whether issues of "toxic load" were considered. - The human studies were described as "preliminary". + The detailed reporting of symptoms at various concentration ranges and durations of exposure provided clear evidence of dose-response + Multiple species evaluated

Review & Assessment - Summary:

Discussion of findings: Large variations in species sensitivity to H_2S intoxication were demonstrated in experiments of whole body exposure of canaries, rats, guinea pigs, dogs, goats and humans. The duration of exposure appeared to have a significant influence on the type and severity of symptoms observed, with most symptoms progressing with continued exposure. Signs and symptoms were also influenced by the exposure concentration.

Deaths of canaries was observed following 8 to 18 hours exposure to 35 to 65ppm H_2S, whereas rat deaths were noted following a 18 to 48 hour exposure to 100ppm H_2S. Similarly, guinea pig and dog mortality was noted following exposure to 103ppm H_2S for a period of 8 to 48 hours and 8-16 hours respectively. Symptoms reported in men exposed to 100-350 ppm H2S for 1 to 4 hours included coughing, eye, throat and respiratory irritation, difficulty breathing, loss of sense of smell and pain in the eyes or head. The authors concluded that the data for men indicate that they react to H_2S in a manner similar to the animals, particularly when considering the similarity of symptoms observed in cases of accidental worker poisoning to those observed in animals exposed to high concentrations of H_2S. Based on this and the results of a study in Germany (Lehman, 1892), the sensitivity of men was concluded to likely be identical to that of the dog. The validity of this conclusion is questionable as it is unclear whether issues of "toxic load" were considered.

Interpretation of the toxicological significance and clinical relevance of the study findings should take into consideration the following:

- The study is dated and was performed long before the development of testing guidelines and the introduction of Good Laboratory Practice (GLP) requirements. The study also relied on equipment and analytical methodology that has been replaced by more advanced technology. The level of confidence that can be assigned to the study findings is undermined by the use of relatively "crude" instrumentation, and the associated uncertainty surrounding the actual exposure concentrations that were tested.
- Exposure concentrations and the time of appearance of symptoms were reported only as ranges. This hinders interpretation of the dose-responsiveness and time-responsiveness of the findings. As noted earlier, however, clear dose and time dependence of symptoms, including death were apparent.
- The number of animals on test was limited. In most instances, group sizes were limited to 1 to 4 animals per specified range of exposure concentrations, below those recommended by testing guidelines. The group sizes for the rat tended to be larger with half of the exposure groups having more than adequate numbers of animals. In guinea pigs and dogs, at least one dose group exposed to very high concentrations of H2S (>1000 ppm) had adequate numbers of animals.
- No pre-trial health examinations were conducted. Given the age of the study, and the general health of animals and animal care practices in place at the time, the possibility is presented that certain of the responses (especially the pathological findings) may have been non-treatment-related.
- The number of control animals/subjects included in the study was not specified.
- Apart from the preliminary human study, the sex of the test animals was not specified. Only male subjects were used in the human study.
- In most cases, the number of deaths per exposure group where death was noted to occur was not reported. Often for a given concentration range and exposure duration both death or unconsciousness were noted (with full recovery of unconscious animals post-exposure) but the numbers dying versus those recovering was not specified.
- No necropsy or histopathological data were provided for the animals exposed to the lower exposure concentrations (i.e., <103 ppm).
- Overall lack of detail pertaining to procedures (e.g., blinding, randomization), equipment and animal/subject characteristics (e.g., pre-trial health, breed, sex, age) undermines the level of confidence that can be assigned to the study findings and conclusions.

- The responses noted among the canary birds may not be representative since the authors contended that canaries are "extremely sensitive" to poisonous gases.

Review & Assessment - Scoring[8] and Rational:

No practical use ☐

Low ☒

Low – Moderate ☐

Moderate ☐

Moderate – High ☐

High ☐

Rational: This study is of limited usefulness only for the development of emergency response criteria in that a number of deficiencies in design, conduct and reporting were evident.

Strengths:

- Use of a wide range of test animal species (*i.e.*, canary birds, rats, guinea pigs, dogs and goats).
- Use of graded exposure concentrations and varying exposure times.
- Good description of clinical signs and necropsy findings (… albeit the latter results were reported only for the dogs and only for selected exposure concentrations.

Weaknesses:

- Use of limited numbers of test animals for certain exposure conditions.
- Failure to distinguish between sexes of test animals.
- Inadequate description of source, strain, pre-study health status, *etc.* of the test animals.
- No information provided with respect to animal housing or husbandry.
- Lack of detail concerning randomization and assignment of test animals to groups.
- Limited description only of gas delivery system and exposure chamber.
- Purity of H_2S gas not provided (… the H_2S was generated in situ by combining FeS and HCl).
- Lack of detail concerning confirmation of nominal exposure concentrations (… test concentrations evidently were measured using the "calcium chloride method", but no details were supplied).
- Lack of detail to allow critical assessment of concentration and time-responsiveness since exposure levels and exposure times often were reported as ranges

[8] Score reflects usefulness of study for development of emergency planning criteria vis-à-vis acute lethality.

only (… effectively precluding calculation of “toxic load”).

- Failure in many instances to report actual numbers of test animals that either died or were afflicted with clinical signs, thereby precluding calculation of LC_{50} values.
- Complete lack of data with respect to control animals.
- Lack of detail concerning manner in which test animals were placed into the exposure chamber and the degree of equilibration achieved.
- Limited necropsy data (… findings were reported for dogs only and only for dogs exposed to selected concentrations).

Reviewers:

DD	☒
RT	☒
CM	☐

Document Review - Non-Clinical Studies

Author:	O'Donoghue		**Study Code:**	NC034
Title:	Hydrogen sulphide poisoning in swine			
Year:	1961			
Paper Description:	Full length paper: ☒ Peer-reviewed ☒ Non-peer reviewed ☐	Abstract: ☐	Review article: ☐	Cited in-review article[9] ☐ Details:
Abstract:	*"The exposure of young swine and rabbits to varying concentrations of hydrogen sulphide indicated that toxicity was related more to the concentration of the gas than to the length of time of exposure. Sudden exposure may reduce the minimum lethal concentration. No chronic effects were observed in animals surviving exposures as great as 1000 P.P.M. of the gas.* *It is unlikely that hydrogen sulphide poisoning would occur in domestic animals under conditions other than those that have been responsible for such fatalities in man; that is sudden exposure to gas concentration of 400 P.P.M. or greater.* *A confirmed diagnosis would have to be based on a known exposure. Pathology and toxicological examination of tissues or organs will not supply confirmatory evidence."*			
Objective:	To assess the symptoms observed in pigs and rabbits following to H_2S exposure under controlled conditions. Exposure concentrations ranged from 50 to 1200 ppm, for varying times. In many cases, exposure concentrations were adjusted upward over defined periods and exposures continued until clinical signs became severe and the animals' health was seriously compromised.			
Primary focus of the study:	Lethality/fatality: ☒	Other: Clinical signs following acute exposures to H2S.		

Overall study design:

Exposure level(s)	Exposure frequency/duration	Species	Strain/ Breed	Age at initiation	Sex	Number of test animals	Pre-study health status
(a) 50-100ppm	(a) 2 hr	(a) Pig	Not specified	Not specified	Not specified	(a) 1	Not specified
(b) 250-1000ppm	(b) 2hr and 10 min	(b) Pig				(b) 1	
(c) 400ppm	(c) 1 sec	(c) Pig				(c) 1	
(d) 350-1200ppm	(d) 44 min	(d) Pig				(d) 1	
(e) 250-970ppm	(e) 3 hr and 50 min	(e) Pig				(e) 1	
(f) 500-1050ppm	(f) 36 min	(f) Pig				(f) 1	
(g) 50 ppm	(g) 16 hrs	(g) Rabbit				(g) 3	
(h) 1000 ppm	(h) momentary	(h) Rabbit				(h) 3	

[9] Refers to a paper describing the original paper that was either unattainable or in a foreign language.

Observations:

General

Did the study follow a standardized test protocol? Yes ☐ No ☒

If yes, which test protocol did the study follow? OECD ☐ USEPA ☐ Other:

Was the study conducted under Good Laboratory Practice (GLP)? Yes ☐ No ☒

Lethality/Fatality

Were deaths observed? Yes ☒ No ☐

If so, were deaths exposure-related? Yes ☒ No ☐

If not, provide an explanation (e.g., trauma, concurrent disease, improper and/or inadequate husbandry, etc.).

If so, were the exposure-related deaths observed within 14 days of the initial exposure? Yes ☒ No ☐

Details:

Exposure Level (ppm)	Exposure Time (min)	Number of Deaths / Number of Animals Tested	Time to Death (min)
50-100 ppm	2 hours	0/1 pig	Not applicable
250-1000 ppm	2 hours and 10 min	1/1 pig	2 hours and 10 min (45 min after 1000 ppm reached)
400 ppm (“accidental”)	1 second	1/1 pig	Immediate
350-1200 ppm	44 minutes	1/1 pig	44 minutes (15 min after 1200 ppm reached)
250-970 ppm	3 hours and 50 minutes	0/1 pig	Not applicable
500-1050 ppm	36 minutes	0/1 pig	Not applicable
50 ppm	16 hours	0/3 rabbits	Not applicable
1000 ppm (“accidental”)	momentary	1/3 rabbits	Two hours post-exposure

Were any exposure-related deaths observed more than 14 days after the initial exposure? Yes ☐ No ☒

Details:

Exposure Level (ppm)	Exposure Time (min)	Number of Deaths / Number of Animals Tested	Time to Death (min)

Were animals that died subjected to gross pathological examination (*i.e.*, necropsy)? Yes ☒ No ☐

If so, were necropsy findings consistent with exposure-related cause of death? Yes ☒ No ☐

List major necropsy findings: No significant pathology in immediate deaths. In pigs gradually exposed, superficial cyanosis, minor haemorrgage in the lungs and hypostatic congestion of ventral lungs were observed. In the rabbit dying two hours after accidental exposure, severe pulmonary haemorrage with

epitaxis and distention of the right ventricle were observed

Were lethal concentrations (LCs) reported? Yes ☐ No ☒

If so, describe:

Were time concentrations (TCs) reported? Yes ☐ No ☒

If so, describe:

Signs & Symptoms

Were clinical signs monitored as part of the study? Yes ☒ No ☐

Were any clinical signs consistent with life-threatening, serious and/or irreversible health outcomes reported as a part of the study (e.g., convulsions, coma, unconsciousness, laboured breathing, abnormal gait, etc.)? Yes ☒ No ☐

If so, were the clinical signs exposure-related? Yes ☒ No ☐

If not, provide an explanation:

If so, were these exposure-related clinical signs observed within 14 days of the initial exposure? Yes ☒ No ☐

Details:

Nature of Symptom	Exposure Level (ppm)	Exposure Time (min)	Number of Animals Affected	Time to Onset (min)	Duration
Dyspnea-labored breathing	900 ppm	1 hour,40 min (previous exposures between 0-900 ppm)	1/1 pig	Immediately when 900 ppm was reached	Until unconsciousness (occurred 20 min later at 970 ppm)
Semi-comatose state	500 ppm	65 minutes (previous exposure between 0 and 500 ppm)	1/1 pig	Immediately when 500 ppm reached	Until removal from exposure.
	700 ppm	10-15 minutes (previous exposure between 0 and 700 ppm)	2/2 pig	Immediately when 700 ppm reached	Until death.
	900 ppm	16 minutes (previous exposure between 0 and 900 ppm)	1/1 pig	Immediately when 900 ppm reached	Until exposure stopped.
Muscular spasms, convulsive movements, cyanosis	1000 ppm	85 minutes (previous exposure between 0 and 1000 ppm)	1/1 pig	Immediately when 1000 ppm reached	Until death.
	1000 ppm	Momentary	3/3 rabbits	Immediately	Not specified – one rabbit died two hours post-exposure and others recovered
	1050 ppm	20 minutes (previous exposure between 0 and	1/1 pig	Immediately when 1050 ppm reached	Until exposure stopped.

		1050 ppm)			
	1200 ppm	30 minutes (previous exposure between 0 and 1200 ppm)	1/1 pig	Within 10 min of 1200 ppm being reached.	Until death.

Did any of these exposure-related clinical signs first appear more than 14 days after the initial exposure? Yes ☐ No ☒

Details:

Nature of Symptom	Exposure Level (ppm)	Exposure Time (min)	Number of Animals Affected	Time to Onset (min)	Duration

Were any other exposure-related clinical signs observed? Yes ☒ No ☐

If yes, list other clinical signs: Discomfort, slight eye irritation, and salivation with periodic swallowing (in order of appearance) with increasing exposure concentrations. Symptoms then progressed to semi-comatose state.

Review & Assessment: Study Design, Conduct & Reporting:

A. Test Animals:	- Only a single animal was exposed in six of the seven experiments. The exception was Experiment (g) in which 3 rabbits were exposed. The number of test animals was limited and did not satisfy guideline recommendations. - The breed, age and sex of exposed animals were not defined. - The pre-test health status of the animals was not indicated. - The breeding facility from which the animals were obtained was not noted. + Two species of animals were evaluated
B. Exposure conditions:	- The source and purity of the H_2S gas was not indicated. - Details concerning the exposure chamber and gas delivery system were minimal. +/- A *"titrolog instrument"* reportedly monitored the actual gas concentration continuously, but details concerning the instrumentation and readings were not provided - The animals were placed within the exposure chamber before equilibration of the gas, thereby, reducing control over exposure conditions. - The duration of exposure was uncertain. There was inconsistency within the report as to actual exposure times. +/- The mode of administration was whole body. - No indication as to whether or not airflow, temperature and humidity within the exposure chamber were monitored.
C. Housing/Feeding	- No information pertaining to housing conditions was provided (*i.e.*, temperature, humidity and photoperiod). - The type of feed and feeding schedule were not defined. - No details respecting water supply were given.
D. Exposure equipment:	- Information respecting the exposure chamber and gas delivery system was lacking. The chamber was described only as *"a specifically designed chamber"*. Details concerning construction, dimensions, gas metering, venting *etc.* were not

	available. No description of the gas delivery system was provided. - Target concentrations evidently were monitored with a *"titrolog instrument"*, but details respecting the instrumentation and readings were lacking.
E. Procedural:	+/- Control animals were reportedly employed, but no details were provided (*e.g.*, number of animals) - No indication of an acclimation period for test animals - No indication that test animals were randomly assigned to exposure groups - Period of observation following exposure was not specified … the authors simply stated that no after-effects were witnessed among animals subjected to non-lethal exposures. - "Accidental" exposures occurred on two occasions, signifying lack of attention and carelessness.
F. Data collection:	+ Information respecting onset, type, duration and severity of clinical signs were reported.
G. Data analysis:	- No statistical analysis of the results was conducted
H. Interpretations:	- Insufficient detail provided on test animals, control animals, exposure equipment and housing/feeding - Limited number of animals tested + Detailed reporting of clinical signs, including time of onset.

Review & Assessment - Summary:

Discussion of findings: In pigs or rabbits exposed to H_2S, death or symptoms consistent with life-threatening, serious and/or irreversible health outcomes were observed at concentrations of 400 ppm and above. No after-effects were evident among animals that survived the exposures. The authors noted that the deaths observed after accidental momentary exposure to H_2S indicate that sudden exposure is associated with a reduced minimal lethal concentration level. For example, in pigs gradually exposed to H_2S death was not observed until a concentration of 1000-1200 ppm was reached, while a pig which died due to accidental exposure was believed to be exposed to only 400 ppm.

Review & Assessment - Scoring[10] and Rational:

No practical use	☐
Low	☒
Low – Moderate	☐
Moderate	☐
Moderate – High	☐
High	☐

Rational: The experimental design was deficient in several respects when compared to guideline recommendations. As a "pilot" study, it did provide some useful information concerning the concentration-time-response of clinical signs, especially at higher exposure concentrations. In addition, information respecting onset, type, severity and duration of symptoms was reported.

[10] Score reflects usefulness of study for development of emergency planning criteria vis-à-vis acute lethality.

Strengths:

- Unique experimental design involving exposure to gradually increasing concentrations of H_2S over varying time periods allows for assessment of onset and/or recovery from clinical signs.
- Use of different exposure concentration-exposure time combinations, permitting assessment of the influence of each parameter on acute toxicity outcomes.
- Clinical signs well documented (*i.e.*, nature, onset, duration and severity).
- Necropsy findings well documented.

Weaknesses:

- Lack of description of exposure chamber and gas delivery system.
- Lack of detail surrounding analytical confirmation of nominal exposure concentrations.
- Use of restricted numbers of test animals.
- Reference to "accidental" exposures leading to death of animals signifies general lack of attention and carelessness, and seriously detracts from the level of confidence that can be assigned to the study.
- Lack of detail concerning test animals (*i.e.*, no information supplied with respect to source, age, sex, pre-test health status, husbandry).
- Lack of detail concerning post-exposure observation period.
- Complete lack of detail concerning control animals.
- Inconsistencies in the reporting of exposure times (*i.e.*, summary statements provided for each test animal vis-à-vis the time required to reach the highest exposure concentration were not always consistent with the time sequence listings shown for the progressive increases in exposure concentrations).

Reviewers:

DD	☒
RT	☒
CM	☐

Document Review - Non-Clinical Studies

Author:	Prior, MG; Sharma, A.K., Yong, S. and Lopez, A.	**Study Code:**	NC035
Title:	Concentration-time interactions in hydrogen sulphide toxicity in rats.		
Year:	1988		

Paper Description:	Full length paper: ☒ Peer-reviewed ☒ Non-peer reviewed ☐	Abstract: ☐	Review article: ☐	Cited in-review article[11] ☐ Details:

Abstract:	*Concentration-time interactions were investigated in young male and female Sprague-Dawley, Long Evans and Fischer-344 rats exposed to hydrogen sulphide for two, four or six hours. Higher concentrations caused more deaths, with no significant difference for duration of exposure. A significant sex effect was noted with 30% mortality in males and 20% in females, with no significant difference among strains. Changes in weight were significant: increasing with concentration, higher in males than in females, different amount strains (Fischer-344 < Sprague Dawley < Long Evans), and affected by duration of exposure. Lethal concentration values (LC_{50} and LC_{10}) were estimated, for the pooled data set (n = 456); the probit equation was Y = 5.74749 + 3.8259X where X is log_{10} does of hydrogen sulphide in parts per million. The LC_{50}/LC_{10} values were 644/298 parts per million (902/417 mg m^{-3}) respectively. Individual probit analyses were also performed for strain, hours of exposure and sex. The LC_{50} and LC_{10} values for male, female and strain were not different. Significant differences were observed among LC_{50}/LC_{10} values for hours of exposure (2 h + 587/549 parts per million, 822/769 mg m^{-3}; 4 h – 501/422 parts per million, 701/591 mg m^{-3}; 6 h = 335/299 parts per million, 469/491 mg m^{-3}). There was no effect of spatial position in the exposure chamber on the distribution of mortality. All rats of all strains dying had severe pulmonary edema.*
Objective:	1) To investigate the effect of sex and strain of rats, duration of exposure, and spatial position in inhalation chamber on the mortality of rats from a single exposure to hydrogen sulphide; and, 2) to utilize the findings to develop an exposure model for future studies on the toxicity of hydrogen sulphide.

Primary focus of the study:	Lethality/fatality: ☒	Other: To also examine the effect of sex and strain of rats, duration of exposure, and spatial position in inhalation chamber on weight loss among rats from a single exposure to H_2S.

Overall study design:

Exposure level(s)	Exposure frequency/duration	Species	Strain/ Breed	Age at initiation	Sex	Number of test animals	Pre-study health status
0 to >600 ppm. (Note that the actual exposure concentrations tested were not specifically stated).	Single exposure lasting 2, 4 or 6 hours. Animals which survived were observed for 14 days post-exposure.	Rat	Sprague-Dawley, Long Evans and Fischer-344	9-10 weeks at time of exposure	Both	A total of 72 males and 72 females were assigned to the 4-hour exposure group, and a total of 72 males and 84 females were assigned to the 2-hour and 6-hour exposure groups. Evidently, 12 rats per sex were exposed to each exposure concentration for each exposure time.	Not specified. (Rats were sourced from a reputable supplier and presume to be healthy).

[11] Refers to a paper describing the original paper that was either unattainable or in a foreign language.

Observations:

General

Did the study follow a standardized test protocol? Yes ☐ No ☒

If yes, which test protocol did the study follow? OECD ☐
USEPA ☐
Other:

Was the study conducted under Good Laboratory Practice (GLP)? Yes ☐ No ☒

Lethality/Fatality

Were deaths observed? Yes ☒ No ☐

If so, were deaths exposure-related? Yes ☒ No ☐

If not, provide an explanation (e.g., trauma, concurrent disease, improper and/or inadequate husbandry, etc.).

If so, were the exposure-related deaths observed within 14 days of the initial exposure? Yes ☒ No ☐

Details:

Exposure Level (ppm)	Exposure Time	Number of Deaths / Number of Animals Tested	Time to Death
299	6 hours	10%	Not specified
335	6 hour	50%	"
422	4 hours	10%	"
501	4 hours	50%	"
549	2 hours	10%	"
587	2 hours	50%	"

Were any exposure-related deaths observed more than 14 days after the initial exposure? Yes ☐ No ☒

Details:

Exposure Level (ppm)	Exposure Time (min)	Number of Deaths / Number of Animals Tested	Time to Death (min)

Were animals that died subjected to gross pathological examination (*i.e.*, necropsy)? Yes ☒ No ☐

If so, were necropsy findings consistent with exposure-related cause of death? Yes ☒ No ☐

List major necropsy findings: Severe pulmonary edema in all rats that died on test; large amounts of foamy fluid in mouths, noses, trachea and bronchi. Proteinaceous fluid found in the conductive airways, alveoli and around the perivascular space of major blood vessels. Edema was extensive enough to incriminate it as most probable cause of death

Were lethal concentrations (LCs) reported? Yes ☒ No ☐

If so, describe: LC50 (6 hours)=335 ppm; LC50 (4 hours)=501 ppm; LC50 (2 hours)=587 ppm
LC10 (6 hours)= 299 ppm; LC10 (4 hours)=422 ppm; LC10 (2 hours)=549 ppm

Were time concentrations (TCs) reported? Yes ☐ No ☒

If so, describe:

Signs & Symptoms

Were clinical signs monitored as part of the study? (Weight loss was the only clinical parameter monitored) Yes ☐ No ☒

Were any clinical signs consistent with life-threatening, serious and/or irreversible health outcomes reported as a part of the study (*e.g.*, convulsions, coma, unconsciousness, laboured breathing, abnormal gait, *etc.*)? Yes ☐ No ☒

If so, were the clinical signs exposure-related? Yes ☐ No ☐

If not, provide an explanation:

If so, were these exposure-related clinical signs observed within 14 days of the initial exposure? Yes ☐ No ☐

Details:

Nature of Symptom	Exposure Level (ppm)	Exposure Time (min)	Number of Animals Affected	Time to Onset (min)	Duration

Did any of these exposure-related clinical signs first appear more than 14 days after the initial exposure? Yes ☐ No ☐

Details:

Nature of Symptom	Exposure Level (ppm)	Exposure Time (min)	Number of Animals Affected	Time to Onset (min)	Duration

Were any other exposure-related clinical signs observed? Yes ☒ No ☐

If yes, list other clinical signs: Concentration-dependent weight loss was observed (higher in males than females, different among strains and affected by duration of exposure). No other clinical signs of toxicity were reported.

Review & Assessment: Study Design, Conduct & Reporting:

A. Test Animals:	+ Group size per exposure concentration and exposure time (*i.e.*, 12 rats per sex) adequate and consistent with guideline recommendations. + Details concerning source, age and acclimation of test animals were supplied + Both sexes were employed - Pre-health status of animals was not reported - Body weights of test animals at initiation were not provided - Control group, if employed, was not described.

