Concentration-time Interactions in Hydrogen Sulphide Toxicity in Rats

Michael G. Prior, Arvind K. Sharma, Shirley Yong and Alfonso Lopez*

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 among 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 $\log_{10}$ dose 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.

RÉSUMÉ

Cette expérience portait sur des jeunes rats et rates Sprague-Dawley, Long Evans et Fischer-344. Elle consistait à leur faire inhaler de l'acide sulfhydrique durant deux, quatre et six heures, pour ensuite analyser le rapport entre la concentration de l'acide précité et la durée de la période d'inhalation. Les concentrations les plus fortes causèrent le plus de mortalités, indépendamment du temps d'inhalation. On enregistra une différence appréciable relative au sexe, puisque 30% des mâles moururent, par rapport à seulement 20% des femelles, indépendamment de leur souche. La perte de poids s'avéra proportionnelle à la concentration d'acide sulfhydrique et au temps d'inhalation; elle se révéla moins élevée chez les sujets Fischer-344 que chez les Sprague-Dawley et les Long Evans; elle subit aussi l'influence du temps d'inhalation. On calcula aussi la valeur des concentrations létales CL_{50} et CL_{10}, d'après les données regroupées des 456 sujets impliqués dans l'expérience; l'équation probit donna: Y = -5,74749 + 3,8259 X, équation dans laquelle X représentait le $\log_{10}$ de la dose d'acide sulfhydrique en p.p.m. Les valeurs CL_{50} et CL_{10} atteignirent respectivement 644 et 298 p.p.m., ou 902 et 417 mg/m^3. On effectua aussi des analyses probit individuelles pour chacune des souches de rats et des périodes d'inhalation, ainsi que pour chacun des sexes. Les valeurs de CL_{50} et CL_{10} n'affichèrent pas de différence entre les mâles et les femelles de l'une ou l'autre souche. Ces valeurs présentèrent toutefois les différences appréciables suivantes, par rapport à la durée de la période d'inhalation: 587 et 549 p.p.m., ou 822 et 769 mg/m^3, pour la période de deux heures; 501 et 422 p.p.m., ou 701 et 591 mg/m^3, pour celle de quatre heures; 335 et 299 p.p.m., ou 469 et 419 mg/m^3, pour celle de six heures. La position spatiale dans la chambre d'inhalation n'exerça pas d'influence sur la distribution des mortalités. Tous les sujets des trois souches affichèrent un oedème pulmonaire marqué.

INTRODUCTION

Hydrogen sulphide occurs naturally, for example, in coal, natural gas, oil and sulphur springs, and is a product of the anaerobic decomposition of sulphur-containing organic matter. Also it is a by-product of several industrial processes, including desulphurization of sour natural gas (1). The two major sources of exposure for livestock are manure gas and sour gas operations. Pigs and cattle have died following the emptying of slurry (manure) tanks, when agitation released the toxic gases (2,3). Gas well blowouts can give rise to elevated concentrations of hydrogen sulphide, with a variety of perceived effects in livestock (4,5,6,7,8). Goats (9), mature cattle (9), calves (10), pigs (11), and chickens (12) have been exposed to hydrogen sulphide under experimental conditions; such studies are rare due to the high costs and specialized facilities required.

The available literature on the toxicity of hydrogen sulphide for rats is very limited (13). The median lethal

*Animal Sciences Wing, Alberta Environmental Centre, Bag 4000, Vegreville, Alberta T0B 4L0.

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concentration (LC_{50}) was 444 parts per million (ppm) (622 mg m^{-3}) when Sprague-Dawley rats of both sexes were exposed by inhalation (14). This value was based on a 4 h exposure followed by a 14 day observation period. In a comparative study, 50% of exposed rats and mice of unspecified strain and sex died after approximately 15 to 30 min of inhalation exposure at 1000 ppm (1400 mg m^{-3}) (15,16). The objectives of the present study were to investigate the effect of sex and strain of rats, duration of exposure, and spatial position in inhalation chamber, on the mortality of rats exposed to a single exposure of hydrogen sulphide; also to utilize the rat to develop an exposure model for future studies on the toxicity of hydrogen sulphide.