B. Exposure conditions:	+/- Animals were exposed to a gradient of H_2S concentrations for 2, 4 or 6 hours under "continuous flow" conditions. H_2S concentrations were monitored (sampled four times per hour). Actual concentrations of H_2S tested were not specified. - It was not stated whether exposure chambers were equilibrated before or after test animals were placed inside. This could potentially alter the duration of exposure. Notations in the Discussion section suggest that the chambers were not equilibrated prior to the introduction of the test animals. + Test animals were acclimated to the exposure chamber for 3 days prior to exposure to reduce stress.
C. Housing/Feeding	+ Details concerning housing environment were judged to be adequate (*i.e.*, temperature, humidity and photoperiod were controlled and within the ranges specified in OECD testing guidelines) + The number of animals grouped in single chambers was outlined and permitted clear observation of each animal (4 rats per cage were housed in each of 3 individual compartments to permit assessment of influence of location within cage) + Animals were fed and watered *ad libitum*. Food and water sources were described.
D. Exposure equipment:	+ The exposure chamber was adequately described (i.e., 70-liter clear acrylic chamber consisting of 3 circular wire-mesh cages, each divided into 4 individual compartments used to hold single test animals). + Gas delivery system adequately described (*i.e.*, H_2S and air separately metered, combined and introduced into exposure chamber). + H_2S concentrations in the exposure chamber were regularly monitored (*i.e.*, 4 times per hour). +/- Flows of H_2S and air through the chamber evidently were controlled, but the flow rates were not stated. + Source and purity of H_2S were provided.
E. Procedural:	+ Animals were acclimated for 10 days prior to exposure, consistent with guideline recommendations +/- Animals were randomly assigned to treatment groups but the method of randomization was not defined. - Unclear whether or not a control group was employed. +/- The period of observation following exposure was appropriate (14 days)
F. Data collection:	- Individual animal data were not supplied, nor were LC_{50}/LC_{10} values segregated by sex or strain of rat. - No clinical responses were recorded with the exception of changes in body weight.
G. Data analysis:	+/- Description of statistical methods was judged to be adequate + Confidence intervals were reported + Statistical significance and significant interactions by sex, strain of rats, duration and position in the chamber were reported.
H. Interpretations:	+ The influence of sex, strain, duration of exposure and position in the exposure chamber was studied. + The large number of animals per exposure group lends confidence to the results.

Review & Assessment - Summary:

Discussion of findings: LC_{50}/LC_{10} values were found to vary significantly by duration of exposure with 2, 4 and 6 hour LC_{50}/LC_{10} values reported to be 587/549 ppm, 501/422 ppm, and 335/299 ppm, respectively. There was little difference between the LC_{10} and LC_{50} values, suggesting an abrupt threshold and a steep concentration-response for lethality. No significant differences on LC_{50}/LC_{10} values were found for sex, strain or spatial location in the exposure chamber. Overall, however, it was reported that exposure to H_2S affected males significantly more than females, with mortality in males of 30% compared to 20% in females. All rats of all strains dying on test showed evidence of severe pulmonary edema.

Probit analysis of the lethality data yielded the following:

Data Set	LC50	95% Confidence Interval	LC10	95% Confidence Interval	
2-hr	587	Not estimated	549	Not estimated	
4-hr	501	477 – 545	422	364 - 447	
6-hr	335	325 – 345	299	284 – 309	
'Pooled'	644	508 – 3743	298	49 – 378	(based all strains, all exposure times, and both sexes)

Review & Assessment - Scoring[12] and Rational:

No practical use ☐

Low ☐

Low – Moderate ☐

Moderate ☒

Moderate – High ☐

High ☐

Rational: This study is useful for the development of emergency planning endpoints (based on the use of lethality as the primary endpoint of interest) in that it is an acute exposure study examining lethality concentrations in both male and female rats for durations of exposure ranging from 2 to 6 hours. Design, conduct and reporting were judged to be adequate for the purposes of the study. The very large number of test animals employed and the use of both sexes and three strains of rats add confidence to the study findings. The exposure chamber and gas delivery system were well described. Added confidence could have been achieved by supplying individual animal results and reporting the LC50/LC10 values segregated by sex or strain of rat. These findings were discussed, but the data were not supplied. A figure was presented (Figure 2) in the results section showing the probit distribution of the concentration-response for each exposure time, but the resolution was not adequate to permit accurate determination of the exposure concentrations tested. Monitoring and reporting of clinical signs observed during and following exposure would have been of benefit.

Strengths:

- Use of adequate numbers of test animals (12 per sex per exposure level).

[12] Score reflects usefulness of study for development of emergency planning criteria vis-à-vis acute lethality.

- Use of both sexes as well as multiple strains of rats.
- Use of a gradient of exposure concentrations (not specified) and exposure times (2, 4, or 6 hours).
- Full description of exposure chamber and gas delivery system.
- Analytical confirmation of exposure concentrations.
- Summary descriptions of weight loss and necropsy findings.

Weaknesses:

- Failure to specify actual exposure concentrations tested. (Figure 2 shows the probit distribution of concentration-response, but the resolution is not adequate to discern the exact exposure levels tested).
- Evident failure to include control group(s) of animals.
- Evident failure to equilibrate the exposure chamber before the introduction of the test animals. (Significance is difficult to assess since the air flow rate through the chamber was not specified; however, significance is likely to be marginal since the shortest exposure time was 2 hours, allowing adequate time for equilibration).
- Reliance on summary data. Individual animal/individual group data were not provided for any of the outcomes reported (*i.e.*, lethality, weight loss, necropsy).
- Failure to report clinical signs.

Reviewers:

DD	☒
RT	☒
CM	☐

Document Review - Non-Clinical Studies

Author:	Tansy, M.F., Kendall, F.M., Fantasla, J., Landin, W.E., Oberly, R.	**Study Code:**	NC047
Title:	Acute and subchronic toxicity studies of rats exposed to vapors of methyl mercaptan and other reduced-sulfur compounds.		
Year:	1981		

Paper Description:	Full length paper: ☒ Peer-reviewed ☒ Non-peer reviewed ☐	Abstract: ☐	Review article: ☐	Cited in-review article[13] ☐ Details:

Abstract:	*Acute inhalation experiments were conducted to determine 24-h LC50 values for adult Sprague-Dawley rats of both sexes exposed to vapors of methyl mercaptan and other reduced-S compounds for 4-h periods. Using calculated gas concentrations, the following LC50 value for each gas and combination was determined: methyl mercaptan 675 ppm; dimethyl sulfide 40,250 ppm; dimethyl disulfide 805 ppm; hydrogen sulfide 444 ppm; and an equimolar mixture of methyl mercaptan, dimethyl sulfide, and dimethyl disulfide 550 ppm. The effects on body and tissue weights, gross metabolic performance, O_2 consumption, systolic blood pressure, various blood parameters, and intestinal transit time associated with 3-mo exposures of young adult male rats to chemically verified concentrations of 2, 17, and 57 ppm methyl mercaptan vapor are summarized in this report. No mortality was experienced by any group. Histopathological findings were essentially nil except for microscopic suggestions of liver damage. The most readily apparent phenomenon was the decrease in body weight. Average values of terminal body weights for all exposed groups were lower than that for the sham control group. This difference was significant in the 57 ppm group and followed a statistically significant dose-related trend.*
Objective:	1) To establish LC_{50} values for methyl mercaptan and other reduced sulphur compounds in rats; and, 2) to determine whether sub-chronic exposure to a methyl mercaptan vapor concentration in air that approached the recommended workplace concentration could be associated with significant differences in the mean values of various functional and metabolic performance parameters when compared to similar data from sham-exposed rats. (Note: The present review is concerned with the portion of the study directed at the first objective only, and specifically at that portion of the study involving exposure to H_2S.)
Primary focus of the study:	Lethality/fatality: ☒ Other:

Overall study design:

Exposure level(s)	Exposure frequency/duration	Species	Strain/ Breed	Age at initiation	Sex	Number of test animals	Pre-study health status
0,400, 440, 475, 500, 525, 554, or 600 ppm	Single exposure lasting 4 hours. (Note that animals were followed for up to 14 days post-exposure).	Rat	Sprague-Dawley	Not specified	Both	10 rats per exposure level, consisting of 5 of each sex.	Not specified. (Rats were sourced from a reputable supplier and presumed to be healthy)

[13] Refers to a paper describing the original paper that was either unattainable or in a foreign language.

Observations:

General

Did the study follow a standardized test protocol? Yes ☐ No ☒

If yes, which test protocol did the study follow? OECD ☐ USEPA ☐ Other:

Was the study conducted under Good Laboratory Practice (GLP)? Yes ☐ No ☒

Lethality/Fatality

Were deaths observed? Yes ☒ No ☐

If so, were deaths exposure-related? Yes ☒ No ☐

If not, provide an explanation (e.g., trauma, concurrent disease, improper and/or inadequate husbandry, etc.).

If so, were the exposure-related deaths observed within 14 days of the initial exposure? Yes ☒ No ☐

Details:

Exposure Level (ppm)	Exposure Time	Number of Deaths / Number of Animals Tested	Time to Death
Sham (0 ppm)	4 hours	0/10	N/A
400	4 hours	3/10	Less than 24 hours
440	4 hours	3/10	Less than 24 hours
475	4 hours	7/10	Less than 24 hours
500	4 hours	8/10	Less than 24 hours
525	4 hours	8/10	Less than 24 hours
554	4 hours	9/10	Less than 24 hours
600	4 hours	10/10	Less than 24 hours

Were any exposure-related deaths observed more than 14 days after the initial exposure? Yes ☐ No ☒

Details:

Exposure Level (ppm)	Exposure Time (min)	Number of Deaths / Number of Animals Tested	Time to Death (min)

Were animals that died subjected to gross pathological examination (*i.e.*, necropsy)? Yes ☒ No ☐

If so, were necropsy findings consistent with exposure-related cause of death? Yes ☐ No ☒

List major necropsy findings: No evidence of external bleeding from any orifice in rats that succumbed or survived. (Note that although the authors reported that all animals were subjected to gross pathological examination, the only reference to the necropsy findings was the absence of external bleeding from any orifice).

Were lethal concentrations (LCs) reported? Yes ☒ No ☐

If so, describe: 4-hour LC50 reported to be 444 ppm (Range: 416 to 473 ppm)

Were time concentrations (TCs) reported? Yes ☐ No ☒

If so, describe:

Signs & Symptoms

Were clinical signs monitored as part of the study? Yes ☒ No ☐

Were any clinical signs consistent with life-threatening, serious and/or irreversible health outcomes reported as a part of the study (*e.g.*, convulsions, coma, unconsciousness, laboured breathing, abnormal gait, *etc.*)? Yes ☐ No ☒

If so, were the clinical signs exposure-related? Yes ☐ No ☐

If not, provide an explanation:

If so, were these exposure-related clinical signs observed within 14 days of the initial exposure? Yes ☐ No ☐

Details:

Nature of Symptom	Exposure Level (ppm)	Exposure Time (min)	Number of Animals Affected	Time to Onset (min)	Duration

Did any of these exposure-related clinical signs first appear more than 14 days after the initial exposure? Yes ☐ No ☐

Details:

Nature of Symptom	Exposure Level (ppm)	Exposure Time (min)	Number of Animals Affected	Time to Onset (min)	Duration

Were any other exposure-related clinical signs observed? Yes ☐ No ☒

If yes, list other clinical signs: Note that the authors reported that as part of the LC50 determinations, any visually apparent behavior such as exploring, huddling, preening and obvious distress was noted during the course of the 4-hour exposure; however, no mention of such clinical signs was included as part of the study results, even for the animals that died on test.

Review & Assessment: Study Design, Conduct & Reporting:

A. Test Animals:	+ The number of animals per sex per exposure level (5) was in accordance with OECD guidelines. + Details concerning source, age, weight variation and acclimation of test animals were supplied. + Both sexes were employed. - It was not reported whether a pre-test health assessment was conducted.

B. Exposure conditions:	+ Exposure concentrations and duration were defined. +/- No indication that the exposure chamber was equilibrated prior to the introduction of the test animals; however, given the volume of the chamber (75 liters) and the duration of exposure (4 hours), failure to equilibrate would likely be of little, if any, consequence. - No indication that airflow, temperature and humidity within the exposure chamber were monitored during exposure - No record that the reported test concentrations of H_2S were analytically confirmed.
C. Housing/Feeding	+/- The number of animals grouped in each exposure chamber was provided (*i.e.*, 5 males and 5 females were combined in one chamber during the exposure period, and then separated for the 14-day observation period). The authors noted that the 75-liter chamber employed permitted continuous observation of each animal during exposure. + The type and source of feed and water were stated and the feeding schedule was appropriate (*i.e.*, *ad libitum* during housing, withheld during exposure). +/- Temperature maintained in the animal room was in compliance with OECD guidelines, but humidity, length of photoperiod, and air exchange rate were not specified.
D. Exposure equipment:	+ Details concerning the type and dimension of the exposure chamber were provided (*i.e.*, customized 75L glass chamber). + A description of the gas delivery system was provided (*i.e.*, metered delivery of H_2S and air into the chamber under vacuum).
E. Procedural:	+ The acclimation period was specified and was in compliance with OECD test guidelines + The test animals were randomly assigned to groups and the method of randomization was referenced. + A control group was employed. + The period of observation following exposure (14 days) was in compliance with OECD test guidelines.
F. Data collection:	+/- Individual mortality data were provided, but exact time of death was not noted. - Clinical signs such as aberrant behaviors were said to monitored during the course of the 4-hour exposures, but not apparently during the 14-day observation period and the presence or absence of these signs were not reported in the results section. The only gross pathology finding mentioned was that there was no evidence of external bleeding from any orifice
G. Data analysis:	+ The statistical methods employed were outlined and 95% confidence intervals reported.
H. Interpretations:	- The design of the study could have been improved by including lower concentrations of H_2S. + The authors' conclusion regarding the implications of the narrow concentration range responsible for minimum and maximum mortality was relevant to the understanding of the concentration-response relationship for H_2S (*i.e.*, the concentration-response was characterized by an abrupt threshold, high response gain, and only a small range of concentration between 0 and 100% mortality).

Review & Assessment - Summary:

Discussion of findings: A 4-hour LC50 of 444 ppm H_2S was determined in Sprague-Dawley rats with a 95% confidence interval of 416-473. Since deaths were observed at the lowest H_2S concentrations tested (3/10 deaths at 400 ppm), the study might have benefited from the use of a larger range of H_2S concentrations, particularly at the low end. A significant jump in mortality was observed at 475 ppm, and 100% mortality was observed at 600 ppm. The authors noted the results to be consistent with an abrupt threshold, a high response gain and a small range of concentrations between 0 and 100% mortality. It was pointed out that the high response gain and narrow range of concentrations associated with minimum and maximum mortality indicates that small errors in estimation of dose can lead to drastic differences in mortality.

Review & Assessment - Scoring[14] and Rational:

No practical use	☐
Low	☐
Low – Moderate	☐
Moderate	☒
Moderate – High	☐
High	☐

Rationale: The study is useful for the development of emergency planning endpoints (based on use of lethality as the endpoint of interest) in that it is an acute exposure study that identified a 4-hour LC_{50} in the rat. The study design, conduct and reporting were judged to be adequate. Added confidence could have been achieved by analytically confirming the test concentrations of H_2S in the exposure chamber, better description of clinical signs, better description of gross pathological findings, broadening the range of concentrations of H_2S tested (… especially at the lower end), and recording the time of death during the exposure period.

Strengths:

- Use of adequate numbers of both sexes of rats.
- Use of gradient of exposures concentrations, albeit range was somewhat narrow (*i.e.*, 400 to 600 ppm).
- Animals monitored for recommended 14-day post-exposure observation period.
- Adequate description of exposure chamber and gas delivery system (… albeit airflow rate not stated).
- Use of control group of animals.

Weaknesses:

- Failure to analytically confirm nominal exposure concentrations.
- Failure to include different exposure concentration-exposure time combinations (… although the use of such combinations is not specified in the testing guidelines, such combinations can permit better understanding of acute lethality of gases vis-à-vis Haber's Law).
- Lack of mention of presence or absence of clinical signs despite the fact that such signs evidently were monitored as part of the study.
- Limited reporting of necropsy findings.
- Failure to report actual time of death of rats that died on test.

[14] Score reflects usefulness of study for development of emergency planning criteria vis-à-vis acute lethality.

Reviewers:

DD	☒
RT	☒
CM	☐

Document Review - Non-Clinical Studies

Author:	Weedon, FR; Hartzell, A; Setterstrom, C.		**Study Code:**	NC054
Title:	Toxicity of ammonia, chlorine, hydrogen cyanide, hydrogen sulphide, and sulphur dioxide gases. V. Animals			
Year:	1940			
Paper Description:	Full length paper: ☒ Peer-reviewed ☐ Non-peer reviewed ☒	Abstract: ☐	Review article: ☐	Cited in-review article[15] ☐ Details:
Abstract:	*Not available*			
Objective:	To examine the toxicity of a series of industrial gases to animals following exposure under controlled conditions involving continuous flow. (Note: The present review is concerned with the portion of the study directed at the examination of the toxicity of hydrogen sulphide to rats and mice. Those portions of the study aimed at the examination of the toxicity of ammonia, chlorine, hydrogen cyanide and sulphur dioxide were not subject to detailed review, apart from information common to all of the gases. A separate portion of the study directed at the examination of the effect of H_2S on houseflies also was not subject to detailed review. Note also that a description of the gas delivery system and exposure chamber was contained in a separate paper entitled *Apparatus for studying effects of low concentrations of gases on plants and animals* by C. Setterstrom and P.W. Zimmerman of the Boyce Thompson Institute for Plant Research, Inc., dated 1938).			
Primary focus of the study:	Lethality/fatality: ☒	Other: Clinical signs and pathology.		

Overall study design:

Exposure level(s)	Exposure frequency/duration	Species	Strain/ Breed	Age at initiation	Sex	Number of test animals	Pre-study health status
16, 63, 250, and 1000 ppm	Until death occurred or up to 16-23 hours (Animals which survived exposure were held up to 5 months for observation).	Rat, mouse	Not specified	Not specified. The animals were described as "young, vigorous, and mature".	Both	Eight rats and four mice per exposure concentration tested (… for at total of 32 rats and 16 mice). Number of animals was not differentiated by sex. Control animals also were included, but numbers were not specified.	Not specified

Observations:

General

Did the study follow a standardized test protocol? Yes ☐ No ☒

If yes, which test protocol did the study follow? OECD ☐ USEPA ☐ Other:

Was the study conducted under Good Laboratory Practice (GLP)? Yes ☐ No ☒

[15] Refers to a paper describing the original paper that was either unattainable or in a foreign language.

Lethality/Fatality

Were deaths observed? Yes ☒ No ☐

If so, were deaths exposure-related? Yes ☒ No ☐

If not, provide an explanation (e.g., trauma, concurrent disease, improper and/or inadequate husbandry, etc.).

If so, were the exposure-related deaths observed within 14 days of the initial exposure? Yes ☒ No ☐

Details:

Exposure Level (ppm)	Exposure Time (min)	Number of Deaths / Number of Animals Tested	Time to Death (min)
Rats			
16 ppm	16 hours	0/8	N/A
63 ppm	16 hours	1/8	Not specified
250 ppm	23 hours	3/8	18-23 hours
1000 ppm	37 minutes	8/8	29-37 minutes
Mice			
16 ppm	16 hours	0/4 mice	N/A
63 ppm	16 hours	4/4 mice	One mouse died within 57 minutes … two mice died within 16 hours … and the remaining mouse died 23 hours post-exposure
250 ppm	7 hours	4/4 mice	6.9-7 hours
1000 ppm	20 minutes	4/4 mice	18-20 minutes

Were any exposure-related deaths observed more than 14 days after the initial exposure? Yes ☐ No ☒

Details:

Exposure Level (ppm)	Exposure Time (min)	Number of Deaths / Number of Animals Tested	Time to Death (min)

Were animals that died subjected to gross pathological examination (*i.e.*, necropsy)? Yes ☒ No ☐

If so, were necropsy findings consistent with exposure-related cause of death? Yes ☒ No ☐

List major necropsy findings: Light to marked congestion of the brain, liver and/or kidneys, dilation of the heart, distention of the stomach, gall bladder and/or intestines, minor to massive hemorrhagic infiltration of the lungs, and/or pale discoloration of the liver, kidneys and/or adrenals. Findings were similar in both rats and mice.

Were lethal concentrations (LCs) reported? Yes ☐ No ☒

If so, describe:

Were time concentrations (TCs) reported? Yes ☒ No ☐

If so, describe: LT50s in rats for 1000, 250, and 63 ppm were: 14 min, >16 hours and >16 hours, respectively
LT50s in mice for 1000, 250 and 63 ppm were: 18 min, 5 hours and 11.5 hours, respectively

Signs & Symptoms

Were clinical signs monitored as part of the study? Yes ☒ No ☐

Were any clinical signs consistent with life-threatening, serious and/or irreversible health outcomes reported as a part of the study (*e.g.*, convulsions, coma, unconsciousness, laboured breathing, abnormal gait, *etc.*)? Yes ☒ No ☐

If so, were the clinical signs exposure-related? Yes ☒ No ☐

If not, provide an explanation:

If so, were these exposure-related clinical signs observed within 14 days of the initial exposure? Yes ☐ No ☐

Details:

Nature of Symptom	Exposure Level (ppm)	Exposure Time (min)	Number of Animals Affected	Time to Onset (min)	Duration
Loss of muscular coordination, staggering, coma. prostration	1000	37 min	8/8 rats	5-11 minutes	24-32 minutes (until death)
Respiratory distress (gasping)	250	7 hours	4/4 mice	2 hours	5 hours (until death)
Lethargy and heavy breathing	63	16 hours	Mice and rats (number not specified)	1-16 hours (rats); (earlier for mice but not specified)	1-15 hours (until death or duration of experiment)

Did any of these exposure-related clinical signs first appear more than 14 days after the initial exposure? Yes ☐ No ☒

Details:

Nature of Symptom	Exposure Level (ppm)	Exposure Time (min)	Number of Animals Affected	Time to Onset (min)	Duration

Were any other exposure-related clinical signs observed? Yes ☒ No ☐

If yes, list other clinical signs: marked lachrymation among mice exposed to 1,000 ppm; mild to marked restlessness initially among rats and mice at all exposure concentrations.(*i.e.*, 16, 63, 250 and 1,000 ppm).

Review & Assessment: Study Design, Conduct & Reporting:

A. Test Animals:	+/- Number of test animals limited to 8 rats and 4 mice (both sexes) for each dose level, as opposed to recommended 5 animals per sex per dose level. Number of male animals versus number of females tested was not specified. However, the number of animals was judged to be sufficient for the purposes of the study. - No information was supplied concerning the strain, source or health status of the test animals prior to study initiation. +/- Age of test animals was not specified; rather they were described as *"young, vigorous and mature"*. - Body weights of test animals at initiation were not provided. - Details concerning control animals were largely lacking (*i.e.*, described only as being of same age as test animals … numbers were not supplied).
B. Exposure conditions:	+/- Animals were exposed to 16, 63, 250 or 1000 ppm H_2S under *"continuous flow"* conditions. - No record that reported test concentrations of the gas were analytically confirmed (... albeit the companion paper by Setterstrom and Zimmerman referred to the use of "autometers" used to record the concentrations of the gases by measuring the conductivity of absorbing solutions … in the case of H_2S, the absorbing solution was listed as lead acetate). +/- Duration of exposure varied up to 16 hours or until time of death. +/- Some evidence that temperature and humidity within the exposure chamber were monitored, but degree of control could not be discerned. - No indication as to whether or not the exposure chamber was equilibrated prior to the introduction of the test animals.
C. Housing/Feeding	+/- Basic details respecting housing during treatment were provided. Animals were housed in wire cages during exposure (but information was not supplied as to whether or not the animals were caged singly or gang-caged). Temperature (73 ^{0}F) and humidity (75%) at time of treatment were recorded (albeit humidity level was higher than generally recommended). - Details concerning bedding materials were not supplied. +/- Details concerning feed supply were provided, including proximate analysis; however no information was supplied respecting contaminant analysis. Feed was provided *ad libitum*. - Details concerning water supply were generally lacking. Water was provided on demand. +/- Limited reference to need to control photoperiod (*i.e.*, measures were taken to minimize light fluctuations).
D. Exposure equipment:	+/- Details concerning the gas delivery system used in the study were provided in a series of companion papers (Setterstrom and Zimmerman, 1938; McCallan and Setterstrom, 1940). + Attention was given to controlling gas flow, maintaining exposure concentrations in the chamber, regulating temperature and humidity, and analytically confirming exposure concentrations. - Equipment was necessarily 'crude' by present day standards, with little automation, and reliance on stopwatches and "warning bells". Accuracy and precision of calibration methods and analytical techniques was judged to be questionable. - Details concerning the equipment were largely for studies involving exposure to SO_2 only. No information was supplied concerning equipment modifications and changes in calibration methods for exposures with H_2S. - Details with respect to the analytical methodology used to measure the concentrations of H_2S were lacking. The available information indicated only that the chamber atmosphere was monitored continuously with an "autometer", and that the concentration of H_2S was determined by measuring the conductivity of an "absorbent" generated by passing the gas through a lead acetate solution.

E. Procedural:	- No indication that test animals were quarantined or acclimatized prior to treatment. - No details supplied concerning randomization of test animals and assignment to test groups. +/- Reference to use of control animals as part of study, but no details supplied. + Evidence that animals that died on test were either subject to necropsy immediately or stored under refrigerated conditions until necropsy could be scheduled in order to avoid tissue autolysis.
F. Data collection:	+ Clinical observations were performed during treatment, and included time of onset of symptoms, duration of symptoms and severity of responses. + Mortality data were provided, including time to death. - Body weight data were <u>not</u> collected. + Necropsies were performed on all animals, and included visual observation of major organ systems. - Organ weight data were <u>not</u> recorded as part of necropsy procedures. + Evidence that major viscera were preserved for possible future histological examination. - Individual animal data were <u>not</u> supplied.
G. Data analysis:	+/- Data analysis consisted of construction of time-mortality curves on logarithmic-probability coordinates. - No further analyses of the study findings were completed.
H. Interpretations:	- The accuracy and precision of the analytical methods used to measure the exposure concentrations of H_2S are questionable. +/- The study was performed using rats and mice, thereby requiring extrapolation of the findings to the human condition.

Review & Assessment - Summary:

<u>Discussion of findings</u>: Rats and mice exposed to graded concentrations of H_2S for up to 16 hours under controlled conditions showed dose-dependent signs of intoxication, ranging from mild restlessness to hyperactivity, coma and death. LT50s were determined for 1000 ppm, 250 ppm and 63 ppm in both species. Mice were more markedly affected than rats. The lowest concentration tested (16 ppm) produced only mild, transient restlessness during the initial stages of exposure, with no other evidence of intoxication despite continued exposure for 16 hours. Necropsy findings at this concentration were uniformly non-remarkable. At 63 ppm, frank evidence of intoxication was presented, especially among the mice, with deaths recorded as early as within one hour of exposure and 100% of the mice dying within 40 hours. The rats appeared more resistant, with only one of 8 animals dying on test. At 250 ppm, all mice died at approximately 7 hours of exposure while only 3 of 8 rats had died by 23 hours when the experiment was discontinued. At 1000 ppm, all mice died within20 minutes and all rats within 37 minutes. Necropsy findings from animals that died showed hemorrhagic infiltration of the lungs and congestion and/or discoloration of the brain, liver and/or kidneys consistent with intoxication. Clinical signs and necropsy findings were more remarkable among the test animals exposed to the highest concentrations (250 and 1000 ppm).

Interpretation of the significance of the findings should take into consideration the following:

- The time to mortality curves for both the rats and mice showed very steep responses, suggesting that concentration is the major determinant of toxicity for H_2S. Once the threshold dose for toxicity was exceeded, the animals quickly succumbed.
- The mice were very markedly affected, with all animals dying within 40 hours of exposure to H_2S. Rats were less severely affected, indicating distinct species differences in response.
- The study is dated and was performed long before the development of testing guidelines and the introduction of Good Laboratory Practice (GLP) requirements. The study also relied on equipment and analytical methodology that has been replaced by more advanced technology. The level of confidence that can be assigned to the study findings is undermined by the use of relatively "crude" instrumentation, and the associated uncertainty surrounding the actual exposure concentrations that were tested.

Much of the description of exposure conditions and the gas delivery system related to sulfur dioxide. There was very little discussion surrounding modifications, if any, that were performed to allow for the controlled delivery and analysis of H_2S. The lack of information acts to erode confidence in the study findings.

Review & Assessment - Scoring[16] and Rational:

No practical use	☐
Low	☐
Low – Moderate	☒
Moderate	☐
Moderate – High	☐
High	☐

Rationale: The study is useful for the development of emergency planning in that it was an acute exposure study in which lethality and clinical signs were monitored in two species exposed to H_2S for durations up to 16 hours. The overall study design and conduct were adequate for the purposes of the investigation, however, reporting was lacking in several respects. Much of the description of the gas delivery system and the analytical methods used to measure the concentration of the gas in the exposure chamber was not specific to H_2S, but rather related to SO_2. Accordingly, some uncertainty surrounds the actual concentrations of H_2S to which the test animals were exposed. Confidence in the study findings could have been improved by 1) better description of exposure conditions and the gas delivery system, as specifically related to the exposures involving H_2S and 2) provision of data for the control group of animals.

Strengths:

- Use of graded concentrations of H_2S, ranging from 16 to 1,000 ppm.
- Use of two species of test animals (*i.e.*, rats and mice).
- Use of limited, but adequate numbers of test animals.
- Use of both sexes.
- Adequate description of gas delivery system and exposure chamber (… in companion paper).
- Good description of concentration-time response for mortalities, clinical signs and necropsy findings.

Weaknesses:

- Lack of detail concerning control animals.
- Questionable health status of some animals at start of study.
- Failure to specifically report on confirmation of nominal test concentrations (… reference only to the use of "autometers" in the companion paper … no confirmation that test concentrations were actually measured as part of the studies).
- Failure to distinguish between the sexes in terms of the reporting of results.
- Use of relatively antiquated equipment for generating test concentrations, with use of manometers, chart recorders, and "warning bells".

[16] Score reflects usefulness of study for development of emergency planning criteria vis-à-vis acute lethality.

Reviewers:

DD	☒
RT	☒
CM	☐

Document Review - Non-Clinical Studies

Author:	Zwart, A., Arts, J.H.E., Klokman-Houweling, J.M.	**Study Code:**	NC056
Title:	Determination of concentration-time-mortality relationships to replace LC50 values		
Year:	1990		

Paper Description:	Full length paper: ☒ Peer-reviewed ☒ Non-peer reviewed ☐	Abstract: ☐	Review article: ☐	Cited in-review article[17] ☐ Details:

Abstract:	*To determine concentration-time-mortality relationships of ammonia (NH_2), Chlorine (Cl_2), hydrogen sulfide (H_2S), and phosgene ($COCl_2$) in acute inhalation toxicity studies with rats and mice, groups of five males and five females each were exposed for different periods of time to different concentrations of the respective test atmospheres. The consequences of a decrease in the number of animals per group on the accuracy of the LC50 values calculated form the estimated relations were studied by analyzing mortality rates in new sets of data obtained by removing one, two, three, or four animals per sex from the original group results in a random fashion, 500 times for each test compound.* *LC50 values for different durations of exposure were calculated with the newly estimated concentration-time-mortality relationships and the 500 LC50 values were characterized by their fifth, fiftieth, and ninety-fifth percentiles. Furthermore, the mean and standard deviations of the coefficients of the calculated relationships were determined. Within the range of exposure times used in these studies, the fiftieth percentiles were scarcely influenced by the number of animals per sex per group, whereas the fifth and ninety-fifth percentiles covered a larger range when decreasing the number of animals, reaching about ±10% when four animals per sex per group were removed. In the latter situation a small number of draws showed no convergence during estimation of the concentration-time-mortality relationship. Standard deviations of the coefficients of the relationships increased considerably when the number of animals per sex per group was decreased from two to one due to a loss of information on the heterogeneity in some draws.* *It is concluded that LC50 values in the range of duration of exposure applied could have been estimated with one animal per sex per group. The resulting fifth and ninety-fifth percentiles in that case compare favorable with the 90% confidence limits when determining an LC50 according to OECD guideline 403. When extrapolation to low mortality rates is needed, two animals per sex per group seem to determine the lower limit of animal use.*
Objective:	To determine concentration-time-mortality relationships for ammonia, chlorine, hydrogen sulphide and phosgene in acute inhalation toxicity studies with rats and mice. Of particular interest was the application of a statistical technique to examine the consequences of reducing the number of animals in each group for the determination of LC50 values.

Primary focus of the study:	Lethality/fatality: ☒	Other:

Overall study design:

Exposure level(s)	Exposure frequency/duration	Species	Strain/ Breed	Age at initiation	Sex	Number of test animals	Pre-study health status
320 to 1308 ppm (… equivalent to 703 to 1831 mg/m^3)	Single exposures for 5, 10, 30 or 60 minutes. Surviving animals observed for 14 days post-exposure, and then sacrificed.	Rats and mice	Wistar rats; Swiss mice	6-7 weeks (rats) 8-9 weeks (mice) at time of exposure.	Both	Actual testing was performed using 5 animals per sex per concentration level. Lethality indices were then calculated on the basis of group sizes of 1,2,3,4 or 5 rats/sex/exposure level.	Rats were specific-pathogen-free. Nothing specified for mice.

[17] Refers to a paper describing the original paper that was either unattainable or in a foreign language.

Observations:

General

Did the study follow a standardized test protocol? Yes ☐ No ☒

If yes, which test protocol did the study follow? OECD ☐ USEPA ☐ Other:

Was the study conducted under Good Laboratory Practice (GLP)? Yes ☐ No ☒

Lethality/Fatality

Were deaths observed? Yes ☒ No ☐

If so, were deaths exposure-related? Yes ☒ No ☐

If not, provide an explanation (e.g., trauma, concurrent disease, improper and/or inadequate husbandry, etc.).

If so, were the exposure-related deaths observed within 14 days of the initial exposure? Yes ☒ No ☐

Details:

Exposure Level (ppm)	Exposure Time (minutes)	Number of Deaths / Number of Animals Tested	Time to Death
Rats			
665	5	0/5 males; 05 females	Not specified
854	5	2/5 males; 0/5 females	"
1308	5	5/5 males; 5/5 females	"
665	10	0/5 males; 0/5 females	"
856	10	3/5 males; 5/5 females	"
1301	10	5/5 males; 5/5 females	"
321	30	0/5 males; 0/5 females	"
504	30	0/5 males; 0/5 females	"
581	30	0/5 males; 0/5 females	"
595	30	0/5 males; 0/5 females	"
629	30	4/5 males; 5/5 females	"
668	30	0/5 males; 1/5 females	"
694	30	2/5 males; 0/5 females	"
737	30	2/5 males; 1/5 females	"
320	60	0/5 males; 0/5 females	"
502	60	0/5 males; 0/5 females	"
553	60	0/5 males; 0/5 females	"
576	60	0/5 males; 0/5 females	"
590	60	0/5 males; 0/5 females	"
671	60	3/5 males; 4/5 females	"
694	60	3/5 males; 4/5 females	"
Mice			

665	5	0/5 males; 0/5 females	Not specified.
854	5	0/5 males; 0/5 females	"
1308	5	1/5 males; 2/5 females	"
665	10	0/5 males; 0/5 females	"
856	10	0/5 males; 0/5 females	"
1301	10	4/5 males; 5/5 females	"
321	30	0/5 males; 0/5 females	"
504	30	0/5 males; 0/5 females	"
581	30	0/5 males; 0/5 females	"
629	30	1/5 males; 1/5 females	"
668	30	0/5 males; 1/5 females	"
694	30	1/5 males; 2/5 females	"
737	30	0/5 males; 0/5 females	"
320	60	0/5 males; 0/5 females	
502	60	0/5 males; 3/5 females	"
553	60	0/5 males; 2/5 females	"
576	60	2/5 males; 1/5 females	"
671	60	3/5 males; 4/5 females	"
694	60	4/5 males; 2/5 females	"

Were any exposure-related deaths observed more than 14 days after the initial exposure? Yes ☐ No ☒

Details:

Exposure Level (ppm)	Exposure Time (min)	Number of Deaths / Number of Animals Tested	Time to Death (min)

Were animals that died subjected to gross pathological examination (*i.e.*, necropsy)? Yes ☒ No ☐

If so, were necropsy findings consistent with exposure-related cause of death? Yes ☐ No ☐

List major necropsy findings: Although the authors reported that all rats were necropsied and subjected to gross pathological examination, no necropsy findings were provided. No indication was provided as to whether or not the test mice were necropsied.

Were lethal concentrations (LCs) reported? Yes ☒ No ☐

If so, describe: LC_{50} values for the rat (combined sexes) for 10, 30 and 50 minute exposure durations were reported to be 829, 721, and 679 ppm, respectively. In mice, the corresponding 10-minute, 30-minute, and 50-minute LC_{50} values were 1150, 793 and 671 ppm, respectively.

Were time concentrations (TCs) reported? Yes ☐ No ☒

If so, describe:

Signs & Symptoms

Were clinical signs monitored as part of the study? Yes ☒ No ☐

Were any clinical signs consistent with life-threatening, serious and/or irreversible health outcomes reported as a part of the study (*e.g.*, convulsions, coma, unconsciousness, laboured breathing, abnormal gait, *etc.*)? Yes ☐ No ☒

If so, were the clinical signs exposure-related? Yes ☐ No ☐

If not, provide an explanation:

If so, were these exposure-related clinical signs observed within 14 days of the initial exposure? Yes ☐ No ☐

Details:

Nature of Symptom	Exposure Level (ppm)	Exposure Time (min)	Number of Animals Affected	Time to Onset (min)	Duration

Did any of these exposure-related clinical signs first appear more than 14 days after the initial exposure? Yes ☐ No ☒

Details:

Nature of Symptom	Exposure Level (ppm)	Exposure Time (min)	Number of Animals Affected	Time to Onset (min)	Duration

Were any other exposure-related clinical signs observed? Yes ☐ No ☒

If yes, list other clinical signs: The authors indicated that clinical signs were monitored at least once per day throughout the 14-day post-exposure observation period. No indication of any clinical signs appearing during the observation period was provided in the Results section.

Review & Assessment: Study Design, Conduct & Reporting:

A. Test Animals:	+/- Details concerning age, weight variation and acclimation of animals was supplied. Source of animals was not provided. +/- The number of test animals in the main study (5 per sex per exposure level per species) complied with OECD guidelines + Both sexes were employed - Pre-test health status was not specified apart from the rats being specific-pathogen-free.
B. Exposure conditions:	+ A gradient of exposure levels was tested ranging from 320 to 1308 ppm for durations of 5,10,30 or 60 minutes +/- H_2S concentrations were reportedly monitored during test exposures, but details were concerning the sampling and analytical methodology were lacking. - It was not stated whether exposure chambers were equilibrated before or after test animals were placed inside. This could potentially alter the actual duration of exposure to the stated levels of H_2S.

C. Housing/Feeding	+/- Details concerning the housing environment were judged to be adequate (*i.e*., temperature and humidity were controlled and within ranges specified in OECD test guidelines). However, photoperiod was not recorded. + Caging details were provided (… including type of caging and number of animals per cage. - The type and source of feed and water were not stated.
D. Exposure equipment:	+/- Basic details concerning the exposure chamber and gas delivery system were provided (*i.e*., type, dimensions, air flow rate). +/- H_2S concentrations were reportedly monitored, but details respecting analytical methodology, frequency of measurements, *etc*. were not supplied.
E. Procedural:	+/- Acclimation period was of acceptable duration (5 days). - No indication that the test animals were randomly assigned to test groups. - No indication that a control group was employed. + The period of observation following exposure was appropriate (*i.e*., 14 days).
F. Data collection:	+ Raw data for individual animals were provided - Clinical signs and body weights evidently were recorded, but the findings were not reported. - Actual time of death for animals dying on test was not provided. There was no indication of whether the animals died during the exposure period and/or during the 14-day post-exposure observation period. - All rats evidently were necropsied and subjected to gross pathological examination, but no findings were reported.
G. Data analysis:	+/- Unusual assessment method was employed to determine consequences of a decrease in the number of animals per group on the accuracy of LC_{50} values. Mortality rates were analyzed in new sets of data obtained by removing one, two, three or four animals per sex from the original group in a random fashion, 500 times for each test compound. + Confidence intervals were reported + Statistical methods employed were adequately described.
H. Interpretations:	+ The original objective was addressed + Study was published in a peer-reviewed journal + Use of novel technique generated data that would have required 200 000 animals per species in a conventional study. + Two test species and both sexes were evaluated.

Review & Assessment - Summary:

Discussion of findings: In the original study using 5 animals/sex/exposure level, LC_{50} values were determined in rats and mice for 10-minute, 30-minute and 50-minute exposure durations. Investigations into the influence of the number of animals per sex per group indicated that LC_{50} values did not appear to be significantly affected by reducing the number of animals, albeit the confidence intervals were greater for the LC_{50} estimates when the number of animals was reduced to 1/sex.

Review & Assessment - Scoring[18] and Rational:

No practical use	☐
Low	☐
Low – Moderate	☐

[18] Score reflects usefulness of study for development of emergency planning criteria vis-à-vis acute lethality.

Moderate	☒
Moderate – High	☐
High	☐

Rational: The study is useful for the development of emergency planning endpoints in that it is an acute exposure study that identified LC50 values in both rats and mice for 10, 30 and 60 minute durations. The study design, conduct and reporting were judged to be adequate. Added confidence could have been achieved by the use of a control group and random assignment of animals to exposure groups. The study also could have been improved by better descriptions of clinical signs and gross pathological findings, as well as the methodology surrounding the monitoring of chamber concentrations. The description of the exposure chamber and gas delivery system was marginal.

Strengths:

- Use of two species (rat and mouse) and use of both sexes.
- Use of multiple exposure concentrations covering a fairly broad range (≈300 to 1300 ppm).
- Use of multiple exposure times (5, 10, 30, and 60 minutes).
- Use of varied concentration-time combinations to permit assessment of comparative effects of exposure concentration and exposure time on lethality outcomes.

Weaknesses:

- Lack of reporting of clinical signs and body weights, despite the fact that these parameters evidently were monitored as part of the study.
- Lack of reporting of gross pathological findings despite the fact that the animals evidently were necropsied at the end of the observation period.
- Lack of a control group(s) of animals.
- Lack of in-depth description of exposure chamber and gas delivery system, as well as failure to describe sampling and analytical methodology used to confirm the exposure concentrations.
- Lack of information concerning whether or not the exposure chamber was equilibrated prior to the introduction of the test animals (… however, since the volume of the exposure chamber was ≈ 16 liters and the air flow rate through the chamber was 25-40 liters/minute, equilibration would have been achieved within 20 to 30 seconds, i.e., a significantly shorter interval than even the shortest exposure time of 5 minutes).

Reviewers:

DD	☒
RT	☒
CM	☐

Document Review - Non-Clinical Studies

Author:	Hays, F.L.		**Study Code:**	NC057
Title:	Studies of the effects of atmospheric hydrogen sulfide in animals.			
Year:	1972			
Paper Description:	Full length paper: ☒ Peer-reviewed ☐ Non-peer reviewed ☒	Abstract: ☐	Review article: ☐	Cited in-review article[19] ☐ Details:
Abstract:				
Objective:	To investigate the general well being of mice, goats and cows exposed to H_2S as indexed by feed and water intake as well as the lethal concentration duration (LCD) at various levels up to 100 ppm.			
Primary focus of the study:	Lethality/fatality: ☐	Other: Effect of H_2S exposure on feed and water intake, body weight, rectal temperature, liver function, RBC carbonic anhydrase activity (mice only), plasma cortisol levels (goats only), blood pressure (goats and cows only), heart rate (goats and cows only), milk production (cows only).		

Overall study design:

Exposure level(s)	Exposure frequency/duration	Species	Strain/ Breed	Age at initiation	Sex	Number of test animals	Pre-study health status
<u>Mice</u> Experiment 1: 0, 10, 50 or 100 ppm. (An "accidental" exposure to 30 ppm also was documented). Experiment 2: 0 or 20 ppm	Experiment 1: Continuous exposure until reaching LCD (50 and 100 ppm) or for up to 5 days (10 ppm). Experiment 2: Continuous exposure for 48 hours.	Mice	Swiss Webster	Not specified	Male mice (20 ppm); sex of mice in other groups was not specified.	6- 8 per exposure group	Not specified.
<u>Goats</u> 0, 10,50 and 100 ppm	Continuous exposure for 4 days.	Goats	Mixed breed (Angora, French Alpine or Toggenburg)	3 to 4 years	Female	3-5 per exposure group.	Not specified.

[19] Refers to a paper describing the original paper that was either unattainable or in a foreign language.

Cows 0 and 20 ppm	Continuous exposure for 21 days.	Dairy cows	Holstein	Not specified	Female	Total of 3 cows	Not specified.

Observations:

General

Did the study follow a standardized test protocol? Yes ☐ No ☒

If yes, which test protocol did the study follow? OECD ☐ USEPA ☐ Other:

Was the study conducted under Good Laboratory Practice (GLP)? Yes ☐ No ☒

Lethality/Fatality

Were deaths observed? Yes ☒ No ☐

If so, were deaths exposure-related? Yes ☒ No ☐

If not, provide an explanation (*e.g.*, trauma, concurrent disease, improper and/or inadequate husbandry, *etc.*).

If so, were the exposure-related deaths observed within 14 days of the initial exposure? Yes ☒ No ☐

Details:

Exposure Level (ppm)	Exposure Time (min)	Number of Deaths Number of Animals Tested	Time to Death (min)
Mice			
10 ppm	120 hours	0/8	Not applicable
20 ppm	48 hours	0/8	Not applicable
30 ppm ("accidental" exposure of so-called "H_2S group")	18.5 hours*	3/8**	18.5 hours (3 mice – all mice died within 15 minutes of each other)
30 ppm ("accidental" exposure of so-called H_2S group")	18.5 hours	2/8	Two of the mice which survived the "accidental" exposure died within 24 hours post-exposure.
20 to 30 ppm (estimated "accidental" exposure of fasted control group)	18.5 hours	1/8	Mouse died 28 hours post-exposure.
50 ppm	16 hours*	4/8	15 hours (calculated)
100 ppm	8 hours*	3/8	7.5 hours (calculated - deaths reportedly occurred within minutes of each other).

Goats			
10 ppm	96 hours	0/4	Not applicable
50 ppm	96 hours	0/4	Not applicable
100 ppm	96 hours	0/5	Not applicable
Cows			
20 ppm	21 days	0/3	Not applicable

*For unspecified reasons, exposure was terminated at these times. Surviving mice from the 50 and 100 ppm groups were subsequently sacrificed for blood analysis, while the surviving mice exposed to 30 ppm were allowed to recover in the exposure chamber for up to an additional 4 days.
**Two additional mice were noted to die 23.5 hours post-exposure (*i.e.*, 42 hours after the start of exposure).