MATERIALS AND METHODS

ANIMALS, CARE AND HOUSING

Male and female Long Evans (LE), Sprague Dawley (SD) and Fischer-344 (F344) rats, seven to eight weeks of age, were obtained from a commercial source (Charles River Inc., St. Constant, Quebec). The animals were housed individually in stainless steel mesh cages and kept in an environmentally controlled room with a temperature of 22 ± 2°C, humidity of 50 ± 20%, and a photoperiod of 12 h light and 12 h dark. A certified laboratory rodent feed (Purina Laboratory Rodent Chow #5002, St. Louis, Missouri) and reverse osmosis water were provided *ad libitum* to the rats. There was a ten day acclimatization period, and rats were exposed to air in the exposure chambers for three days prior to exposure. Body weights were recorded immediately before and after the exposure period. The guidelines of the Canadian Council on Animal Care (17) were followed throughout the study.

GAS EXPOSURE SYSTEM

The inhalation system is shown in Fig. 1. The exposure chambers were constructed of a clear, acrylic cylinder, with two removable stainless steel cones, and had a volume of 69.3 ± 0.2 L. Each chamber held three circular stainless steel mesh cages, holding four rats per cage in individual compartments. Flows of hydrogen sulphide, 99.5% CP (Matheson Gas Products, Edmonton, Alberta), and air were adjusted to maintain the target concentration in the inhalation chambers. Exhaust air from the chambers was passed through a portable fume scrubber (Mystaire, Plainview, New York) containing a 6% sodium hypochlorite solution, before release into the atmosphere.

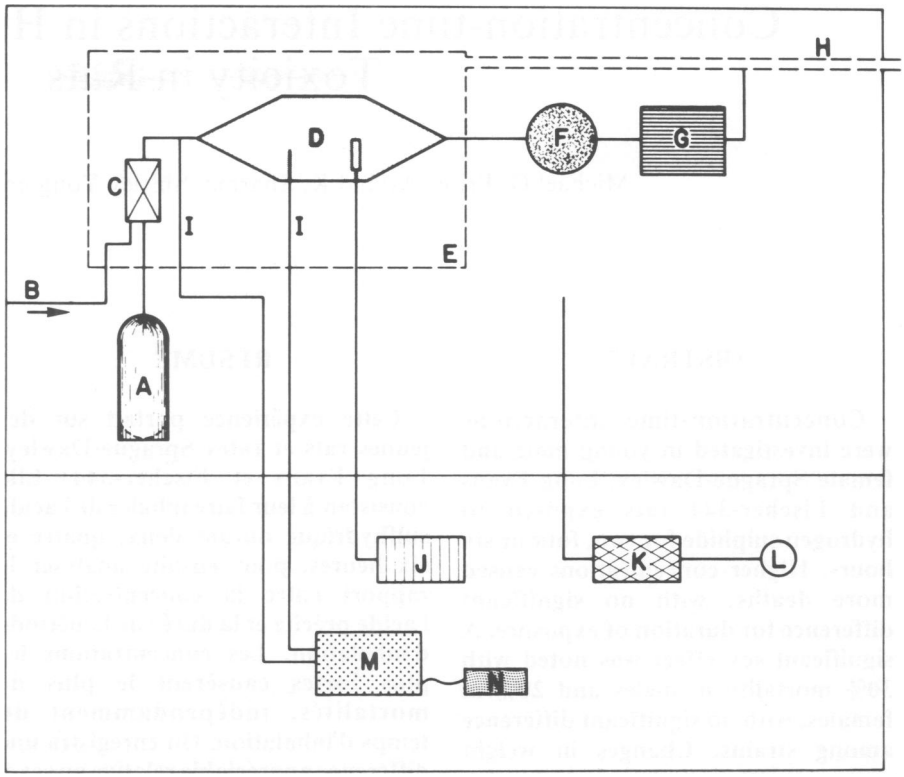

Fig. 1. Schematic of acute inhalation exposure system. (Only one of the two exposure chambers is depicted). A — gas cylinder; B — air; C — flow controllers; D — exposure chamber; E — fume hood; F — vacuum pump; G — scrubber; H — exhaust to outside; I — sample line; J — relative humidity and temperature monitor; K — H_2S monitor; L — alarm; M — gas chromatograph; N — computer.

HYDROGEN SULPHIDE MONITORING SYSTEM

The test (hydrogen sulphide) chambers were maintained at a pressure of −74.6 pascals (Pa) for the duration of the exposures. A pneumatically operated system collected samples automatically from three lines in each chamber, which were sampled approximately four times per hour. The gas chromatograph and monitoring system instruments were located in an adjacent room isolated from the exposure chambers. The samples were analyzed for hydrogen sulphide using a gas chromatograph with flame photometric detector (Hewlett Packard, Edmonton, Alberta), and a 2 x 0.003 m, 80/100 mesh, silanized porous ethylvinyl benzenedivinylbenzene copolymer bead column (Poropak QS™, Chromatographic Specialities, Brockville, Ontario). Exposure room air was monitored continuously for hydrogen sulphide using a hydrogen sulphide monitor (Interscan Corp., Chatsworth, California).