Were any exposure-related deaths observed more than 14 days after the initial exposure? Yes ☐ No ☒

Details:

Exposure Level (ppm)	Exposure Time (min)	Number of Deaths / Number of Animals Tested	Time to Death (min)

Were animals that died subjected to gross pathological examination (*i.e.*, necropsy)? Yes ☐ No ☒
If so, were necropsy findings consistent with exposure-related cause of death? Yes ☐ No ☐
List major necropsy findings:
Were lethal concentrations (LCs) reported? Yes ☐ No ☒
If so, describe:
Were time concentrations (TCs) reported? Yes ☒ No ☐
If so, describe: LT50s for 30, 50 and 100 ppm were estimated as 18.5, 15 and 7.5 hours, respectively

Signs & Symptoms

Were clinical signs monitored as part of the study? (only body weight and food and water consumption) Yes ☐ No ☒
Were any clinical signs consistent with life-threatening, serious and/or irreversible health outcomes reported as a part of the study (e.g., convulsions, coma, unconsciousness, laboured breathing, abnormal gait, etc.)? Yes ☐ No ☐
If so, were the clinical signs exposure-related? Yes ☐ No ☐
If not, provide an explanation:
If so, were these exposure-related clinical signs observed within 14 days of the initial exposure? Yes ☐ No ☐

Details:

Nature of Symptom	Exposure Level (ppm)	Exposure Time (min)	Number of Animals Affected	Time to Onset (min)	Duration

Did any of these exposure-related clinical signs first appear more than 14 days after the initial exposure? Yes ☐ No ☐

Details:

Nature of Symptom	Exposure Level (ppm)	Exposure Time (min)	Number of Animals Affected	Time to Onset (min)	Duration

Were any other exposure-related clinical signs observed? Yes ☒ No ☐

If yes, list other clinical signs: mice: decreased feed and water intake (20-100 ppm) and decreased thermoregulatory ability as evidenced by decreased rectal temperatures (20-100 ppm); goats: decreased feed and water intake (10-100 ppm) and increased rectal temperatures (50-100 ppm), but recovery was observed with continued exposure

Review & Assessment: Study Design, Conduct & Reporting:

A. Test Animals:	+ Adequate numbers of test animals used (*i.e.*, 6-8 mice per exposure concentration, 3-5 goats per exposure concentration, and 3 cows per exposure concentration). + Control groups of animals included as part of studies with mice and goats. For the former studies, both fasted and non-fasted control groups of mice were used. - Due to an equipment failure, the control groups of mice were compromised and "accidentally" exposed to H_2S during one of the series of studies performed. + Details concerning the source, strain, weight variation and acclimation of test animals were supplied. +/--Age was specified only for the goats; sex was specified only for goats, cows and mice in the 20 ppm group. - Health status of animals at study initiation was not indicated.
B. Exposure conditions:	- Exposure concentrations were defined, but in the case of the mice exposed to 50 and 100 ppm, it was unclear why exposure was terminated once a certain number of deaths occurred. - "Accidental" exposure occurred as part of one of the series of tests due to failure of the infusion pump that formed part of the gas delivery system. Exposure was "estimated' to be 30 ppm. Control chambers were also affected by the accident (*i.e.*, the gas infused into the entire animal room). - No indication that there was an equilibration period in the exposure chamber prior to placement of test animals. +/- Airflow, temperature and pressure were monitored during exposure and in compliance with OECD guidelines, but there was no indication that humidity was monitored. +/- H_2S concentrations in the exposure chambers were reportedly measured using "Kitigawa" detection kits (… the detector kit readings were validated by a fluorometric method for the 10 ppm and 50 ppm groups). The frequency of readings was not stated. The testing revealed measured values that were close to the nominal values, but testing was noted to be "not rigorous". It is unclear what this means. - An accidental gas leakage due to failure of the infusion pump that formed part of the gas delivery system resulted in contamination of the laboratory, including the control chambers. Concentrations in the laboratory were "estimated" to be 30 ppm, with a range of 20-30 ppm, but how these concentrations were estimated was not specified.

C. Housing/Feeding	+ Animal husbandry adequately described. + The type and source of feed and water were stated and the feeding schedules outlined. + Animal room temperature, humidity and photoperiod reported.
D. Exposure equipment:	+ Details concerning construction and dimensions of exposure chambers (mice and goats) and exposure "hood" (cows) provided. - Exposure chamber was of somewhat makeshift design (*i.e.*, retrofitted and modified version of earlier constructed chamber used for other purposes). + Source of H_2S gas indicated (*i.e.*, commercially supplied). + Gas delivery system adequately described. + Chamber air flow rates, temperature, *etc.* regularly monitored. +/- Exposure concentrations were reportedly routinely monitored. Methodology relied on use of "Kitigawa detector kit" (*i.e.*, colorimetric analysis with reported ±10% sensitivity), combined with selective use of fluorometric method. - Gas delivery system relied on use of infusion pump, which failed leading to "accidental" contamination of animal room, including control chambers.
E. Procedural:	+/- An acclimation period to permit the test animals to adjust to the exposure chamber/exposure "hood" prior to exposure was included for the goats and cows. No acclimation period was included for the mice. - No indication that test animals were randomly assigned to exposure groups +/- Separate control groups were included as part of the studies with the mice and goats, while the cows served as their own controls. - Accidental contamination of the animal room, including the control chambers occurred during the course of the mouse studies as a result of the failure of the infusion pump that formed part of the gas delivery system. Investigation continued, with mice in the test chamber estimated to be exposed to 30 ppm of H_2S and mice in the control chambers estimated to be exposed to 20 to 30 ppm of H_2S. The basis of the estimated concentrations was not provided. +/- Mice in certain exposure groups were kept for a post-exposure observation period of 14 days (30 ppm group and control groups that were "accidentally" exposed),but most mice (100,50 and 10 ppm groups) were not held for observation. - No indication that any animals were necropsied as part of the studies. +/- Body weights and feed and water intake were monitored, but no indication that conventional clinical signs of toxicity were monitored.
F. Data collection:	+ Individual mortality data were provided and times of deaths noted. - Clinical signs were not monitored or reported during the exposures or post-exposure observation period – with the exception of changes in body weight or feed and water consumption
G. Data analysis:	- The statistical methods employed were not outlined + Good graphical presentation of results
H. Interpretations:	- Time course of deaths for mice "accidentally" exposed to 30 ppm is somewhat suspect (*i.e.*, mice died within 15 minutes of each other at 18.5 hours). - Results from "accidental" exposure should be discarded since control groups were compromised and exposure levels were not confirmed.

Review & Assessment - Summary:

Discussion of findings: Time to 50% lethality concentrations (*i.e.*, Lethal Concentration Duration values or LCDs) were reported for mice exposed to 30, 50 and 100 ppm H2S. The LCDs ranged from 18.5 hours (30 ppm) to 7.5 hours (100 ppm). No deaths were reported in goats exposed to H_2S at concentrations up to 100 ppm for 4 days, or in cows exposed to 20 ppm of H_2S for 3 weeks.

Strengths

- Use of multiple test species (mice, goats, cows).
- Use of gradient of exposure concentrations for studies with mice and goats (0 to 100 ppm).
- Detailed description of gas delivery system and exposure chamber/ exposure "hood".
- Analytical confirmation of exposure concentrations (… albeit methodology relied on colorimetric analysis of limited sensitivity).
- Adequate descriptions of animal husbandry (*i.e.*, feed and water supply, caging, animal room conditions).

Weaknesses

- "Accidental" exposure resulting in contamination of animal room, including control chambers, suggests lack of care and attention to detail.
- Reliability of findings from "accidental" exposure portion of study highly questionable.
- Lack of monitoring of conventional clinical signs.
- No necropsy records.
- Time course of deaths witnessed among certain groups of mice (30 ppm) judged to be questionable because of unusual pattern (*i.e.*, sudden collapse and death after 18 hours of exposure).

Review & Assessment - Scoring[20] and Rational:

No practical use	☐
Low	☐
Low – Moderate	☒
Moderate	☐
Moderate – High	☐
High	☐

[20] Score reflects usefulness of study for development of emergency planning criteria vis-à-vis acute lethality.

Reviewers:

DD	☒
RT	☒
CM	☐

Document Review - Non-Clinical Studies

Author:	Haggard, H.W.	**Study Code:**	NC067
Title:	The Toxicology of Hydrogen Sulphide		
Year:	1925		
Paper Description:	Full length paper: ☒ Peer-reviewed ☐ Non-peer reviewed ☒	Abstract: ☐	Review article: ☒ Cited in-review article[21] ☐ Details:
Abstract:	*not available*		
Objective:	To review the toxicology of hydrogen sulphide and present results of an experiment on toxic H_2S concentrations in dogs		
Primary focus of the study:	Lethality/fatality: ☒	Other: General toxicity of H_2S in dogs	

Overall study design:

Exposure level(s)	Exposure frequency/duration	Species	Strain/ Breed	Age at initiation	Sex	Number of test animals	Pre-study health status
100-150 ppm 200-300 ppm 500-700 ppm 900 ppm 1500 ppm 1800+ ppm	Several hours or until death	Dog	Not specified	Not specified	Not specified	Not clearly indicated, but presumably one dog per exposure level.	Not specified

Observations:

General

Did the study follow a standardized test protocol? Yes ☐ No ☒

If yes, which test protocol did the study follow? OECD ☐ USEPA ☐ Other:

Was the study conducted under Good Laboratory Practice (GLP)? Yes ☐ No ☒

Lethality/Fatality

Were deaths observed? Yes ☒ No ☐

If so, were deaths exposure-related? Yes ☒ No ☐

If not, provide an explanation (e.g., trauma, concurrent disease, improper and/or inadequate husbandry, *etc.*).

If so, were the exposure-related deaths observed within 14 days of the initial exposure? Yes ☒ No ☐

[21] Refers to a paper describing the original paper that was either unattainable or in a foreign language.

Details:

Exposure Level (ppm)	Exposure Time (min)	Number of Deaths / Number of Animals Tested	Time to Death (min)
500-700 ppm	Until death	1/1	Several hours
900 ppm	Until death	1/1	30 minutes to 1 hour
1500 ppm	Until death	1/1	15 to 30 minutes
1800 ppm	Until death	1/1	Immediate

Were any exposure-related deaths observed more than 14 days after the initial exposure? Yes ☐ No ☒

Details:

Exposure Level (ppm)	Exposure Time (min)	Number of Deaths / Number of Animals Tested	Time to Death (min)

Were animals that died subjected to gross pathological examination (*i.e.*, necropsy)? Yes ☐ No ☒
If so, were necropsy findings consistent with exposure-related cause of death? Yes ☐ No ☐
List major necropsy findings:
Were lethal concentrations (LCs) reported? Yes ☐ No ☒
If so, describe:
Were time concentrations (TCs) reported? Yes ☐ No ☒
If so, describe:

Signs & Symptoms

Were clinical signs monitored as part of the study? Yes ☒ No ☐
Were any clinical signs consistent with life-threatening, serious and/or irreversible health outcomes reported as a part of the study (*e.g.*, convulsions, coma, unconsciousness, laboured breathing, abnormal gait, *etc.*)? Yes ☐ No ☒
If so, were the clinical signs exposure-related? Yes ☐ No ☐
If not, provide an explanation:
If so, were these exposure-related clinical signs observed within 14 days of the initial exposure? Yes ☐ No ☐

Details:

Nature of Symptom	Exposure Level (ppm)	Exposure Time (min)	Number of Animals Affected	Time to Onset (min)	Duration

Did any of these exposure-related clinical signs first appear more than 14 days after the initial exposure? Yes ☐ No ☐

Details:

Nature of Symptom	Exposure Level (ppm)	Exposure Time (min)	Number of Animals Affected	Time to Onset (min)	Duration

Were any other exposure-related clinical signs observed? Yes ☒ No ☐

If yes, list other clinical signs: local irritation and systemic symptoms were noted at various concentrations (see table in discussion of findings)

Review & Assessment: Study Design, Conduct & Reporting:

A. Test Animals:	- Only one animal per dose level was employed. (The guidelines generally recommend use of 5 animals per sex per treatment level). - No details with respect to the source, sex, age, weight, or pre-test health status of the test animals were provided.
B. Exposure conditions:	+ A gradient of exposure concentrations was tested. +/- Exposure concentrations evidently were analytically confirmed, but no details concerning the sampling or analytical methodology were provided. - No indication that the exposure chamber was equilibrated with the gas prior to exposure of the test animals. - No indication that airflow, temperature and humidity in the exposure chamber were monitored
C. Housing/Feeding	- No details provided on the housing or feeding of test animals (*e.g*., type and source of food and water; room temperature, and humidity, photoperiod).
D. Exposure equipment:	- The only detail provided regarding the exposure chamber was that it was a glass chamber. Information respecting dimensions, air flow rates, *etc*. was not provided. - No details concerning the gas delivery system were supplied. - The source of H_2S was not provided. - No description of the sampling or analytical methodology that was evidently used to confirm the exposure concentrations was given.
E. Procedural:	- No indication that a control group was employed - No indication that test animals were acclimated to the laboratory environment and/or the exposure chamber. - No indication that test animals were randomly assigned to treatment groups - No indication that there was a period of observation following exposure
F. Data collection:	+/- Clinical signs/symptomatology was evidently monitored but these were only reported in a general manner (*i.e*., systems were listed simply as being "systemic" or "irritant" in nature). Details concerning the exact nature, duration, and severity of the symptoms were not provided. - Raw data for individual animals were not provided
G. Data analysis:	- No indication that statistical methods were employed

H. Interpretations:	- Data were limited to a tabular summary of lethality and clinical signs following exposure to graded concentrations for varying periods of time. A lack of details concerning design and conduct precluded critical interpretation of the findings. +/- Authors noted that their results are in complete agreement with those found by Lehman, 1892 (NC070).

Review & Assessment - Summary:

Discussion of findings: A table presenting the toxic concentrations of H_2S determined in dogs re-produced directly from the paper is presented below.

Toxic Effect	**H_2S Concentration**
Symptoms of local irritation after many hours of exposure	100-150 ppm
Causes local irritation if inhaled for one hour and slight general symptoms if inhaled longer.	200-300 ppm
Causes local irritation and slight systemic symptoms within one hour. May cause death in less than one hour.	500-700 ppm
Causes systemic symptoms in less than 30 minutes. May cause death in less than one hour.	900 ppm
Causes death after 15-30 minutes of exposure	1500 ppm
Causes almost immediate death through paralysis of breathing	1800 + ppm

Review & Assessment - Scoring[22] and Rational:

No practical use ☐

Low ☒

Low – Moderate ☐

Moderate ☐

Moderate – High ☐

High ☐

Rational: This study is of limited usefulness for the development of emergency response endpoints. Although a tabular summary was provided showing the concentration-time-response for lethality and clinical signs in dogs following exposure to H_2S, a lack of detail concerning study design, conduct and reporting renders the data inadequate and of limited usefulness.

[22] Score reflects usefulness of study for development of emergency planning criteria vis-à-vis acute lethality.

Strengths:

- Use of gradient of exposure concentrations (100-1800 ppm) over durations up to several hours to permit assessment of the influence of each parameter on lethality and other health endpoints.
- Observation of test animals for both mortality and symptomatology
- Test concentrations of H_2S were apparently analytically confirmed.

Weaknesses:

- No description of exposure chamber or H_2S monitoring device.
- Use of only one test animal per exposure concentration.
- No description of test animals apart from the species.
- Only general description of clinical signs (*i.e.*, classified only as "systemic" or "irritant").
- No indication that animals were randomly assigned to dose groups.
- No indication that test animals were necropsied.
- Lack of post-exposure observation period.
- No indication that study was subjected to independent peer review.

Reviewers:

DD	☒
RT	☒
CM	☐

Document Review - Non-Clinical Studies

Author:	Lopez, A., Prior, M.G., LeBlanc, D., Yong, S., Albassam, M. and Lillie, L.E.	**Study Code:**	NC069
Title:	Alberta Environmental Centre Series on Inhalation Toxicology. 1. Morphological observations in rats exposed for six hours to an atmosphere of 0, 56, or 420 mg/m^3 hydrogen sulphide		
Year:	1986		

Paper Description:	Full length paper: ☒ Peer-reviewed ☐ Non-peer reviewed ☒	Abstract: ☐	Review article: ☐	Cited in-review article[23] ☐ Details:
Abstract:	*Forty eight male Long Evans rats were exposed to nominal concentrations of 0, 56 or 420 mg m^{-3} (actual 0, 57 ± 15 or 420 ± 1.4 mg m^{-3}) hydrogen sulphide for six hours. Weight loss was observed in all rats exposed to hydrogen sulphide, as was agitation, hypoaesthesia, panting and lacrimation. All rats exposed to 300 ppm died within the six hour exposure period. Necrosis of the nasal epithelium was more marked in the intermediate (sectors 2, 3) than the most rostral (sector 1) and most caudal (section 4) parts of the nasal cavity. The lateral aspects of the nasal turbinates revealed more necrosis when compared to the median aspects, especially the epithelium covering the nasal septum. Mild pulmonary oedema was observed in all animals exposed and killed by 420 mg m^{-3} hydrogen sulphide and those treated with 56 mg m^{-3} and killed at the end of the exposure. Rats exposed to 56 mg m^{-3} did not show pulmonary oedema at 18 or 42 hours post exposure. The oedema had a perivascular distribution, and fluid was rarely seen within the alveoli.*			
Objective:	To examine histopathological findings in rats exposed to a concentration of 0, 40 or 300 ppm of hydrogen sulphide for six hours, with particular emphasis on the lesions observed in the nasal mucosa.			
Primary focus of the study:	Lethality/fatality: ☒	Other: Histopathological lesions within the respiratory tree, particularly in the nasal mucosa, following short-term exposure to H_2S.		

Overall study design:

Exposure level(s)	Exposure frequency/duration	Species	Strain/ Breed	Age at initiation	Sex	Number of test animals	Pre-study health status
0, 40 or 300 ppm. Note that two control groups (0 ppm) were employed, specifically a "room air" control group and an "exposure chamber" control group.	Single exposure lasting 6 hours. Surviving rats were sacrificed at 0 hours, 18 hours or 42 hours post-exposure (4 animals per group)	Rats	Long-Evans	Not specified	Male	Total of 12 rats per exposure group, sacrificed at different intervals post-exposure.	Not specified (Rats were sourced from a reputable commercial source and assumed to be healthy)

[23] Refers to a paper describing the original paper that was either unattainable or in a foreign language.

Observations:

General

Did the study follow a standardized test protocol? Yes ☐ No ☒

If yes, which test protocol did the study follow? OECD ☐ USEPA ☐ Other:

Was the study conducted under Good Laboratory Practice (GLP)? Yes ☐ No ☒

Lethality/Fatality

Were deaths observed? Yes ☒ No ☐

If so, were deaths exposure-related? Yes ☒ No ☐

If not, provide an explanation (e.g., trauma, concurrent disease, improper and/or inadequate husbandry, etc.).

If so, were the exposure-related deaths observed within 14 days of the initial exposure? Yes ☒ No ☐

Details:

Exposure Level (ppm)	Exposure Time (min)	Number of Deaths / Number of Animals Tested	Time to Death (min)
300 ppm	6 hours	12/12	Reported to have been between 5 and 6 hours
40 ppm	6 hours	0/12	Not applicable
0 ppm ("room air" control	6 hours	0/12	Not applicable
0 ppm ("exposure chamber" control)	6 hours	0/12	Not applicable

Were any exposure-related deaths observed more than 14 days after the initial exposure? Yes ☐ No ☒

Details:

Exposure Level (ppm)	Exposure Time (min)	Number of Deaths / Number of Animals Tested	Time to Death (min)

Were animals that died subjected to gross pathological examination (*i.e.*, necropsy)? Yes ☒ No ☐

If so, were necropsy findings consistent with exposure-related cause of death? (some of them) Yes ☒ No ☐

List major necropsy findings: Froth in the upper airways, lungs congestion and haemorrhage were observed in the 300 ppm group. No gross lesions were reportedly observed among the control animals or the rats exposed to 40 ppm of H_2S. Histological findings included acute necrosis of the nasal epithelium in all H_2S-exposed rats, which was more severe in 300 ppm group. Mild pulmonary edema was observed histologically in rats exposed to 300 ppm as well as rats exposed to 40 ppm and sacrificed at 0 h post-exposure (n=4), but not in rats exposed to 40 ppm and sacrificed at 18 (n=4) and 42 hours (n=4) post-exposure.

Non-treatment related findings: focal erosive rhinitis in control groups, focal hepatic necrosis in two rats exposed to 40 ppm H_2S and one rat exposed to 300 ppm H_2S. Hyperplasia of the prostatic acini was observed in one control rat and two rats exposed to 300 ppm.

Were lethal concentrations (LCs) reported? Yes ☐ No ☒

If so, describe:

Were time concentrations (TCs) reported? Yes ☐ No ☒

If so, describe:

Signs & Symptoms

Were clinical signs monitored as part of the study? Yes ☒ No ☐

Were any clinical signs consistent with life-threatening, serious and/or irreversible health outcomes reported as a part of the study (*e.g.*, convulsions, coma, unconsciousness, laboured breathing, abnormal gait, *etc.*)? Yes ☒ No ☐

If so, were the clinical signs exposure-related? Yes ☒ No ☐

If not, provide an explanation:

If so, were these exposure-related clinical signs observed within 14 days of the initial exposure? Yes ☒ No ☐

Details:

Nature of Symptom	Exposure Level (ppm)	Exposure Time (min)	Number of Animals Affected	Time to Onset (min)	Duration
Severe dyspnea	300 ppm	6 hours	12/12	"throughout exposure"	Until death

Did any of these exposure-related clinical signs first appear more than 14 days after the initial exposure? Yes ☐ No ☒

Details:

Nature of Symptom	Exposure Level (ppm)	Exposure Time (min)	Number of Animals Affected	Time to Onset (min)	Duration

Were any other exposure-related clinical signs observed? Yes ☒ No ☐

If yes, list other clinical signs: During the first two hours of exposure to 40 ppm of H_2S, rats were agitated and showed a moderate degree of hypoaesthesia (?), panting and lacrimation. Rats exposed to 300 ppm were agitated until death. Body weight loss was observed in rats exposed to both 40 and 300 ppm H_2S. Rats exposed to compressed air in the chamber (controls) also lost body weight but not to the same degree.

Review & Assessment: Study Design, Conduct & Reporting:

A. Test Animals:	+/- Details concerning source, weight, and acclimation of animals were supplied. Age of animals was not provided. +/- The number of test animals (12 per exposure level) complied with OECD guidelines - Only male rats were employed - Pre-test health status was not specified and non-treatment related pathology findings in some rats suggested that not all test animals may have been healthy prior to exposure
B. Exposure conditions:	+/- Two concentrations of H_2S (40, 300 ppm) were tested for a single duration of 6 hours + The actual gas concentrations were determined and recorded. Gas concentrations in both test and control atmospheres were monitored 4 times an hour and analyzed by gas chromatography. Actual gas concentrations were determined to be 300 ± 1.0 ppm (range: 298 -300 ppm) and 41 ± 11 ppm (range: 14 to 60 ppm). + The exposure chamber was maintained at negative pressure in compliance with guideline recommendations + Test animals were acclimated to the exposure chambers prior to initiation of exposures. - It was not stated whether exposure chambers were equilibrated before or after test animals were placed inside. This could potentially alter the actual duration of exposure to the stated levels of H_2S.
C. Housing/Feeding	+ Details concerning the housing environment were judged to be adequate (*i.e.*, temperature, humidity and photoperiod were controlled and within ranges specified in OECD test guidelines). +/- Caging details were provided (e.g., stainless steel mesh caging, 4 animals per cage, 3 cages per exposure + The type and source of feed and water were stated and the feeding schedule was appropriate (i.e., *ad libitum* during housing, presumably withheld during 6-hour exposure).
D. Exposure equipment:	+/- Basic details concerning the exposure chamber and gas delivery system were provided (*i.e.*, type, dimensions, air flow rate). + The actual gas concentrations were determined and recorded. Gas concentrations in both test and control atmospheres were monitored 4 times an hour and analyzed by gas chromatography
E. Procedural:	+ Acclimation period was of acceptable duration (2 weeks). +/- Animals were randomly assigned to exposure groups, but the method of randomization was not stated. + Two control groups were employed: chamber controls exposed to compressed air and room controls +/- Surviving rats were held for sacrifice for 0 hours (n=4), 18 hours (n=4) or 42 hours (n=4) post-exposure. The period of observation following exposure was thus less than that specified by OECD guidelines (*i.e.*, 14 days). However, the objective of the study was to examine respiratory tract histopathology rather than acute toxicity per se.
F. Data collection:	+ Raw data for individual animals were provided with respect to histopathological findings. + Clinical signs and body weights were recorded and reported. + Approximate time of death in animals dying on test was provided (between 5 and 6 hours following initiation of exposure). + All rats were necropsied and subjected to gross and histopathological examination. + Surviving rats were sacrificed at 3 different times post-exposure, presumably to assess the time-course of recovery, if any, from histopathological lesions.
G. Data analysis:	+ Confidence intervals were reported - Statistical methods employed were not outlined specifically and in some cases did not appear to be employed (*e.g.*, the difference in body weight loss between control groups and exposed groups was not analyzed statistically)
H. Interpretations:	+ Good discussion of findings and review of relevant literature

Review & Assessment - Summary:

Discussion of findings: All rats exposed to 300 ppm died within 5-6 hours of exposure and were observed to have severe pulmonary edema upon necropsy. The deaths were unexpected since the LC_{50} for H_2S had previously been reported to be 444 ppm for 4-hour exposure in Sprague-Dawley rats (Tansy et al., 1981). Since all rats were observed to be alive at the end of four hours exposure, the authors noted that it is likely the two additional hours had a significant cumulative toxic effect, despite the fact that they were exposed to a lower concentration than in Tansy et al. (1981). This indicates that both concentration and duration of exposure are important determinants of lethality. It is also possible that the different strain of rats employed in this study had an influence (Long-Evan rats vs. Sprague-Dawley rats). No rats exposed to 40 ppm died during the six hour exposure or up to 42 hours post-exposure. Clinical signs in this group (agitation, hypoaesthesia, panting and lacrimation) were observed only in the first two hours of exposure. Mild pulmonary edema was observed in 40 ppm-exposed rats sacrificed immediately post-exposure, but not in those sacrificed 18 or 42 hours post-exposure. This indicates that the survivors recovered without any noticeable residual signs of toxicity. Acute necrosis of the nasal epithelium was observed in both the 40 ppm and 300 ppm exposed rats, but was more severe in the 300 ppm group. In the 40 ppm exposed rats, the nasal necrosis was observed only in rats sacrificed at 0 and at 18 hours post-exposure, with no lesions noted at 42 hours.

Review & Assessment - Scoring[24] and Rational:

No practical use	☐
Low	☐
Low – Moderate	☐
Moderate	☐
Moderate – High	☒
High	☐

Rational: The study is useful for the development of emergency planning endpoints in that it is an acute exposure study in which lethality was monitored in rats exposed to 40 or 300 ppm H_2S for 6 hours. The study design, conduct and reporting were judged to be adequate. Added confidence could have been achieved by the use of both sexes and a longer observation period for mortality post-exposure.

Strengths:

- Use of two exposure levels (40 ppm and 300 ppm) as well as two separate control groups (0 ppm)
- Use of relatively large numbers of rats per exposure level in terms of mortality assessment (n=12)
- Use of three different time intervals post-exposure for sacrifice of surviving rats to assess potential recovery from exposure-related effects
- Direct monitoring of H_2S during exposure in both test and control atmospheres (four times per hour)
- Detailed reporting of gross and histopathologic findings upon necropsy
- Monitoring of clinical signs during exposure, including weight loss
- Use of a 2-week acclimation period for test animals as well as acclimation of the test animals to the exposure chambers before initiation of exposures.

[24] Score reflects usefulness of study for development of emergency planning criteria vis-à-vis acute lethality.

Weaknesses:

- Use of only male rats
- Lack of information concerning whether or not the exposure chamber was equilibrated prior to the introduction of the test animals (… judged to be of little consequence in light of consideration of exposure chamber size (i.e., approx. 69 liters), chamber airflow rate (i.e., 17 L/min), and exposure duration (i.e., 6 hours).
- Insufficient post-observation period in surviving rats with respect to mortality (<42 hours versus 14 days). This is likely because the objective of the study was to examine histopathology findings rather than mortality specifically.
- The non-treatment related pathological findings in individual rats from each group raises questions as to the pre-health status of the rats employed. A pre-health assessment was not conducted but rats were sourced from a reputable commercial source (Charles River Inc., Quebec) and likely presumed healthy.

Reviewers:

DD	☒
RT	☒
CM	☐

Document Review - Non-Clinical Studies

Author:	Lehmann, K.B.		**Study Code:**	NC070 (see also CL011)
Title:	Experimental studies on the effects of technically and hygienically important gases and vapours on organisms. Part V. Hydrogen sulphide.			
Year:	1892			
Paper Description:	Full length paper: ☒ Peer-reviewed ☐ Non-peer reviewed ☒	Abstract: ☐	Review article: ☐	Cited in-review article[25] ☐ Details:
Abstract:	*Not available*			
Objective:	To investigate the acute and subacute toxicity of hydrogen sulphide in different animal species at concentrations relevant to occupational health and safety considerations at the time. (Note that the original paper was published in German. An English version of the paper was obtained from NIOSH. Note also that the paper included the findings from a series of clinical investigations. The review of the clinical portion of the paper can be found in Document Review Form CL011).			
Primary focus of the study:	Lethality/fatality: ☒	Other: Clinical symptoms, necropsy findings		

Overall study design:

Exposure level(s)	Exposure frequency/duration	Species	Strain/ Breed	Age at initiation	Sex	Number of test animals	Pre-study health status
Series 1 130-3250 ppm	Single exposures lasting from 2.5 minutes to 10 hours. Eleven experiments conducted.	Cats, Rabbits, Guinea pigs	Not specified	Not specified. Three cats were referred to as "young"	Not specified	5 cats, 4 rabbits, 2 guinea pigs employed. For each experiment, n=1-2 for cats and n=1 for rabbits and guinea pigs (when employed). Three of the 5 cats and 3 of 4 rabbits used repeatedly for different experiments.	Not specified. In some cases, animals were described as "strong" or "weak".
Series 2 380-5200 ppm	Single exposures lasting from 1 ½ min to 65 minutes. Five experiments conducted.	Dogs, Cats, Rabbit	One dog referred to as a terrier. Not specified for cats or rabbit.	Dogs were referred to as "fully grown". One cat was referred to as "old".	Not specified	3 dogs, 3 cats, 1 rabbit employed. Only 1 dog and cat tested per exposure level, with the same dog and cat employed for three of the 5 exposure levels tested. Rabbit was exposed only to the highest concentration.	Not specified with the exception of the cats being referred to as "strong".

[25] Refers to a paper describing the original paper that was either unattainable or in a foreign language.

Observations:

General

Did the study follow a standardized test protocol? Yes ☐ No ☒

If yes, which test protocol did the study follow? OECD ☐ USEPA ☐

Was the study conducted under Good Laboratory Practice (GLP)? Yes ☐ No ☒

Lethality/Fatality

Were deaths observed? Yes ☒ No ☐

If so, were deaths exposure-related? (with the possible exception of rabbit from the first series of experiments that was reportedly found half-eaten by the cat) Yes ☒ No ☐

If not, provide an explanation (*e.g*., trauma, concurrent disease, improper and/or inadequate husbandry, *etc*.).

If so, were the exposure-related deaths observed within 14 days of the initial exposure? Yes ☒ No ☐

Exposure Level (ppm)	Exposure Time (min)	Number of Deaths / Number of Animals Tested	Time to Death (min)
Series 1			
Cats 130 ppm	8 hours	0/1	Not applicable
140 ppm	10 hours	0/1	Not applicable
220 ppm	8 hours	0/2	Not applicable
360 ppm	3 hours, 30 min	0/1	Not applicable
490 ppm	2 hours, 40 min	0/2	Not applicable
700 ppm	4 hours, 15 min	0/1	Not applicable
720 ppm	5 hours, 30 min	1/1	~5 hours
710 ppm	8 hours, 9 min	1/1	15 min post-exposure
760 ppm	1 hour, 49 min	0/1	Not applicable
3250 ppm	10 min	1/1	10 min
Rabbits 130 ppm	8 hours	0/1	Not applicable
140 ppm	10 hours	1/1	Animal found 1 day post-exposure half eaten by a cat.
220 ppm	8 hours	0/1	Not applicable
360 ppm	3 hours, 30 min	0/1	Not applicable
470 ppm	6 hours, 15 min	1/1	6 hours, 15 min.
490 ppm	2 hours, 40 min	0/1	Not applicable
750 ppm	4 hours, 25 min	1/1	4 hours, 25 min
710 ppm	3 hours, 50 min	1/1	5 min post-exposure
760 ppm	10 min	0/1	Not applicable
1300 ppm	3 min	0/1	Not applicable
3250 ppm	2 ½ min	0/1	Not applicable
Guinea Pigs			
470 ppm	8 hours, 50 min	1/1	Several hours post-exposure
1300 ppm	90 min	1/1	90 min

Series 2			
Dogs 380 ppm	65 min	0/1	Not applicable
560 ppm	41 min	0/1	Not applicable
1880 ppm	1 ½ min	1/1	1 ½ min
3400 ppm	2 min	0/1	Not applicable
5200 ppm	4 min	1/1	1 min
Cats 380 ppm	65 min	0/1	Not applicable
560 ppm	41 min	0/1	Not applicable
1880 ppm	1 ½ min	1/1	1 ½ min
3400 ppm	2 min	1/1	2 min post-exposure
5200 ppm	4 min	1/1	Just after removal
Rabbit – 5200 ppm	4 min	1/1	Just after removal

Were any exposure-related deaths observed more than 14 days after the initial exposure? Yes ☐ No ☒

Details:

Exposure Level (ppm)	Exposure Time (min)	Number of Deaths / Number of Animals Tested	Time to Death (min)

Were animals that died subjected to gross pathological examination (*i.e.*, necropsy)? Yes ☒ No ☐

If so, were necropsy findings consistent with exposure-related cause of death? Yes ☒ No ☐

List major necropsy findings: cat (720 ppm, 5 ½ hours): tracheal & lung hyperaemia, severe lung oedema, pleural transudat, perivascular lymph spaces of thorax very full; cat (320 ppm, 10 min): foamy fluid & small blood coagulation coming from larynx; rabbits : tracheal & lung hyperaemia, lung edema, large amount of foamy tracheal contents; guinea pigs: pulmonary oedema, dark red lungs, punctuated blood effusions in the lungs and border emphysema also found in the guinea pig exposed to the highest dose; dogs: intensive lung edema, lung heaemorrhages

Were lethal concentrations (LCs) reported? Yes ☐ No ☒

If so, describe:

Were time concentrations (TCs) reported? Yes ☐ No ☒

If so, describe:

Signs & Symptoms

Were clinical signs monitored as part of the study? Yes ☒ No ☐

Were any clinical signs consistent with life-threatening, serious and/or irreversible health outcomes reported as a part of the study (e.g., convulsions, coma, unconsciousness, laboured breathing, abnormal gait, etc.)? Yes ☒ No ☐

If so, were the clinical signs exposure-related? Yes ☒ No ☐

If not, provide an explanation:

If so, were these exposure-related clinical signs observed within 14 days of the initial exposure? Yes ☒ No ☐

Details:

Nature of Symptom	Exposure Level (ppm)	Exposure Time (min)	Number of Animals Affected	Time to Onset (min)	Duration
Semi-narcotization[1]	220 ppm (cats)	8 hours	2/2	3-8 hours	Until shortly after removal
	490 ppm (cats)	2 hours, 40 min	2/2	2 hours, 40 min	Until several hours post exposure
	720 ppm (cat)	5 hours, 20 min	1/1	4.5 hours	Until death at 5 hours, 30 min
	710 ppm (cats)	4 hours, 15 min	1/1	3 hours	Until several hours post-exposure
	710 ppm (cat)	8 hours, 9 min	1/1	5 hours, 16 min	Until death at 8 hours, 9 min
	760 ppm (cat)	1 hour, 49 min	1/1	1 hour, 40 min.	Several hours post-exposure
	490 ppm (rabbit)	2 hours, 40 min	1/1	2 hours	>30 hours post-exposure
	470 ppm (rabbit)	6 hours, 15 min	1/1	5-6 hours	Until death at 6 hours
	720 ppm (rabbit)	4 hours, 25 min	1/1	3 hours, 50 min	Until death at 4 hours, 25 min
	760 ppm (rabbit)	10 min	1/1	10 min	< ½ hour post-exposure
	1300 ppm (rabbit)	3 min	1/1	1 min	Recovered rapidly post-exposure
	1300 ppm (guinea pig)	90 min	1/1	12-17 min	Until death at 90 min
	560 ppm (dog)[2]	41 min	1/1	Immediately	Episodic until 20 min post-exposure
Dyspnea/laboured breathing					
	720 ppm (cat)	5 hours, 20 min	1/1	~3 hours	Until death at 5 hours, 20 min
	760 ppm (cat)	1 hour, 49 min	1/1	1 – 2 hours	Until several hours post-exposure
	3250 ppm (cat)	10 min	1/1	4 min	Until death at 10 min
	5200 ppm (cat)[2]	4 min	1/1	1 min	Until respiration ceased at 2 min
	130 ppm (rabbit)	8 hours	1/1	6-8 hours	Until removal from exposure
	140 ppm (rabbit)	10 hours	1/1	8 hours	Not specified (rabbit eaten by cat several hours after exposure)
	220 ppm (rabbit)	8 hours	1/1	4-5 hours	Until removal from exposure
	470 ppm (rabbit)	6.5 hours	1/1	3 hours, 45 min	Until death at 6.5 hours
	490 ppm (rabbit)	2 hours, 40 min	1/1	30 min	Not specified
	720 ppm (rabbit)	4 hours, 25 min	1/1	40 min	Until death at 4 hours, 5 min
	710 ppm (rabbit)	3 hours, 50 min	1/1	2.5 hours	Until death 5 min post-exposure
	1300 ppm (rabbit)	3 min	1/1	2 min	Until minutes post-exposure
	3250 ppm (rabbit)	1.5-2 min	1/1	Immediately	Until < 10 min post-exposure
	1300 ppm (guinea pig)	90 min	1/1	9 min	Until death at 90 min
	560 ppm (dog)[2]	41 min	1/1	Immediately	Episodic until removal from exposure
Convulsions/abnormal movements	720 ppm (cat)	5 hours, 30 min	1/1	5 hours	Until death at 5 hours, 30 min
	3250 ppm (cat)	10 min	1/1	4 min	Until death at 10 min
	3400 ppm (cat)[2]	2 min	1/1	Several seconds	2 min
	470 ppm (rabbit)	6 hours, 15 min	1/1	~6 hours	Until death at 6 hours 15 min

	490 ppm (rabbit)	2 hours, 40 min	1/1	2 hours	Until >30 hours post-exposure
	720 ppm (rabbit)	4 hours, 25 min	1/1	4 hours, 10 min	Until death at 4 hours, 25 min
	710 ppm (rabbit)	3 hours, 50 min	1/1	3 hours, 10 min	Until death 5 min post-exposure
	760 ppm (rabbit)	10 min	1/1	2 min	Until removal from exposure
	1300 ppm (rabbit)	3 min	1/1	2 min	Until removal from exposure
	1300 ppm (guinea pig)	90 min	1/1	20 min	Until 44 min exposure
	560 ppm (dog) [2]	41 min	1/1	2 min	Episodic until removal from exposure
	3400 ppm (dog) [2]	2 min	1/1	Several seconds	Until 12 min post-exposure

[1] staggering, difficulty standing, or dull reaction to provocation
[2] from series 2 experiments

Did any of these exposure-related clinical signs first appear more than 14 days after the initial exposure? Yes ☐ No ☒

Details:					Duration
Nature of Symptom					

Were any other exposure-related clinical signs observed? Yes ☒ No ☐

If yes, list other clinical signs: secretions from nose and mouth, decreased respiration, cough, sleepiness, sneezing, weak heartbeat, moaning, crying, cauterized cornea with purulent mucous secretion, restlessness, intestinal peristalsis, vomiting, nystagmus, wretching movements, decreased intelligence

Review & Assessment: Study Design, Conduct & Reporting:

A. Test Animals:	- Inadequate numbers of test animals (only 1 or 2 per species per exposure-time combination). - Test animals were not adequately described (*i.e.*, no details concerning sex, weight, age provided in most cases) - Source of test animals was not provided. - It was not reported whether a pre-test health assessment was conducted. - No indication of whether or not test animals were acclimated to the laboratory environment prior to exposure. - In many instances, the same test animals were repeatedly exposed in different exposure-time concentrations. The influence of prior acute exposure to H_2S on responses to subsequent exposure complicates the interpretation of results.

B. Exposure conditions:	+ Durations of exposure were clearly defined + A whole body exposure chamber was used and animals were "usually" placed in the chamber after an equilibration period. + Exposure chamber design allowed for clear observation of test animals. +/-An attempt was made to analytically confirm the maintenance of H_2S concentrations in the chamber but the accuracy of this determination is questionable. - No evidence that temperature, pressure, humidity or oxygen content within the exposure chamber were monitored. - Source and purity of H_2S were not provided. - Some indication that more than one species was placed into the exposure chamber for certain experiments (i.e., rabbits may have been placed into the chamber along with cats).
C. Housing/Feeding	- Details concerning animal housing (*i.e.*, temperature and humidity of animal room, and photoperiod) were lacking. - Information pertaining to animal caging (*i.e.*, type and dimensions) was not provided. - Bedding material was not specified. - The type and source of feed was not reported. The feeding schedule was also omitted. - Water supply was not indicated.
D. Exposure equipment:	+/- Description of the exposure chamber was limited for Series 1 experiments (described only as a "glass box"). For Series 2 experiments in dogs and cats, it was described as 635-liter sealed zinc-plate box with large windows +/- A small so-called "Pettenkofer-Voit's apparatus" was employed to generate the H_2S gas. - For Series 1 experiments, Will-varretrapps' bulbs filled with copper sulphate and a mercury air pump were employed to obtain H_2S air samples during exposure and then the iodide method used to determine the gas concentration. For Series 2 experiments, H_2S concentrations were measured directly via the iodine method (2 aspirators sucked out simultaneous samples through a solution of iodine in aqueous potassion iodide). Comparative studies of these methods of H_2S determination by Lehman (1892) indicated they were in accord; however, both methods were judged to provide limited sensitivity compared to modern analysis.
E. Procedural:	- No evidence that a control group was employed - No random assignment of test animals to groups - In many instances, the same test animals were repeatedly exposed in different exposure-time concentrations. The influence of prior acute exposure to H_2S on responses to subsequent exposure complicates the interpretation of results. - Survivors were observed for several hours following exposure for additional deaths and clinical signs. OECD test guideline recommends a post-exposure observation period of 14 days or longer +/- Following death, necropsies were conducted on test animals. +/- Study pre-dated Good Laboratory Practice (GLP) guidelines
F. Data collection:	+ All symptoms were noted, as well as the time of occurrence. + Time to recovery was noted. + Individual data were provided for each test animal where more than one animal was employed +/- In most instances, necropsy data were provided for animals which died on test.
G. Data analysis:	- Data were not statistically analyzed
H. Interpretations:	+ Multiple species evaluated - Use of only 1-2 animals per exposure time concentration and use of animals previously exposed to H_2S severely limits interpretation of results

Review & Assessment - Summary:

Discussion of findings: This report describes in detail the symptoms of H_2S poisoning in different species over a wide range of concentrations (130 ppm-5200 ppm) and exposure times (1 ½ min to 10 hours). The exposure concentration and duration of exposure appeared to have a significant influence on the type and severity of symptoms observed, with most symptoms progressing with continued exposure or higher concentrations.

Death of cats was observed following 5 to 8 hours exposure to 720-760 ppm H_2S or 1 ½ - 10 minute exposure to 1880-3250 ppm H_2S. Death of rabbits was observed following 3 to 6 hour exposure to 470-710 ppm or 4 minute exposure to 5200 ppm. Death of guinea pigs was observed following almost 9 hour exposure to 470 ppm and following 90 minute exposure to 1300 ppm. Finally, death of dogs was observed following 1- 1 ½ minute exposure to 1880 or 5200 ppm H_2S.

Comparison of symptoms in animals who had not been previously exposed to H_2S versus those repeatedly exposed indicated that the fresh animals were more resistant to the effects of H_2S. In general, the recovery of animals, even from very high doses, occurred more quickly than expected based on descriptions in the literature of the time on the slow convalescence of afflicted sewer workers.

Interpretation of the toxicological significance and clinical relevance of the study findings should take into consideration that the study is dated and was performed long before the development of testing guidelines and the introduction of Good Laboratory Practice (GLP) requirements. The study also relied on equipment and analytical methodology that has been replaced by more advanced technology. The level of confidence that can be assigned to the study findings is undermined by the use of relatively "crude" instrumentation, and the associated uncertainty surrounding the actual exposure concentrations that were tested. There were also a number of notable weaknesses in the experimental design (see below).

Interesting remarks made by the study investigator included:

In reviewing the existing literature on the acute toxicity of H_2S: *"I restrict myself in the discussion on the literature to collecting the scattered, often contradictory quantitative data in the literature on the toxicity of inhaled hydrogen sulphide"*.

In maintaining uniform test concentrations of H^2S in exposure chambers: *"…there is absolutely no guarantee of a proper mixing nor for the absence of air in the gas flow. These conditions most probably cause irregularities in the results from individual experiments …"*.

In commenting on earlier remarks made by Eulenberg: *"In any case a hydrogen sulphide content in the atmosphere of 0.6% (i.e., 6,000 ppm)does not cause such severe symptoms in human beings, it can at the most contribute to the aggravation. How Eulenberg justifies this sentence, in which a human being is declared to be ten times more resistant than an experimental animal, is incomprehensible to me"*.

In assessing the adequacy and reliability of one the analytical methods that was used during the course of the experiments to measure the concentration of H_2S in the exposure chamber: *"I would no longer choose this method. These [experiments] showed that the method was not completely faultless, that the airstream, in faxct, lost its hydrogen sulphide in the iodine solution"*.

In commenting on the necropsy of a rabbit used in one of the experiments: *"The rabbit was obviously killed the following day by the cat and was found half-eaten, such that no dissection was carried out"*.

Review & Assessment - Scoring[26] and Rational:

No practical use	☐
Low	☒
Low – Moderate	☐
Moderate	☐
Moderate – High	☐
High	☐

Rationale: This study is of limited usefulness only for development of an emergency planning endpoints. Although several animal species were included and several exposure concentration/exposure time combinations were examined, serious weaknesses in experimental design, conduct and/or reporting were judged to undermine the level of confidence that could be assigned to the study findings and conclusions. Increased confidence in the study findings could have been obtained through the use of larger numbers of test animals and the inclusion of control groups. The use of the same test animals for different exposure/time combinations also severely limits interpretation of the results.

Strengths:

- Use of gradient of exposure concentrations and exposure times to permit assessment of comparative influence of each parameter on lethality and other health endpoints.
- Use of multiple animal species (guinea pig, rabbit, cat, dog).
- Detailed observations of clinical signs and symptoms.
- Regular attempts to measure H_2S concentrations in the chamber during exposure (albeit methods were suspect in terms of reliability).
- Necropsy findings reported and summarized for animals which died on test.

Weaknesses

- Use of limited number of test animals (*i.e.*, only 1-2 test animals for each exposure concentration/exposure time combination).
- Repeated use of the same test animals in different experiments (i.e., animals which survived exposures were often subsequently exposed to a different exposure concentration/exposure time combination).
- Inadequate description of test animals (*e.g.*, source, age , sex, strain, pre-study health status).
- Failure to include control animals
- Limited description of gas delivery system and exposure chamber.
- Uncertainty with respect to actual exposure concentrations used (i.e., study investigator admitted lack of confidence in several of the analytical methods employed).
- Complete lack of detail concerning animal housing and husbandry
- Lack of details concerning randomization and assignment of test animals to groups
- Failure to observe surviving animals for 14 days post-exposure

[26] Score reflects usefulness of study for development of emergency planning criteria vis-à-vis acute lethality.

Reviewers:

DD	☒
RT	☒
CM	☐

Document Review - Non-Clinical Studies

Author:	MacEwen, J.D., Vernot, E.H.	**Study Code:**	NC072
Title:	Toxic Hazards Research Unit Annual Technical Report: 1972		
Year:	1972		

Paper Description:	Full length paper: ☒ Peer-reviewed ☐ Non-peer reviewed ☒	Abstract: ☐	Review article: ☐	Cited in-review article[27] ☐ Details:
Abstract:				
Objective:	To review the activities of the U.S. Air Force Toxic Hazards Research Unit for the period of June 1971 through May 1972. Acute inhalation toxicity experiments were conducted on hydrogen sulphide as well as a number of other compounds. H_2S tests were conducted to "*clarify ambiguities in literature sources and to precisely define one-hour LC50 values for rats and mice*". Only the results pertaining to H_2S are described in this Document Review Form.			
Primary focus of the study:	Lethality/fatality: ☒	Other:		

Overall study design:

Exposure level(s)	Exposure frequency/duration	Species	Strain/ Breed	Age at initiation	Sex	Number of test animals	Pre-study health status
400,504, 635, 800 ppm	Single exposure/ 1-hour	Rats Mice	Sprague-Dawley ICR	Not stated	Male	10 per exposure level	Quality Control examinations indicated all test animals were in good health.