HYDROGEN SULPHIDE EXPOSURE

Rats were assigned randomly to exposure groups, which were distributed randomly within the chambers. Seventy-two males and 84 females were assigned to the 2 and 6 h exposure groups, and 72 males and 72 females to the 4 h exposure groups, and exposed to various concentrations of hydrogen sulphide. Postmortem examinations were carried out on all animals dying during the exposure or during the 14 day postexposure observation period.

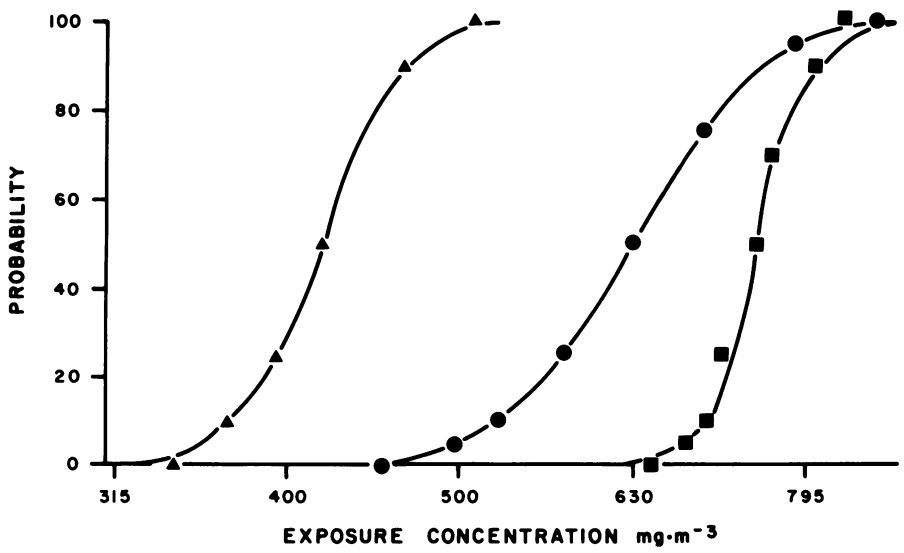

Fig. 2. Probit distribution of concentration-response for rats exposed to various concentrations of hydrogen sulphide for 2, 4 or 6 h. Legend: ▲ = 6 h, ● = 4 h, ■ = 2 h.

STATISTICAL ANALYSIS

Standard regression and analysis of covariance techniques were used to estimate the effects of hydrogen sulphide concentration, sex, hours of exposure and strain on the weight loss of animals (18). Lethal concentration (LC_{10} and LC_{50}) values were estimated by probit analysis using a maximum likelihood iteration technique. Hydrogen sulphide concentrations were transformed to the $\log_{10}$ scale for the analysis. Additional statistics on the standard deviation of the estimate, 95% confidence intervals, and the chi-square goodness of fit were also estimated (19).

RESULTS

The probit distribution of mortality in rats of all strains exposed to various concentrations of hydrogen sulphide for 2, 4 or 6 h is given in Fig. 2, and the respective probit equations in Table I. The effect of hydrogen sulphide concentration on weight loss is summarized in Table II.

Exposure to hydrogen sulphide affected males significantly more than females. Mortality in males was about 30% compared to 20% in females.

Probit analysis for the pooled data and for various subsclasses showed that for the 4 and 6 h exposure periods, the probit model provided a satisfactory fit to the data as evidenced by the nonsignificant ($P > 0.05$) chi-square statistics (Table II). Probit equations were statistically different among all the exposure periods, but were not different between male and female, and among LE, SD and F344 strains. The shape of the probit distribution for the exposure periods is shown in Fig. 2. The LC_{50}/LC_{10} values for the 2, 4 and 6 h exposures periods were 587/549 (822/769), 501/422 (701/591), and 335/299 (469/419) ppm (mg m^{-3}), respectively. The steepness of the slope of the response curves (Fig. 2) for the three periods of exposure suggests a possible overload of the detoxification mechanisms for hydrogen sulphide.