Observations:

General

Did the study follow a standardized test protocol? Yes ☐ No ☒

If yes, which test protocol did the study follow? OECD ☐ USEPA ☐ Other:

Was the study conducted under Good Laboratory Practice (GLP)? Yes ☐ No ☒

Lethality/Fatality

Were deaths observed? Yes ☒ No ☐

If so, were deaths exposure-related? Yes ☒ No ☐

If not, provide an explanation (*e.g.*, trauma, concurrent disease, improper and/or inadequate husbandry, *etc.*).

If so, were the exposure-related deaths observed within 14 days of the initial exposure? Yes ☒ No ☐

[27] Refers to a paper describing the original paper that was either unattainable or in a foreign language.

Details:

Exposure Level (ppm)	Exposure Time (min)	Number of Deaths / Number of Animals Tested	Time to Death (min)
Rats			
400 ppm	1 hour	0/10	Not stated
504 ppm	"	0/10	"
635 ppm	"	1/10	"
800 ppm	"	9/10	"
Mice			
400 ppm	1 hour	2/10	Not stated
504 ppm	"	0/10	"
635 ppm	"	5/10	"
800 ppm	"	8/10	"

Were any exposure-related deaths observed more than 14 days after the initial exposure? Yes ☐ No ☒

Details:

Exposure Level (ppm)	Exposure Time (min)	Number of Deaths / Number of Animals Tested	Time to Death (min)

Were animals that died subjected to gross pathological examination (*i.e.*, necropsy)? (Apparently, only surviving animals were subject to necropsy at the end of the 14-day observation period) Yes ☐ No ☒

If so, were necropsy findings consistent with exposure-related cause of death? (some of them) Yes ☐ No ☐

List major necropsy findings: One surviving mouse each from the 800 ppm and 635 ppm groups had a blocked urethral opening due to encrustation of the external orifice, and consequently their bladders were distended. Surviving rats showed congestion and mottling of kidney and liver, with moderate to severe fatty changes in the liver.

Were lethal concentrations (LCs) reported? Yes ☒ No ☐

If so, describe: LC50 (rats): 712 ppm (95% confidence limits: 662 – 765 ppm); LC50 (mice): 634 ppm (95% confidence limits: 576 – 698 ppm)

Were time concentrations (TCs) reported? Yes ☐ No ☒

If so, describe:

Signs & Symptoms

Were clinical signs monitored as part of the study? Yes ☒ No ☐

Were any clinical signs consistent with life-threatening, serious and/or irreversible health outcomes reported as a part of the study (*e.g.*, convulsions, coma, unconsciousness, laboured breathing, abnormal gait, *etc.*)? Yes ☒ No ☐

If so, were the clinical signs exposure-related? Yes ☒ No ☐

If not, provide an explanation:

If so, were these exposure-related clinical signs observed within 14 days of the initial exposure? Yes ☒ No ☐

Details:

Nature of Symptom	Exposure Level (ppm)	Exposure Time (min)	Number of Animals Affected	Time to Onset (min)	Duration
Rats					
Gasping	Not stated	< 1 hour	Not stated	Not stated	Not stated
Mice					
Gasping	Not stated	< 1 hour	Not stated	Not stated	Not stated
Convulsions	"	"	"	"	"

Did any of these exposure-related clinical signs first appear more than 14 days after the initial exposure? Yes ☐ No ☒

Details:

Nature of Symptom	Exposure Level (ppm)	Exposure Time (min)	Number of Animals Affected	Time to Onset (min)	Duration

Were any other exposure-related clinical signs observed? Yes ☐ No ☒

If yes, list other clinical signs:

Review & Assessment: Study Design, Conduct & Reporting:

A. Test Animals:	+/- Details concerning the species, sex and weight of test animals were supplied. Source and age of animals were not provided. + The number of test animals (10 per exposure level) was in accordance with OECD guidelines - Only male rats were employed + Pre-test health status was determined to ensure all test animals were in good health
B. Exposure conditions:	+/- Multiple doses of H_2S (400, 504,635, 800 ppm) were tested for a single duration of 1 hour + The chamber exposure concentrations were monitored continuously and were reported to be unchanged from nominal levels + The exposure chamber was maintained at negative pressure in compliance with guideline recommendations + Rapid transfer of animals in and out of each H_2S concentration increased the accuracy of exposure durations +/- It was not stated whether exposure chambers were equilibrated before or after test animals were placed inside. This could

	potentially alter the actual duration of exposure to the stated levels of H_2S ... however, because of the chamber size (30 L) and the air flow rate (30 L/min), any lack of equilibration would have been of little consequence.
C. Housing/Feeding	- Details concerning animal husbandry (*e.g.*, room temperature and humidity, type of caging and bedding, source of feed and water, etc.) were not provided. (Details evidently are available in earlier annual reports issued by the Toxic Hazards Research Unit).
D. Exposure equipment:	+/- Basic details concerning the exposure chamber and gas delivery system were provided (*i.e.*, type, dimensions, air flow rate). The exposure chamber was reported to be a 30-liter glass bell jar, with an airflow rate of 30 liters per minute. +/- High purity H2S gas was reportedly sourced from a commercial supplier (… not further details supplied). + The actual gas concentrations were determined and recorded using an ion specific sulfide electrode technique.
E. Procedural:	+/- Preconditioning chambers were used to prepare and stabilize animals in a controlled environment, but the length of acclimation period was not noted. - No indication that animals were randomly assigned to exposure groups. - No indication that a control group was employed. + Surviving animals were held for 14 days for observation prior to sacrifice.
F. Data collection:	+/- Raw data for individual animals were provided with respect to mortality +/- Clinical signs and body weights were recorded and reported, but not on an individual animal basis. - Approximate time of death in animals dying on test was not provided - Only surviving rats appear to have been necropsied and subjected to examination.
G. Data analysis:	+ Confidence intervals were reported for LC50 values - Statistical methods employed were not outlined
H. Interpretations:	- No discussion of findings in light of a review of relevant literature and other published LC50s. + Two species of test animals employed.

Review & Assessment - Summary:

Discussion of findings: A 1-hour LC50 in the rat of 712 ppm was determined with a 95% confidence interval of 662 to 765 ppm. In mice, a 1-hour LC50 of 634 ppm was determined with a 95% confidence interval of 576 to 698 ppm. Toxic signs observed included gasping in both species and convulsions in the mice only. Necropsy evaluations appear to have been conducted only on surviving animals after a 14-day post-exposure observation period. Surviving rats were noted to show congestion and mottling of the kidney and liver with moderate to severe fatty changes in the liver.

Review & Assessment - Scoring[28] and Rational:

No practical use	☐
Low	☐
Low – Moderate	☐
Moderate	☒
Moderate – High	☐
High	☐

[28] Score reflects usefulness of study for development of emergency planning criteria vis-à-vis acute lethality.

Rational: The study is useful for the development of emergency planning endpoints (based on use of lethality as the endpoint of interest) in that it is an acute exposure study that identified a 1-hour LC_{50} in both the rat and the mouse. The study design, conduct and reporting were judged to be adequate. Added confidence could have been achieved by the use of both sexes, necropsy evaluations on animals which died during test exposures, and use of a control group.

Strengths:

- Use of graded exposure concentrations (400, 504, 635 and 800 ppm)
- Use of adequate numbers of animals (10 per exposure concentration).
- Use of two test species.
- Animals monitored for recommended 14-day post-exposure observation period.
- Direct monitoring of H_2S during exposure to confirm nominal concentrations.
- Monitoring of clinical signs during and after exposure, including weight loss, albeit reporting was limited (see below).
- Adequate description of exposure chamber and gas delivery system.

Weaknesses:

- Use of only male rats.
- Use of a single exposure time only.
- Use of one-hour exposure time vs. 4 hour recommended by OECD.
- No control group employed, and no indication that test animals were randomly assigned to exposure groups.
- Lack of details concerning animal husbandry.
- Lack of information concerning whether or not the exposure chamber was equilibrated prior to the introduction of the test animals (… although probably of little, if any, consequence given the chamber size and air flow rate).
- Failure to include different exposure concentration-exposure time combinations (… although the use of such combinations is not specified in the testing guidelines, such combinations can permit better understanding of acute lethality of gases vis-à-vis Haber's Law).
- Limited reporting of clinical signs (e.g., number of animals exhibiting signs was not indicated, nor were signs segregated by exposure concentration).
- No reporting of necropsy findings in animals that died on test.
- Overall reporting of experimental details was somewhat limited (… although the entire annual report was lengthy, only 2 to 3 pages were devoted to the discussion of H_2S).

Reviewers:

DD	☒
RT	☒
CM	☐

Document Review - Non-Clinical Studies

Author:	Lund, O.E. and Wieland, H.	**Study Code:**	NC073
Title:	Pathologisch-anatomische befund bei experimenteller schwefelwasserstoff-vergiftung (Pathologic-anatomic findings in experimental hydrogen sulphide poisoning: a study with Rhesus monkeys).		
Year:	1966		

Paper Description:	Full length paper: ☒ Peer-reviewed ☒ Non-peer reviewed ☐	Abstract: ☐	Review article: ☐	Cited in-review article[29] ☐ Details:
Abstract:	*Not available*			
Objective:	To determine the nature, extent and site of pathological alterations in tissues from Rhesus monkeys acutely exposed to H_2S. (The brain, heart, liver, kidneys and adrenals were examined).			
Primary focus of the study:	Lethality/fatality: ☐	Other: Pathologic alterations in selected tissues.		

Overall study design:

Exposure level(s)	Exposure frequency/duration	Species	Strain/ Breed	Age at initiation	Sex	Number of test animals	Pre-study health status
500 ppm	Monkey A - Single exposure lasting 35 minutes. Monkey B – Two exposures separated by a 3-day interval for 25 minutes and 17 minutes, respectively. Monkey C – Single exposure lasting 22 minutes.	Monkey	Rhesus	Not stated	Not stated	3	Not indicated

Observations:

General

Did the study follow a standardized test protocol? Yes ☐ No ☒

If yes, which test protocol did the study follow? OECD ☐ USEPA ☐ Other:

Was the study conducted under Good Laboratory Practice (GLP)? Yes ☐ No ☒

Lethality/Fatality

Were deaths observed? Yes ☒ No ☐

If so, were deaths exposure-related? Yes ☒ No ☐

If not, provide an explanation (e.g., trauma, concurrent disease, improper and/or inadequate husbandry, etc.).

If so, were the exposure-related deaths observed within 14 days of the initial exposure? Yes ☒ No ☐

[29] Refers to a paper describing the original paper that was either unattainable or in a foreign language.

Details:

Exposure Level (ppm)	Exposure Time (min)	Number of Deaths / Number of Animals Tested	Time to Death (min)
Monkey A - 500 ppm	35 minutes	1/1	35 minutes
Monkey B -500 ppm	Up to 25 minutes	0/1	Not applicable
Monkeyt C – 500 ppm	22 minutes	0/1	Not applicable

Were any exposure-related deaths observed more than 14 days after the initial exposure? Yes ☐ No ☒

Details:

Exposure Level (ppm)	Exposure Time (min)	Number of Deaths / Number of Animals Tested	Time to Death (min)

Were animals that died subjected to gross pathological examination (*i.e*., necropsy)? Yes ☒ No ☐

If so, were necropsy findings consistent with exposure-related cause of death? Yes ☐ No ☒

List major necropsy findings: Monkey which died on test (Monkey A) showed no morphologically detectable changes in the brain, heart, kidney and adrenals. Moderate hyperemia of the liver vessels was noted. Monkeys which survived exposure, but were sacrificed post-treatment showed necroses of the cerebral cortex and basal ganglia of the brain as well as a reduction in the number of Purkinje cells in the cerebellum.

Were lethal concentrations (LCs) reported? Yes ☐ No ☒

If so, describe:

Were time concentrations (TCs) reported? Yes ☐ No ☒

If so, describe:

Signs & Symptoms

Were clinical signs monitored as part of the study? Yes ☒ No ☐

Were any clinical signs consistent with life-threatening, serious and/or irreversible health outcomes reported as a part of the study (*e.g*., convulsions, coma, unconsciousness, laboured breathing, abnormal gait, *etc*.)? Yes ☒ No ☐

If so, were the clinical signs exposure-related? Yes ☒ No ☐

If not, provide an explanation:

If so, were these exposure-related clinical signs observed within 14 days of the initial exposure? Yes ☒ No ☐

Details:

Nature of Symptom	Exposure Level (ppm)	Exposure Time (min)	Number of Animals Affected	Time to Onset (min)	Duration
Gasping for air	500 ppm	Up to 35 minutes	3/3	Within approx. 13 minutes	Until loss of consciousness

Sudden collapse and loss of consciousness	500 ppm	Up to 35 minutes	3/3	Within 15 to 17 minutes	Until death or termination of exposure. (Monkey C reportedly regained consciousness 140 minutes following the termination of exposure. The recovery time for Monkey B was not stated).

Did any of these exposure-related clinical signs first appear more than 14 days after the initial exposure? Yes ☐ No ☒

Details:

Nature of Symptom	Exposure Level (ppm)	Exposure Time (min)	Number of Animals Affected	Time to Onset (min)	Duration

Were any other exposure-related clinical signs observed? Yes ☐ No ☐

If yes, list other clinical signs: Rubbing of the eyes, yawning, deep respiration preceded loss of consciousness. Somnolescence, uncoordianted movements and loss of appetite reported among one of the surviving monkeys post-exposure (Monkey C). No details provided for the remaining surviving monkey (Monkey B).

Review & Assessment: Study Design, Conduct & Reporting:

A. Test Animals:	+/- Testing was confined to 3 monkeys. - Details concerning age, sex, weight, pre-study health status of test animals were not provided. - No indication that monkeys were acclimated to the laboratory prior to testing. - No details concerning source or supplier of monkeys. - No control group of monkeys employed.
B. Exposure conditions:	+/- Exposure to single concentration of H_2S (500 ppm) either once or twice for a period up to 35 minutes. - Details concerning equilibration of exposure chamber unclear.
C. Housing/Feeding	- No details concerning animal husbandry given (*i.e*., feed supply, water supply, bedding, caging, animal room temperature and humidity, photoperiod, *etc*.).
D. Exposure equipment:	- Stated only to be "a closed respiration system". No details given concerning the gas delivery system or exposure chamber. - Purity and/or source of H_2S not provided. - No indication that exposure concentration in the "closed respiratory system" was analytically confirmed. - No details provided concerning air flow, temperature, pressure, *etc*. within the exposure chamber.
E. Procedural:	+/- Two monkeys were exposed to H_2S on a single occasion for a period up to 35 minutes. The remaining monkey was exposed twice, with a 3-day interval between exposures, for a period up to 25 minutes. + Clinical signs were monitored during and after exposure. +/- Surviving monkeys were monitored for up to 10 days post-exposure. +/- All monkeys were subjected to pathological examination, with evidence of morphologic alterations examined in the brain, heart, liver, kidneys and adrenals. The lungs were not examined.

F. Data collection:	+ Individual animal data provided for clinical signs and pathological findings. + Time to appearance of clinical signs recorded.
G. Data analysis:	- No statistical analysis of the data was performed (… presumably owing to restricted number of animals used).
H. Interpretations:	+/- Authors concluded that acute exposure to "high concentrations" of H_2S is capable of causing pathologic-anatomic damage to the cerebral cortex and basal ganglia in Rhesus monkeys. Nature of pathologic damage was similar to that caused by anoxia. Damage does not appear immediately, rather the morphologic features require time to develop post-exposure.

Review & Assessment - Summary:

Discussion of findings: The time course of clinical signs as well as the nature, extent and site of morphologic alterations were followed among Rhesus monkeys exposed to 500 ppm of H_2S on one or two occasions for up to 35 minutes. One of the three monkeys tested died on test … the remaining two monkeys survived. All monkeys lost consciousness within 15 minutes of exposure. Pathologic examination of selected tissues (*i.e.*, brain, heart, liver, kidneys and adrenals) revealed necrotic lesions in the cerebral cortex and basal ganglia of the brain as well as reduced numbers of Purkinje cells in the cerebellum among the two surviving monkeys that were sacrificed post-exposure. The changes resembled those caused by anoxia. No such changes were observed in the tissues from the single monkey which died on test since, according to the authors, sufficient time was not allowed for the morphologic features to develop.

Review & Assessment - Scoring[30] and Rational:

No practical use	☐
Low	☒
Low – Moderate	☐
Moderate	☐
Moderate – High	☐
High	☐

Rational:

The study was judged to be of limited usefulness in advancing understanding of the concentration-time-response characteristics of H_2S vis-à-vis lethality owing to a number of weaknesses in experimental design, conduct and reporting.

Strengths:

- Use of a higher order test species (*i.e.*, monkey), bearing a comparatively close resemblance to man.
- Use of different acute exposure regimens (*i.e.*, 500 ppm exposure delivered one or twice for periods ranging from 17 to 35 minutes).
- Study design included monitoring and recording of clinical signs both during and following exposure.
- Study design included detailed pathological examination of selected tissues, including the brain and heart.

[30] Score reflects usefulness of study for development of emergency planning criteria vis-à-vis acute lethality.

Weaknesses:

- Number of test animals (n=3) was somewhat limited.
- Use of single exposure concentration.
- Complete lack of detail concerning test animals and animal husbandry (*i.e*., information respecting source, age, sex, body weight, pre-study health status, caging, feed supply, etc. was lacking).
- Lack of detail concerning source and purity of H_2S, as well as lack of information respecting the gas delivery system and exposure chamber.
- No indication that exposure concentration (*i.e*., 500 ppm nominal) was analytically confirmed.
- Post-exposure monitoring period was somewhat limited (*i.e.* confined to 5-10 days for surviving monkeys).
- Pathological assessment did not include examination of the lungs (*i.e*., one of the primary target tissues).

Reviewers:

DD	☒
RT	☒
CM	☐

Clinical Review Forms

Document Review – Clinical Studies

Author:	Mitchell, C.W. and Yant, W.P. 1925	**Study Code:**	CL010 (see also NC032 and CR066)
Title:	Correlation of the data obtained from refinery accidents with a laboratory study of H_2S and its treatment.		
Year:	1925		

Paper Description:	Full length study: ☒ Peer-reviewed ☒ Non-peer reviewed ☐	Abstract: ☐	Review article: ☐	Cited in-review article[31]: ☐ Details:
Abstract:	*"In the laboratory study, the symptoms of hydrogen sulphide (H_2S) poisoning in animals and men were found to be almost identical with those caused by gases in the refineries. The need for a definite method of treating H_2S poisoning was evident. The medical findings, the study on toxicity of H_2S, and the treatment for H_2S poisoning will be discussed in turn."*			
Objective:	To investigate the toxicity of hydrogen sulphide in various laboratory animal species as a possible means to further understanding of the onset, progress and treatment of H_2S poisoning among refinery workers. A preliminary study involving exposure of human subjects to H_2S under controlled conditions was also performed and the results of this preliminary are described herein. (Note that the review of the non-clinical studies described in the paper can be found in Document Review Form NC032. A description of the case reports outlined in the paper can be found in Document Review Form CR066).			
Primary focus of the study:	Lethality/fatality: ☐	Other: Clinical symptoms associated with H_2S exposure		

Overall study design:

Exposure level(s)	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health status
100-150 ppm, 150-200 ppm, or 250-350 ppm 350-450 ppm	Single exposure lasting 4 hours Single exposure lasting 1 hour	Male	Not stated	Not stated	Stated to be "healthy"

Observations:

	Yes	No
General		
Did the study follow a standardized clinical protocol?	☐	☒
If yes, which protocol did the study follow?		
Was the study conducted in accordance with Good Clinical Practices (GCP)?	☐	☒
Lethality/Fatality		
Were deaths observed	☐	☒
If so, were deaths exposure related?	☐	☐
If no, provide an explanation		
Were exposure related deaths observed within 14 days of the initial exposure?	☐	☐

[31] Refers to a paper describing the original paper that was either unattainable or in a foreign language.

Details:			
Exposure Level (ppm)	Exposure Time (min)	Number of Deaths Number of Subjects Tested	Time to Death (min)

Were exposure-related deaths observed more than 14 days of the initial exposure? ☐ ☒

Details:			
Exposure Level (ppm)	Exposure Time (min)	Number of Deaths Number of Subjects Tested	Time to Death (min)

Signs & Symptoms

Were clinical symptoms monitored as part of the study? ☒ ☐

Were any symptoms consisted with life threatening, serious and/or irreversible health outcomes reported as port of the study (*e.g.*, convulsions, coma, unconsciousness, laboured breathing, *etc*.)? ☒ ☐

If so, were signs or symptoms exposure related? ☒ ☐

If not, provide an explanation

Were exposure related signs or symptoms observed within 14 days of the initial exposure? ☒ ☐

Details:					
Nature of Symptom	Exposure Level (ppm)	Exposure Time (min)	Number of Subjects Affected	Time to Onset (min)	Duration (min)
Difficult and/or disturbed breathing	150-350 ppm	1-4 hours	Not stated	Not stated	Not stated
”	350-450 ppm	15-30 min	Not stated	Not stated	Not stated

Did any exposure-related signs or symptoms first appear more than 14 days of the initial exposure? ☐ ☒

Details:					
Nature of Symptom	Exposure Level (ppm)	Exposure Time (min)	Number of People Affected	Time to Onset (min)	Duration (min)

If yes, list other signs and symptoms: coughing, eye and respiratory irritation, pain in eyes and head, sleepiness, loss of sense of smell, light shy, nasal catarrh, salivation and mucous secretion

Review & Assessment: Study Design, Conduct & Reporting:

A. Subjects:	- Principle characteristics of subjects were not defined beyond that they were male and healthy (*e.g.*, age, occupation, prior H_2S exposure, health status) - Number of test subjects was not provided. - It is unknown whether subjects provided informed consent - Unclear whether a control group of unexposed subjects was employed.
B. Exposure conditions:	- The H_2S was produced in situ by combining FeS and HCl (*i.e.*, the H_2S was not sourced from a commercial supplier). The purity of the gas was not indicated. - The precise exposure concentration(s) was not stated. Only a series of ranges of concentrations were reported. +/- The duration of exposure was defined; however, only time intervals were listed for the reporting of symptoms. + Subjects were reportedly placed into the exposure chamber after the concentration of the gas has been allowed to equilibrate + The distribution of the gas within the chamber was maintained through the use of a fan to ensure homogeneous mixing. +/- At intervals during the exposure period, the H_2S concentration in the chamber was determined by the so-called "cadmium chloride" method. Details concerning the exact sampling and analytical methodology were lacking. +/- Exposures were stated to be "continuous"
C. Exposure equipment:	- Exposure was completed in a 1000-cubic foot gas chamber. +/- Description of the exposure chamber was limited. Further description was reported to be provided in a companion report (US Public Health Reports, vol. 37(19), May 12, 1922 pp.1127-1142). +/- A so-called "Kipp generator" was employed to generate the H_2S gas by combining FeS and HCl. - The cadmium chloride method was used to measure the gas concentration within the chamber. The method was judged to provide limited sensitivity.
D. Procedural:	+/- Study pre-dated Good Clinical Practice (GLP) guidelines - Unclear whether a control group was employed and whether subjects were randomly assigned to test groups - No indication that subjects were held for a post-exposure observation period.
E. Data collection:	+/- All symptoms were noted, as well as the time of occurrence. However, only a time range was provided. - Individual data were not provided for each test subject, thereby limiting the independent assessment of the findings. - All observational data was generalized in tables. - Reversibility of symptoms was not discussed specifically with respect to the human test subjects
F. Data analysis:	- Data were not statistically analyzed.

G. Interpretations:	- The authors believed that based on the results of human exposure trials up to 350 ppm for 4 hours or 450 ppm for 1 hour and data from canine studies, it is possible to predict the reaction of men to higher concentrations. - The human studies were described by the authors as being "preliminary" in nature only.

Review & Assessment - Summary:

Discussion of findings:	Observed clinical symptoms in male subjects exposed to H_2S concentrations of 100-450 ppm for 1 to 4 hours. Symptoms included eye and respiratory irritation, breathing disturbances, sleepiness, loss of sense of smell and pain in the eyes and head. Symptoms appeared within minutes of exposure and progressed in severity with time. The duration and reversibility of symptoms was not noted. There was no indication that the subjects were observed post-exposure for recovery. No deaths occurred.

Review & Assessment - Scoring[32] and Rational:

No practical use	☐
Low	☒
Low to Moderate	☐
Moderate	☐
Moderate to High	☐
High	☐
Rationale:	This study is of limited usefulness for the development of emergency response planning guidelines. Weaknesses in experimental design, conduct and/or reporting were judged to undermine the level of confidence that could be assigned to the study findings and conclusions The onset of symptoms at various durations of exposure was described but significant detail regarding the test subjects was lacking; for example, the number of subjects, their age and any prior occupational exposure to H_2S. The duration or reversibility of symptoms was not described and subjects were not apparently observed post-exposure. It was unclear whether a control group was employed. Interpretation of the toxicological significance and clinical relevance of the study findings should also take into consideration that the study is dated and was performed long before the development of testing guidelines and the introduction of Good Clinical Practice (GLP) guidelines. It relied on equipment and analytical methodology that has been replaced by more advanced technology. The level of confidence that can be assigned to the study findings is undermined by: 1) the lack of detail provided on test subjects and 2) the use of relatively "crude" instrumentation, and the associated uncertainty surrounding the actual exposure concentrations that were tested.
Strengths:	• Use of human subjects (… thereby avoiding uncertainties associated with extrapolating findings from test animals to humans). • Use of gradient of exposure concentrations (100 to 450 ppm). • Regular monitoring and recording of clinical symptoms during exposure.

[32] Score reflects usefulness of study for development of emergency planning criteria vis-à-vis acute lethality.

Weaknesses	• Study was "preliminary" in nature only (… by authors' admission). • Lack of detail concerning test subjects (*i.e.*, age, weight, health status, occupation, smoking history, *etc*.) • Use of male subjects only. • Lack of detail concerning number of test subjects used. • Limited description only of gas delivery system and exposure chamber. • Inadequate detail concerning analytical confirmation of exposure concentrations. • Lack of detail concerning exact exposure concentrations and times examined (… concentrations and times were reported as ranges only). • No records with respect to post-exposure observations. • Lack of control group of subjects. • Clinical symptoms recorded evidently included data from earlier clinical investigation performed by Lehmann (1892 – CL011), but no distinction was made as to which symptoms corresponded to which study.

Reviewers:

DD	☒
RT	☒
CM	☐

Document Review - Clinical Studies

Author:	Lehmann, K.B.	**Study Code:**	CL011 (see also NC070)
Title:	Experimental Studies on the effects of technically and hygienically important gases and vapours on organisms. Part V. Hydrogen sulphide.		
Year:	1892		
Paper Description:	Full length study: ☒ Peer-reviewed ☐ Non-peer reviewed ☒	Abstract: ☐	Review article: ☐	Cited in-review article[33]: ☐ Details:
Abstract:	*Not available*		
Objective:	To extend investigations into the acute and subacute toxicity of hydrogen sulphide from animals to humans using similar exposure methodology. (Note that much of the paper was devoted to non-clinical investigations of the acute inhalation toxicity of H_2S. The results of these investigations are summarized in Document Review Form NC070).		
Primary focus of the study:	Lethality/fatality: ☐	Other: Clinical symptoms following acute exposures to H_2S	

Overall study design:

Exposure level(s)	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health status
Series 1 100-575 ppm	Single exposures (same subject) to various concentrations of H_2S for durations ranging from 40 min to almost 4 hours. (Total of nine separate experiments conducted).	Male	Stated to be young	1[1]	Described as well nourished, completely healthy, with a tendency to corpulence.
Series 2 20-280 ppm	Single exposures (same subjects) to various concentrations of H_2S for durations ranging from 30 min to 1 hour. (Total of five separate experiments conducted).	Male	Not specified. One subject was a student so was presumably young.	3	Not specified other than that one subject (the author) had "easily irritated membranes"
Series 3 100-532 ppm	Single exposures (same subject) to various concentrations of H_2S for durations from 30 min to 3 hours. Five separate experiments conducted with several days in between. Last experiment involved two 3-hour exposure periods with a 3 h, 45 min rest in between.	Male	Stated to be young	1	Described as fully fit and in perfect health.

[1] Various other persons often took part in the experiments, but results were not reported for these individuals other than that on the whole they reacted with greater sensitivity

[33] Refers to a paper describing the original paper that was either unattainable or in a foreign language.

Observations:

General	Yes	No
Did the study follow a standardized test protocol?	☐	☒
Was the study conducted under Good Clinical Practice (GCP) guidelines?	☐	☒
Lethality/Fatality		
Were deaths observed	☐	☒
If so, were deaths exposure related?	☐	☒
If no, provide an explanation (e.g., trauma, concurrent disease)		
Were exposure related deaths observed within 14 days of the initial exposure?	☐	☒

Details:

Exposure Level (ppm)	Exposure Time (min)	Number of Deaths / Number of Subjects Tested	Time to Death (min)

Were exposure-related deaths observed more than 14 days of the initial exposure?	☐	☒	

Details:

Exposure Level (ppm)	Exposure Time (min)	Number of Deaths / Number of Subjects Tested	Time to Death (min)

Signs & Symptoms		
Were clinical symptoms monitored as part of the study?	☒	☐
Were any symptoms consisted with life threatening, serious and/or irreversible health outcomes reported as port of the study (*e.g.*, convulsions, coma, unconsciousness, laboured breathing, abnormal gait, etc.)?	☒	☐
If so, were these symptoms exposure related?	☒	☐
If not, provide an explanation (*e.g.*, trauma, disease, husbandry):		
Were these exposure-related signs or symptoms observed within 14 days of the initial exposure?	☒	☐

Details[1]:					
Nature of Symptom	Exposure Level (ppm)	Exposure Time (min)	Number of Subjects Affected	Time to Onset (min)	Duration (min)
Difficult breathing	124-196 ppm	3 hours	1/1	2 hours, 26 min	< 9 min
	210-230 ppm	52 min	1/1	Immediate	Momentary
	247-411 ppm	1 hour, 50 min	1/1	1 hour, 21 min	Not stated
	373-493 ppm	2 hour, 35 min	1/1	15 min	1 hour
	532 ppm	30 min	1/1	13 min	Until exit from exposure
Trembling/numbness of extremities	532 ppm	22 min	1/1	22 min	Until 2 hours post-exposure
	575 ppm[2]	3 hours, 19 min	1/1	>2 hours, 41 min	Until exit from exposure
Uncertain gait	532 ppm	22 min	1/1	22 min	Until 2 hours post-exposure

1 Unless otherwise stated, exposures were from Series 3 experiments

2 From Series 1 experiments

Did any serious, life-threatening exposure-related symptoms first appear more than 14 days of the initial exposure? ☐ ☒

Details:					
Nature of Symptom	Exposure Level (ppm)	Exposure Time (min)	Number of People Affected	Time to Onset (min)	Duration (min)

Were any less serious, non-life-threatening symptoms reported?

If yes, list other signs and symptoms: eye and respiratory irritation, heart palpitations, inflamed swollen conjunctivas, tears, nasal secretions and catarrh, intense headaches, pains in eyes and nose (sometimes described as severe tingling or pricking pains) paleness, cold sweats, difficulty opening eyes, intolerance of light, coughing, pain and pressure in epigastrum, itching in eyes.

Post-exposure symptoms: continued irritation, light shy, eye and nasal catarrhs, exhaustion, giddiness, pain in eyes and head, difficulty opening eyes, unpleasant odour from mouth, belching, poor appetite, painful diarrhea, bladder tenesmus, disturbance of sleep, bronchitis, rhinitis, severe watering of the eyes, pain in lower body, shivering fits, fever, sweating, sleepiness , nausea and roseola-like exanthema on fingers.

NB: symptoms varied significantly with level and duration of exposure

Review & Assessment: Study Design, Conduct & Reporting:

A. Subjects:	+/- Subject sex and general health and age were described in most cases, but characteristics such as occupation and any prior exposure to H_2S were not supplied. - Male subjects only were tested - The number of test subjects was limited. In the Series 1 and 3 experiments, only one subject was employed, while Series 2 experiments involved 3 subjects who were poorly described. - The same subject(s) were repeatedly exposed in different exposure-time combinations. The results cannot therefore be generalized to people with no prior exposure to H_2S. - It is unknown whether subjects provided informed consent - A control subject/group was not employed
B. Exposure conditions:	+ Durations of exposure were clearly defined - No evidence that temperature, pressure, humidity or oxygen content within the exposure chamber were monitored. - Source and purity of H_2S were not provided - The concentration of H_2S varied over the course of the exposures by as much as 20-120 ppm. An average could be calculated, but in at least one instance the iodine solution spilled over the last 17 minutes and the concentration over that period could not be determined. The investigator noted that the gas concentration was not determined during each single part of the experiment, but only the average content. - In the Series 1 experiments, the subject did not enter the room after an equilibration period but rather "made" some hydrogen sulphide himself in 2-3 places in the washhouse which served as an exposure chamber. It is unclear for the Series 2 and Series 3 experiments whether there was an equilibration period. +/- A large fan was employed in an attempt to distribute the H_2S throughout the warehouse (Series 1) - The amount of gas in the room could not be kept completely constant since, even with doors closed, there is some ventilation through crevices
C. Exposure equipment:	+/- The exposure "chamber" (washhouse) and methods of generating and quantitating H_2S gas were for the most part adequately described, although it is unclear whether the Series 2 and 3 experiments were conducted in the same "washhouse". - The exposure "chamber" was described as a 29 m^3 washhouse, by no means air tight. No windows were mentioned whereby subjects could be observed. Rather, symptoms were recorded by subjects themselves in a notebook - The method used to generate and quantitate H_2S gas varied with each series of experiments. For Series 1, generation involved slightly warming a mixture of iron sulphide with pure, diluted sulphuric acid and quantitation involved drawing the H_2S containing air through a solution of iodine in aqueous potassium iodide. Later studies showed that the airstream in fact lost its H_2S in the iodine solution but took some iodine vapour away with it such that the concentrations listed for Series 1 experiments were too high. Series 2 experiments attempted to correct this error (by attaching another two Peligot's pipes with potassion idide solution onto the apparatus behind the iodine solution) but the accuracy of the concentration data is still questionable. Series 3 experiments involved further precautions to prevent any loss of iodine as well as a "new" apparatus for H_2S determination which provided the ability to take air samples continuously throughout exposure. Compared to modern methods, even this "new" apparatus was likely to have provided limited sensitivity.

D. Procedural:	+/- Study pre-dated Good Clinical Practice (GLP) guidelines - A control group was not employed. - The same subject(s) were repeatedly exposed in different exposure-time combinations. The results cannot therefore be generalized to people with no prior exposure to H_2S. +/- Post-exposure symptoms were recorded for 1 to 4 days after exposure, presumably until the last day that symptoms likely attributable to the exposure were experienced. It is not apparent that subjects were followed up for any residual or chronic health problems potentially attributable to the H_2S exposures.
E. Data collection:	+/- All symptoms were noted, as well as, the time of occurrence. The duration of various symptoms was not always indicated but often the time of symptom reversal was noted. - In Series 2, where more than one subject was employed, individual data were not provided for each test subject. - Only subjective symptoms were recorded. No objective measurements of clinical signs were performed
F. Data analysis:	- Data were not statistically analyzed.
G. Interpretations:	- Use of only 1-3 subjects per exposure time concentration with the same subjects repeatedly exposed to H_2S confounds and limits interpretation of results - Generation and quantitation of H_2S in the exposure chamber (washhouse) appeared to be quite crude in most instances. This lowers confidence in the accuracy of the exposure data. +/- Based on results in his experiments with animals (NC070), the author concluded that there is a very strong correspondence in the sensitivity of cats and humans to H_2S, at least for doses in which they completed dared experiments with humans, i.e., up to 500 ppm. He suggested that the limit for humans as for cats, above which an exposure period of a few hours becomes life-threatening is likely to be 700-800 ppm. 1000-1500 ppm is likely to be rapidly fatal in humans.

Review & Assessment - Summary:

Discussion of findings:	Observed clinical symptoms in male subjects exposed to H_2S concentrations of 20-575 ppm for durations ranging from 30 minutes to 4 hours. The exposure concentration and duration of exposure appeared to have a significant influence on the type and severity of symptoms observed, with most symptoms progressing with higher concentration and/or continued exposure. No symptoms or only slight eye and respiratory irritation were observed at concentrations below 150 ppm. Notable symptoms at higher concentrations, depending on the duration of exposure, included a painful stinging of the eyes, nose and/or pharynx, severe headaches, pain in the eyes, difficulty breathing, eye and nose secretions, light intolerance and heart palpitations. Symptoms observed post-exposure included: light intolerance, exhaustion, giddiness, pain in eyes and head, difficulty opening eyes, unpleasant odour from mouth, belching, poor appetite, painful diarrhea, bladder tenesmus, disturbance of sleep, bronchitis and rhinitis and pain in the lower body. The study provided evidence that prior exposure to moderately high concentrations of H_2S increase sensitivity to subsequent exposures. Interpretation of the toxicological significance and clinical relevance of the study findings should take into consideration that the study is dated and was performed long before the development of harmonized testing protocols and the introduction of Good Clinical Practice (GCP) guidelines. The study also relied on equipment and analytical methodology that has been replaced by more advanced technology. The level of confidence that can be assigned to the study findings is undermined by the use of relatively "crude" instrumentation, and the associated uncertainty surrounding the actual exposure concentrations that were tested. There were also a number of notable weaknesses in the experimental design (see below). The studies were described in a first person narrative. Interestingly remarks made by the study investigator included: Commenting on the recruitment of subjects: *"I prevailed upon Mr. Kwilecki to undertake a large series of experiments on himself"*,

	and thereafter, *" … in several short experiments that I carried out on myself, a student Mr. Z., and my servant, W."*. Commenting on maintaining uniform concentrations of H_2S in the exposure chamber: *"As far as was possible the gas content of the room [a washhouse] was kept constant throughout the whole experimental period – although this was only guessed at by subjective feeling. It is obvious the amount of gas in the room could not be completely constant since, with doors closed, there is some ventilation through crevices and since the production of hydrogen sulphide was measured subjectively. Unfortunately, the gas concentration was not determined during each single part of the experiment, but only the average content"*. Commenting on the reliability of the first series of experiments: *"These experiments were still not quite sufficient for me. I have therefore arranged for them to be repeated with Mr. Greulich during last summer and took care to exclude all known sources of error"*.

Review & Assessment - Scoring[34] and Rational:

No practical use	☐
Low	☒
Low to Moderate	☐
Moderate	☐
Moderate to High	☐
High	☐
Rational:	Weaknesses in experimental design, conduct and/or reporting were judged to undermine the level of confidence that could be assigned to the study findings and conclusions. Increased confidence in the findings could have been obtained through the use of control subjects and a larger number of test subjects. The use of the same test subjects for different exposure/time combinations also severely limits the generalizability of the results.
Strengths	• Use of gradient of exposure concentrations and exposure times to permit assessment of comparative influence of each parameter on acute toxicity. • Detailed observations of clinical symptoms, including duration and/or reversibility of symptoms in most instances.
Weaknesses	• Use of limited numbers of subjects (1-3 test subjects for each exposure concentration/exposure time combination) • Use of test subjects repeatedly exposed to acute H_2S exposures. • Inadequate description of test subjects (*e.g.*, occupation, exact age, prior exposures to H_2S). • Failure to include control subjects. • Unusual chamber selection (Chamber was described as a "washroom" in which the H_2S was produced by combining ferrous sulphate with acid). • Significant uncertainty surrounding the actual exposure concentrations that were tested.

[34] Score reflects usefulness of study for development of emergency planning criteria vis-à-vis acute lethality.

Reviewers:

DD	☒
RT	☒
CM	☐

Case Reports

Document Review – Case Reports

Author:	Winek, C.L., Collom, W.D. and Wecht, C.H.	**Study Code:**	CR002
Title:	Death from hydrogen sulphide fumes		
Year:	1968		

Paper Description:	Full length paper: ☐ Peer-reviewed ☐ Non-peer reviewed ☒	Abstract: ☐	Review article: ☐	Cited in-review article[35]: ☐ Details: …
Abstract:	*Not available*			
Objective:	Paper consisted of a "Letter to the Editor" describing the circumstances surrounding the death of a middle-aged man overcome by hydrogen sulphide fumes while working in a confined tank used to store coal-tar resins.			
Primary focus of the study:	Lethality/fatality: ☒	Other:		

Overall case report features:

Nature and Circumstances of Exposure	Exposure level(s)	Exposure frequency/duration	Subject details			Pre-exposure health status
			Number	Age	Sex	
Industrial accident involving exposure to H_2S while working in a tank containing coal-tar residues.	1,900 ppm (near top of tank). 6,100 ppm (near middle of tank).	Reported to be 5 minutes.	1	55	Male	Reported to have had "no history of disease".

Observations:

Lethality/Fatality

Were deaths observed? Yes ☒ No ☐

Details:

Exposure Level (ppm)	Exposure Time (min)	Number of Deaths	Time to Death (min)
Reported to be 1,900 ppm near the top of the tank, and 6,100 ppm near the middle of the tank.	5 minutes	1 (only a single individual was involved).	Approximately 45 minutes from discovery.

Were autopsies performed on subjects who died? Yes ☒ No ☐

If so, were autopsy findings consistent with exposure-related cause of death? Yes ☒ No ☐

[35] Refers to a paper describing the original paper that was either unattainable or in a foreign language.

List major autopsy findings: acute bilateral pulmonary edema, chronic passive congestion of lungs, extremely wet frothy congested surfaces on lungs, with diffuse red to reddish brown or purple appearance, great amount of mucus in tracheo-bronchiolar tree, brain was somewhat edematous.

Signs & Symptoms

Were clinical signs and symptoms reported? Yes ☒ No ☐

Were any symptoms consistent with life-threatening, serious and/or irreversible health outcomes reported (e.g., convulsions, coma, unconsciousness, laboured breathing, etc.) reported? Yes ☒ No ☐

Details:

Nature of Symptom	Exposure Level (ppm)	Exposure Time (min)	Number of Subjects Affected	Time to Onset (min)	Duration
Unconsciousness	Reported to be 1,900 ppm near top of tank and 6,100 ppm near middle of tank.	Reported to be 5 minutes.	1	Within 5 minutes	Until death (approx. 45 minutes post-discovery).

Did any latent exposure-related symptoms appear after the exposure? Yes ☐ No ☒

Details:

Nature of Symptom	Exposure Level (ppm)	Exposure Time (min)	Number of Subjects Affected	Time to Onset (min)	Duration

Were any other exposure-related symptoms observed? Yes ☐ No ☒

If yes, list other symptoms:

Review & Assessment: Case Report Features:

A. Subjects:	+/- Age, sex, race and prior health status indicated. No other details given.
B. Exposure conditions:	+/- Measurements of H_2S taken near top and middle of storage tank. - Time elapsed between incident and measurements not stated. Also, no indication was given as to whether the tank was open to atmosphere or closed during this interval. - Manner in which H_2S samples were obtained and analyzed not provided. - Concentration of H_2S actually encountered by subject unknown. Concentration may have been higher than 6,100 ppm since tank was reported to be 15 feet in height and the subject was likely exposed to fumes at a height lower than the mid-point of the tank.

	+ Reasonable indication of exposure time (5 minutes).
C. Data collection:	+/- Some description of exposure conditions, including exposure time. + At least some attempt was made to measure exposure concentrations that might have been encountered. + Good description of autopsy findings. + Post-mortem examination included analysis of body tissues for presence of H_2S (…presence confirmed in brain, liver and kidney).
D. Data analysis:	+/- No analysis of data.
E. Interpretations:	+/- Based on available evidence , including H2S measurements and autopsy findings, the authors concluded that the subject died from over-exposure to hydrogen sulphide.

Review & Assessment - Summary:

Discussion of findings:	Case report concerns death of a worker exposed to H_2S while working in a storage tank used to hold coal-tar resins. Measurements taken inside the tank post-incident as well as autopsy findings point to H_2S over-exposure as being the cause of death. Uncertainty exists as to the actual concentration of H_2S that may have been encountered by the worker.

Review & Assessment - Scoring[36] and Rational:

No practical use	☐
Low	☒
Low – Moderate	☐
Moderate	☐
Moderate – High	☐
High	☐
Rational:	Usefulness of study for characterizing concentration-time-response for lethality is limited due to lack of information concerning the actual concentration of H_2S to which the worker may have been exposed. Note that Table 1 of Appendix 2 of the AEUB Discussion Paper (October 2004) lists the concentration of H_2S causing the fatality as 6,100 ppm, with an exposure time of 5 minutes (Record 108). Based on the available evidence, this concentration cannot be absolutely substantiated. The possibility exists that the worker may have been exposed to a higher concentration(s) given the dimensions of the storage tank as well as events that may have transpired between the time of the incident and the time at which the H_2S measurements were taken (*i.e.*, H_2S may have escaped from the tank if it was left open during the interval).

[36] Score reflects usefulness of study for development of emergency planning criteria vis-à-vis acute lethality.

Strengths:	• Description of "real world" incident involving over-exposure to H_2S leading to death. • Some indication of potential exposure concentration(s) that might have been encountered as well as indication of exposure time. • Good description of autopsy findings, including results from analysis of tissues for the presence of H_2S. • Good correlation between symptoms (unconsciousness), eventual outcome (death) and autopsy findings
Weaknesses.	• Actual concentration of H_2S to which subject may have been exposed unknown. Evidence points to possibly higher concentration than that measured and reported. • Details concerning measurements of H_2S taken in relation to the incident were limited. The time interval between the incident and the measurements was not indicated, nor were details given concerning the sampling and analytical methodology employed.

Reviewers:

DD	☒
RT	☒
CM	☐

Document Review – Case Reports

Author:	Mitchell, C.W. and Yant, W.P.		**Study Code:**	CR066 (see also NC032 and CL010)
Title:	Correlation of the data obtained from refinery accidents with a laboratory study of H_2S and its treatment			
Year:				
Paper Description:	Full length paper: ☒ Peer-reviewed ☐ Non-peer reviewed ☒	Abstract: ☐	Review article: ☐	Cited in-review article[37]: ☐ Details: …
Abstract:	*In the laboratory study, the symptoms of hydrogen sulphide (H2S) poisoning in anaimals and men were found to be almost identical with those caused by gases in the refineries. The need for a definite method of treating H2S poisoning was evident. The medical findings, the study on toxicity of H2S, and the treatment for H2S poisoning will be discussed in turn.*			
Objective:	The study was intended to examine the health effects of short-term exposures to hydrogen sulphide in test animals and volunteer human subjects, and to determine the degree of correlation between the findings and the symptoms reported among refinery workers poisoned by the gases from high-sulphur crude oil. The case reports compiled by the authors relating to the worker poisoning incidents are summarized herein. Emphasis was given to cases involving asphyxiation from the gases. The sections of the paper detailing the non-clinical and clinical studies are summarized in Document Review Forms NC032 and CL010, respectively.			
Primary focus of the study:	Lethality/fatality: ☐	Other: Investigations of clinical symptoms and recovery among refinery workers poisoned by gases from high-sulphur crude oil.		

Overall case report features:

Nature and Circumstances of Exposure	Exposure level(s)	Exposure frequency/duration	Subject details			Pre-exposure health status
			Number	Age	Sex	
Case 1: Maintenance worker (i.e., tinsmith) repairing line at the "receiving house" of the refinery was overcome by fumes.	Not reported	Single exposure. Subject was reportedly rendered unconscious within one minute.	1	27	Male	Not reported.
Case 2: Worker was measuring level of crude oil in a tank and was	Not reported	Single exposure. Subject was reportedly rendered unconscious in less than one minute.	1	30	Male	Not reported

[37] Refers to a paper describing the original paper that was either unattainable or in a foreign language.

quickly overcome by fumes after lifting the hatch cover.						
Case 3. Worker was attempting to block a hatch cover on a tank containing Mexican crude oil and was overcome by the fumes, subsequently falling to the ground.	Not reported	Single exposure of unknown duration.	1	21	Male	Not reported
Case 4. A labourer cleaning scrubbers at a gas plant was overcome by fumes from Mexican oil.	Not reported	Single exposure of unknown duration.	1	31	Male	Not reported
Case 5. Two workers were overcome by sewer gas while cleaning a "condenser box".	Not reported. (The sewer gas reportedly carried " a high percentage of H_2S").	Single exposure lasting approximately 5 minutes	2	Not stated	Males	Not reported.

Observations:

Lethality/Fatality

Were deaths observed? Yes ☒ No ☐

Details:

Exposure Level (ppm)	Exposure Time (min)	Number of Deaths	Time to Death (min)
Unknown (Case 5)	Approximately 5 minutes	2	Within 5 minutes of exposure

Were autopsies performed on subjects who died? Yes ☐ No ☒
If so, were autopsy findings consistent with exposure-related cause of death? Yes ☐ No ☐
List major autopsy findings:

Signs & Symptoms

Were clinical signs and symptoms reported? Yes ☒ No ☐
Were any symptoms consistent with life-threatening, serious and/or irreversible health outcomes reported (e.g., convulsions, coma, unconsciousness, laboured breathing, etc.) reported? Yes ☒ No ☐

Details:

Nature of Symptom	Exposure Level (ppm)	Exposure Time (min)	Number of Subjects Affected	Time to Onset (min)	Duration
Unconsciousness	Unknown (all cases)	One to 5 minutes in most cases	6	Immediately (within one to 5 minutes in most cases).	Recovery in non-fatal cases was reportedly rapid (within 24 hours in most cases).

Did any latent exposure-related symptoms appear after the exposure? Yes ☐ No ☒

Details:

Nature of Symptom	Exposure Level (ppm)	Exposure Time (min)	Number of Subjects Affected	Time to Onset (min)	Duration

Were any other exposure-related symptoms observed? Yes ☒ No ☐
If yes, list other symptoms: Case 2 worker complained of headache, nausea and stomach pain. No other complaints were registered by the other surviving workers, except for the Case 3 worker who evidently sustained injuries because of the fall.

Review & Assessment: Case Report Features:

A. Subjects:	+/- Subject information limited to age, sex, job category and years of service. No other details provided. +/- All cases involved male workers.

B. Exposure conditions:	- Descriptions of incidents were very brief. - No indication of concentrations of H_2S encountered in any of the cases reported. +/- Duration of exposure noted in 3 of 5 cases. Exposure times for remaining 2 cases were unknown.
C. Data collection:	- Limited descriptions of symptoms and recovery. - No indication of any follow-up medical attention, with the exception of immediate treatment in the dispensary or hospital for Cases 2 and 3.
D. Data analysis:	+/- No analysis other than attempt by authors to correlate symptoms with findings from controlled animal studies (NC032) and preliminary clinical studies with human subjects (CL010).
E. Interpretations:	+/- Authors offered that cases suggest poisoning can occur regardless of nationality, age and length of service. +/- Authors assigned cause of poisoning to carelessness or failure to understand hazards involved.

Review & Assessment - Summary:

Discussion of findings:	A series of case reports were presented, all of which involved the poisoning of refinery workers from the fumes associated with high-sulphur Mexican crude oil. The descriptions of the cases were limited, with no indication of the concentrations of H_2S that might have been encountered. The poisoning outcomes varied from unconsciousness followed by full recovery to death. Reporting of symptoms was limited, and no medical follow-up of any of the subjects occurred.

Review & Assessment - Scoring[38] and Rational:

No practical use	☒
Low	☐
Low – Moderate	☐
Moderate	☐
Moderate – High	☐
High	☐

Rational: The case reports were deemed to be of no practical use owing to the limited information provided, most notably the lack of information respecting the concentrations of H_2S to which the workers may have been exposed.

Strengths:

- Some attempt made to correlate findings with observations from non-clinical and clinical investigations described as part of same paper.
- "Real world" incidents involving over-exposure of humans to H_2S.

[38] Score reflects usefulness of study for development of emergency planning criteria vis-à-vis acute lethality.

Weaknesses:

- No information respecting concentrations of H2S to which workers may have been exposed.
- Limited reporting of symptoms.
- Limited medical intervention only. No indication of medical follow-up.

Reviewers:

DD	☒
RT	☒
CM	☐

Document Review – Case Reports

Author:	Prouza, Z.	**Study Code:**	CR067
Title:	Group poisoning with hydrogen sulphide in an unusual situation at a viscose plant		
Year:	1970		

Paper Description:	Full length paper: ☒ Peer-reviewed ☐ Non-peer reviewed ☐	Abstract: ☐	Review article: ☐	Cited in-review article[39]: ☐ Details: …

Abstract:	*The author describes one case of fatal poisoning, seven cases of acute poisoning and two cases of irritation with hydrogen sulphide which occurred when the heaters in a spinning bath tank were repaired. The accumulation of hydrogen sulphide was caused by a leak from the overflow pipe inter-connecting three tanks, at a place where hydrogen sulphide had never occurred before (the above repair works were carried out on the plant for more than 11 years). The fatal poisoning occurred within a few seconds in an atmosphere where the NPK concentration was exceeded by more than 160 times. In the case of the poisoned men who survived, no after-effects were observed either immediately afterwards or after two years. The great number of the poisoned was caused by neglect of the faxctory rules concerning health protection during work, which were known by all. A spontaneous attempt to rescue a comrade in trouble led to a loss of judgement by the rescuers.*
Objective:	To review and comment on the circumstances surrounding an industrial accident in a viscose rayon plant in which a number of workers were exposed to hydrogen sulphide during the repair of a tank heater. The accident resulted in the death of one worker and the poisoing of several other individuals involved in the rescue attempt.

Primary focus of the study:	Lethality/fatality: ☒	Other: Clinical symptoms among survivng workers.

Overall case report features:

Nature and Circumstances of Exposure	Exposure level(s)	Exposure frequency/duration	Subject details			Pre-exposure health status
			Number	Age	Sex	
During repair procedures, a factory worker entered a "spinning bath" tank in order to disentangle the heating elements and was immediately overcome by H2S fumes. He fell	The following levels of H2S were measured approximately 4.5 hours after the incident: i) 11 ppm in the near vicinity of the "spinning	The stricken worker was reported to have been in the tank for "a few minutes". He was reported to have fallen almost immediately after entering the tank. The two initial rescue workers also reportedly began to lose consciousness shortly after entering the tank.	Stricken worker First rescue worker Second rescue worker Supervisor Other rescue workers (n=6)	23 years 31 years 26 years 44 years 27 to 52 years	Male Male Male Male Male	Not given Not given Not given Not given Not given

[39] Refers to a paper describing the original paper that was either unattainable or in a foreign language.

unconscious while still in the tank. Two other workers entered the tank in an attempt to rescue the first worker and were also overcome. A supervisor who remained outside the tank managed to help the two rescuers to safety; however, the first worker still remained unconscious in the tank. Several other workers were summoned and assisted in finally removing the first worker from the tank.	bath" tank ii) 25 ppm just above the tank iii) 2850 ppm inside the tank at the height of the heater elements.					

Observations:

Lethality/Fatality

Were deaths observed? Yes ☒ No ☐

Details:

Exposure Level (ppm)	Exposure Time (min)	Number of Deaths	Time to Death (min)
Reported to be greater than 2,850 ppm.	Reported to be "a few minutes".	One	Not specified

Were autopsies performed on subjects who died? Yes ☒ No ☐

If so, were autopsy findings consistent with exposure-related cause of death? Yes ☒ No ☐

List major autopsy findings: grayish-green discoloration of the grey matter of the brain, green-colored urine, and "posthumous yellow-green spots".

Signs & Symptoms

Were clinical signs and symptoms reported? Yes ☒ No ☐

Were any symptoms consistent with life-threatening, serious and/or irreversible health outcomes reported (e.g., convulsions, coma, unconsciousness, laboured breathing, etc.) reported? Yes ☒ No ☐

Details:

Nature of Symptom	Exposure Level (ppm)	Exposure Time (min)	Number of Subjects Affected	Time to Onset (min)	Duration
Unconsciousness, symptoms consistent with cyanosis (i.e., blue extremities, cold to touch)	Greater than 2,850 ppm	Total of "a few minutes".	One	Almost immediately	Until death
Pending unconsciousness	Greater than 2,850 ppm	Not specified … but presumably for a few minutes	Two	Almost immediately	Not specified … but workers had recovered by the time a doctor was summoned and arrived on scene.

Did any latent exposure-related symptoms appear after the exposure? Yes ☐ No ☒

Details:

Nature of Symptom	Exposure Level (ppm)	Exposure Time (min)	Number of Subjects Affected	Time to Onset (min)	Duration

Were any other exposure-related symptoms observed? Yes ☒ No ☐

If yes, list other symptoms: Nausea, weakness, pain in chest were reported by four of the rescue workers shortly after the incident. As a precaution, these men were hospitalized, and then released in good health after seven days. Mild residual “neurotic” effects evidently appeared among some of the rescue workers in the months that followed the incident. The exact nature of the neurotic effects was not indicated.

Review & Assessment: Case Report Features:

A. Subjects:	+/- Age of subjects reported. No other details provided. (Presumably, all subjects were males).
B. Exposure conditions:	+/- General description of incident (including estimates of exposure concentrations and/or exposure times) was provided. - Exact exposure times were not specified. The stricken worker was reported to have been exposed in the tank for “a few

	minutes". The exposure times for the remaining workers were not indicated. - Actual exposure concentrations to which the workers were exposed were unknown. Measurements were taken 4.5 hours after the incident. +/- Measurements of H_2S concentrations involved use of detector tubes and "a Jelinek apparatus".
C. Data collection:	+ Clinical symptoms experienced by the stricken worker, the first two rescuers, and the supervisor were reported. + Clinical condition of stricken worker upon removal from the tank was described (*i.e.*, unconscious and cyanotic). +/- General description of autopsy findings provided. + Medical follow-up was completed for surviving workers for a period up to two years.
D. Data analysis:	+/- No data analysis was performed
E. Interpretations:	+/- Death of stricken worker was ascribed to over-exposure to H2S. +/- Concentration causing death (*i.e.*, concentration to which the stricken worker was exposed) was stated to be greater than 2,850 ppm (or 4,000 mg/m^3).

Review & Assessment - Summary:

Discussion of findings:	Case report describes circumstances surrounding death of repair worker from over-exposure to H_2S. Stricken worker was exposed to H_2S at a concentration greater than 2,850 ppm for "a few minutes". Clinical symptoms preceding death included: not feeling well, followed by collapse and unconsciousness occurring almost immediately upon entry into the "spinning bath" tank containing the H_2S fumes. Rescue workers who entered the tank also experienced near unconsciousness within a few minutes. Other workers involved in the rescue reported nausea, weakness and/or chest pains. Symptoms cleared within seven days. No latent effects were reported based on medical follow-up over a period of two years.

Review & Assessment - Scoring[40] and Rational:

No practical use ☐

Low ☒

Low – Moderate ☐

Moderate ☐

Moderate – High ☐

High ☐

Rational: The study is of limited usefulness for defining the concentration-time-response for lethality from H_2S exposure. The exact concentration to which the stricken worker was exposed was reported only be "greater than 4,000 mg/m^3" (or 2,850 ppm). The exact exposure time also was unknown (*i.e.* "a few minutes").

Note that Table 1 of Appendix 2 of the AEUB Discussion Paper indicates that the stricken worker was exposed to 1,000 ppm of H_2S for one minute (record 16).

[40] Score reflects usefulness of study for development of emergency planning criteria vis-à-vis acute lethality.

This exposure concentration-exposure time combination does not agree with the information provided in the case report (see above). In addition, the table suggests that 10 workers were exposed to this combination, and 1 of the 10 workers died. The basis of this record is unknown. Although the case report includes reference to 10 workers (including the stricken worker who died), the workers were exposed to varying exposure concentration-exposure time combinations … most of which were unknown, but less severe than the conditions experienced by the stricken worker. Thus, Record 16 is somewhat misleading.

Strengths:

- Case report describing circumstances surrounding "real world" incident involving the death of a worker over-exposed to H2S.
- Some indication of approximate exposure concentration (i.e., greater than 2,850 ppm) and exposure time (i.e., "a few minutes") resulting in death.
- Good correlation between clinical symptoms, death and autopsy findings.

Weaknesses:

- Actual exposure concentration and exposure time leading to death not known.
- Actual exposures received by rescue workers who survived the incident not known.
- Measurement of H_2S concentrations involved use of detector tubes with limited sensitivity.

Reviewers:

DD	☒
RT	☒
CM	☐

Review Articles

Document Review – Review Articles

Author:	Lefaux, R.		**Study Code:**	RE001
Title:	Practical toxicology of plastics. III. Health and safety			
Year:				
Paper Description:	Full length paper: ☐ Peer-reviewed ☐ Non-peer reviewed ☐	Abstract: ☐	Review article: ☒	Cited in-review article[41]: ☐ Details:
Abstract:	*Not available*			
Objective:	The chapter of the paper devoted to health and safety discusses a number of industrial concerns related to the manufacture of plastics, including the health effects associated with combustion by-products. A table (Table 33) is presented listing the concentration-response characteristics of a number of combustion by-products, including hydrogen sulphide. The source of the information contained in the table is not indicated. Apart from the table, there is no reference or mention of H_2S elsewhere in the chapter.			
Primary focus of the study:	Lethality/fatality: ☐	Other: General discussion of the health hazards associated with the manufacture of plastics, including combustion by-products.		

Review & Assessment – Summary

Discussion of findings: The only reference to H_2S in the paper is contained in a table in which brief descriptions of health effects (… including lethality) resulting from various exposure concentration-exposure time combinations are listed. The source of the information was not indicated. The descriptions are summarized below:

20 ppm … no effect over several hours
100 ppm … minimum amount causing throat irritation
200 ppm … dangerous in ½ to 1 hour
600 ppm … fatal in ½ hour
1000 ppm … rapidly fatal

Review & Assessment – Scoring and Rational:

No practical use	☒
Low	☐
Low – Moderate	☐
Moderate	☐
Moderate – High	☐
High	☐

[41] Refers to a paper describing the original paper that was either unattainable or in a foreign language.

Rational:

The review article was deemed to be of no practical use in advancing understanding of the concentration-time-response characteristics of H_2S vis-à-vis lethality. The descriptions of health effects were brief and could not be substantiated. The source of the information relating to H_2S contained in the article was unknown.

Strengths:

- The paper provides a listing of health effects according to both exposure concentration and exposure time, with concentration-time combinations associated with lethality indicated.

Weaknesses:

- Source of health effects information was not provided (i.e., the information could not be substantiated).
- Descriptions of health effects were very brief.
- Technical quality of the information could not be determined.

Reviewers:

DD	☒
RT	☒
CM	☐

Document Review – Review Articles

Author:	Haggard, H.W.		**Study Code:**	RE002 (see also NC067)
Title:	The toxicology of hydrogen sulphide			
Year:	1925			
Paper Description:	Full length paper: ☒ Peer-reviewed ☒ Non-peer reviewed ☐	Abstract: ☐	Review article: ☐	Cited in-review article[42]: ☐ Details:
Abstract:	*Not available*			
Objective:	The majority of the paper is devoted to a review of the toxicology of hydrogen sulphide, with reference to fate in the body, mechanism of action, irritant properties, systemic poisoning, and treatment of poisoning. A separate section of the paper describes the findings from a series of acute inhalation exposures of dogs to H_2S performed by the author. This form is concerned with the summary information. A separate Document Review Form discussing the experiments with dogs is available (see NC067).			
Primary focus of the study:	Lethality/fatality: ☐	Other: General review of the toxicology of H_2S.		

Review & Assessment – Summary

Discussion of findings: The paper provides an overview of the toxicology of H_2S, with reference to fate in the body, mechanism of action, irritant properties, systemic poisoning, and treatment of poisoning. Much of the information is "dated", and the bibliography is very limited (*i.e.*, consisting of 9 citations only, with very few specific to H_2S).

Review & Assessment – Scoring and Rational:

No practical use ☒

Low ☐

Low – Moderate ☐

Moderate ☐

Moderate – High ☐

High ☐

Rational:

The paper is deemed to be of no practical use in advancing understanding of the concentration-time-response characteristics of H_2S vis-à-vis lethality. It provides a general overview of the toxicology of H_2S; however, the information is "dated" and the extent of the literature review was very limited based on the small number of original articles cited.

[42] Refers to a paper describing the original paper that was either unattainable or in a foreign language.

Strengths:

- Provides a general overview of the toxicology of H_2S, with reference to systemic poisoning.

Weaknesses:

- Information is “dated”
- Extent of literature review was very limited.
- Reliability of the information could not be readily established (i.e., information from other sources was simply summarized, with very little detail provided).
- No information provided specific to concentration-time-response characteristics of H_2S vis-à-vis lethality or any other health endpoint.

Reviewers:

DD	☒
RT	☒
CM	☐

Document Review – Review Articles

Author:	Back, K.C., Thomas, A.A. and MacEwen, J. D.	**Study Code:**	RE003 (see also NC072)
Title:	Reclassification of materials listed as transportation health hazards		
Year:	1972		
Paper Description:	Full length paper: ☐ Peer-reviewed ☐ Non-peer reviewed ☐	Abstract: ☐	Review article: ☒
			Cited in-review article[43]: ☐ Details:
Abstract:	*This study was performed to provide technical background and recommendations for assisting the Department of Transportation in considering a revised health hazards classification system. The study consisted of three phases. Phase I – An extensive literature search was conducted for pertinent human and acute animal toxicity data for about 200 materials, classed as Poison A, B or C in the Commodity List, Section 172.5, Title 49 CFR, and/or as Toxic (Class 6.1) in the Subsidiary Risk Category in the United nations publication, Volume I, Transportation of Dangerous Goods, 1966. Materials were classified according to the proposed classification criteria, if valid data were adequate for evaluation. Tests were recommended for the materials for which data were missing or inadequate. Phase II – Inhalation (LC_{50}) toxicity tests were run on mice and rats for five materials and oral toxicity (LD_{50}) tests were run on mice and rats for 40 other materials. The phosphine evolution rate for aluminium phosphide in air (55% relative humidity) and in water were determined. The results have been summarized and the materials classified. Phase III – Verification inhalation toxicity (LC_{50}) tests were run on mice and rats exposed to chlorine, anhydrous ammonia and hydrogen sulphide. Results have been included and reflected in the classification of these materials. One other material was classified from the literature data.*		
Objective:	The paper describes the results of work aimed at re-classifying a number of chemicals for transportation purposes based on health effects data sourced from the literature and/or developed in-house at the Aerospace Medical Research Laboratory, Wright-Patterson Air Force Base, OH. Hydrogen sulphide was among the more than 200 chemicals examined. The information specific to H_2S contained in the paper is limited to a table listing the LC_{50} values for mice and rats determined from an acute inhalation toxicity test involving one-hour exposures to the gas. It was subsequently discovered that the LC_{50} values shown in the table were taken directly from the acute inhalation study performed by MacEwan and Vernot (1972 – NC072).		
Primary focus of the study:	Lethality/fatality: ☐	Other: Re-classification of chemicals for transportation purposes on the basis of new and/or existing health effects data in accordance with U.S. and international regulatory requirements.	

Review & Assessment – Summary

Discussion of findings: The paper simply provides a summary of the LC_{50} values determined by MacEwan and Vernot (1972 – NC072). The LC_{50} values listed in the paper are shown below:

LC_{50} Mouse: 673 ppm (925 mg/m^3)
LC_{50} Rat: 713 ppm (990 mg/m^3)

[43] Refers to a paper describing the original paper that was either unattainable or in a foreign language.

Review & Assessment – Scoring and Rational:

No practical use	☒
Low	☐
Low – Moderate	☐
Moderate	☐
Moderate – High	☐
High	☐

Rational:

The paper was deemed to be of no practical use in advancing understanding of the concentration-time-response characteristics of H_2S vis-à-vis lethality since it simply summarized the earlier work performed by MacEwan and Vernot (1972 – NC072). No original data were presented.

Strengths:

- None

Weaknesses:

- Nothing more than a brief summary of work performed by others, and limited to a listing of one-hour LC_{50} values for rats and mice from a single study.
- Technical quality of the data could only be confirmed through retrieval and review of the original study conducted by MacEwan and Vernot (1972 – NC072).

Reviewers:

DD	☒
RT	☒
CM	☐

Document Review – Review Articles

Author:	Tabulae Biologicae Periodicae		**Study Code:**	RE004
Title:	Naturliche reichstoffe (in German)			
Year:	1933			
Paper Description:	Full length paper: ☐ Peer-reviewed ☐ Non-peer reviewed ☐	Abstract: ☐	Review article: ☒	Cited in-review article[44]: ☐ Details:
Abstract:	*Not available*			
Objective:	The paper consists entirely of a table listing the physical-chemical properties of a number of naturally-occurring chemicals. The properties include molecular formula, molecular weight, melting point, vapour pressure, density, solubility in alcohol, *etc*. Much of the listing is devoted to alcohols, aldehydes and ketones. There is no listing for hydrogen sulphide. (Note that the paper was referenced by NIOSH as part of the development of the Immediately Dangerous to Life and Health guideline for H_2S).			
Primary focus of the study:	Lethality/fatality: ☐	Other: Physical-chemical properties.		

Review & Assessment – Summary

Discussion of findings: The paper consists only of a table listing the physical-chemical properties of a series of naturally-occurring chemicals, with much of the listing devoted to alcohols, aldehydes and ketones. There is no listing for hydrogen sulphide.

Review & Assessment – Scoring and Rational:

No practical use ☒

Low ☐

Low – Moderate ☐

Moderate ☐

Moderate – High ☐

High ☐

Rational:

The paper was deemed to be of no practical use in advancing understanding of the concentration-time-response characteristics of H_2S vis-à-vis lethality. It consisted only of a listing of the physical-chemical properties of a series of naturally-occurring chemicals, with no mention of H_2S. Accordingly, the information was deemed to be irrelevant.

[44] Refers to a paper describing the original paper that was either unattainable or in a foreign language.

Strengths:

- None

Weaknesses:

- The paper contains no information relating to H_2S.

Reviewers:

DD	☒
RT	☒
CM	☐

Document Review – Review Articles

Author:	National Institute for Occupational Safety and Health (NIOSH)	**Study Code:**	RE005
Title:	Criteria for a Recommended Standard … Occupational Exposure to Hydrogen Sulphide		
Year:			

Paper Description:	Full length paper: ☐ Peer-reviewed ☐ Non-peer reviewed ☐	Abstract: ☐	Review article: ☒	Cited in-review article[45]: ☐ Details:

Abstract:	*The recommended standard is given limiting employee exposure to less than 15 milligrams of hydrogen sulphide per cubic meter of air (10 ppm) during a 10-minute sampling period for up to a 10-hour work shift in a 40-hour workweek, with evacuation of the area if the concentration equals or exceeds 70 milligrams per cubic meter. In addition, standards are given for medical surveillance, labelling and posting, personal protective equipment, work practices, sanitation, and monitoring and recordkeeping. The criteria for the standards are also given, including extent of exposure in the United States, historical reports of exposure, effects on humans, epidemiological studies, animal toxicity, correlation of exposure and effect, carcinogenicity, mutagenicity, teratogenicity, effects on reproduction, environmental concentrations to which workers have been exposed, various attempts at controlling exposure, environmental sampling and analytical methods, biological monitoring, work practices and safety precautions for handling hydrogen sulphide, the bases upon which previous and the present standards are recommended, and research needs.*
Objective:	The paper represents a comprehensive review of the health effects information on H_2S for the purposes of establishing standards for workplace exposure.

Primary focus of the study:	Lethality/fatality: ☐	Other: Comprehensive overview of the toxicology of H_2S.

Review & Assessment – Summary

Discussion of findings: The paper provides a summary of the health effects information on H_2S, including results from case reports involving systemic poisonings, epidemiological studies, and animal testing.

Review & Assessment – Scoring and Rational:

No practical use	☒
Low	☐
Low – Moderate	☐
Moderate	☐
Moderate – High	☐
High	☐

[45] Refers to a paper describing the original paper that was either unattainable or in a foreign language.

Rational:

The paper represents a review article, with no original data provided. The reliability and technical quality of the original information was not apparent and was not determined.

Strengths:

- Comprehensive review of the toxicology of H_2S, including summary of findings from case reports involving systemic poisonings, epidemiological studies and animal toxicity tests.

Weaknesses:

- Reliability and technical quality of original studies were not readily apparent and were not determined.

Reviewers:

DD	☒
RT	☒
CM	☐