The regression equation of hydrogen sulphide concentration on weight loss (Table II) showed a significant decrease in weight due to toxic gas concentration. For every 10 ppm (14 mg m^{-3}) increase in gas concentration, the weight loss increased by 0.21 g in the pooled data. Heterogeneity of regression coefficients was noted between hours of exposure but not for the sex or strain. For this reason individual regression equations for 2, 4 and 6 h are also presented in Table II. The effect of gas concentration was highly significant on the weight loss for the 2 and 4 h exposure groups, possibly due to higher concentrations.

The effects of hours, sex, strain and their interactions on weight loss were studied by the analysis of covariance technique with hydrogen sulphide concentrations as the covariate. Results are presented in Table III and showed that except for the hours x strain x sex interaction term, all other effects were highly significant ($P < 0.01$). Weight loss was higher in males; highest in the LE and lowest in the F344 strain; and highest in the 6 h and lowest in the 2 h exposure periods. Significant ($P < 0.01$) hours x sex interaction was due to the fact that weight loss in males at 2, 4 and 6 h exposure was 61, 67 and 79% higher than in the females in the corresponding groups.

Similarly, the sex x strain interaction was influenced by the weight loss in males being 72, 85 and 54% higher than the females in the LE, SD and F344 strains, respectively. Further, the hours x strain effect was evident because the difference in weight loss between the 4 and 6 h exposure periods was much less in the F344 strain compared to the other two strains.

TABLE I. Probit Analysis of the $\log_{10}$ Hydrogen Sulphide Concentration

Data Set	Probit Equation	Standard Deviation	LC_{10} values $Cl^a<LC_{10}<Ch^b$	LC_{50} values $Cl^a<LC_{10}<Ch^b$	χ^2
Pooled	-5.7479 + 3.8259X	0.2613	49<298<378	508<644<3743	192.81
2 h	-118.3300 + 44.5478X	0.0224	[c]-<549<597	[c]-<587<-[c]	78.96[d]
4 h	-41.6105 + 17.2645X	0.0579	364<422<447	477<501<545	19.16
6 h	-62.4003 + 26.6980X	0.0374	284<299<309	325<335<345	3.28
LE	-6.2369 + 4.0157X	0.2490	18<301<385	491<628<10086	73.04[d]
SD	-2.8743 + 2.7597X	0.3623	[c]-<244<362	493<713<-[c]	85.69[d]
F344	-9.4625 + 5.1946X	0.1925	60<344<422	499<608<3317	86.18[d]
Male	-3.5296 + 3.0565X	0.3271	[c]-<235<357	433<617<-[c]	90.08[d]
Female	-9.6517 + 5.2194X	0.1915	0<364<-[c]	506<641<-[c]	108.25[d]

[a]Lower limit of 95% confidence interval
[b]Higher limit of 95% confidence interval
[c]Nonestimable confidence interval values
[d]($P < 0.01$) for χ^2 test of goodness to fit

TABLE II. Effect of Hydrogen Sulphide Concentration on Weight Loss of Animals

Group	n	Intercept	Slope ± Standard Error	Mean Standard Error	% Coefficient of Determination
Pooled	456	-21.632	0.0219[a] ± 0.0022	5.60	17.89
2 h	156	-21.564	0.0240[a] ± 0.0048	3.53	13.89
4 h	144	-26.034	0.0279[a] ± 0.0070	5.27	10.11
6 h	156	-13.282	0.0063 ± 0.0097	6.88	0.27

[a]Highly significant ($P < 0.01$)

The mouths and noses of all animals dying from hydrogen sulphide exposure contained considerable amounts of foamy fluid. The lungs failed to collapse when the thorax was opened and had severe pulmonary edema. The trachea and bronchi also contained large amounts of foamy fluid. Histological examination of the affected lungs revealed considerable amounts of proteinaceous fluid in the conductive airways, alveoli, and around the perivascular space of major blood vessels. These lesions were extensive enough to account for death and were similar to those described in humans killed by accidental exposure to hydrogen sulphide (20).

The mean hydrogen sulphide concentration was almost identical at all 12 sampling positions, indicating a uniform flow and distribution of hydrogen sulphide within the chamber. As stated in the methods section, there were 12 positions in the inhalation chamber to which animals were assigned during the study. With respect to the distribution of dead animals within the chambers, the calculated chi-square value of 13.19 was not significant ($P > 0.05$), therefore the mortality of animals was concluded to be independent of their position within the chamber.

DISCUSSION

The magnitude of the dose in inhalation exposures varies directly as the concentration C of hydrogen sulphide to which the rats were exposed, the duration t of their exposure, together with α the retention factor. Thus, dose = αC.t.MV, where MV is the minute volume. Haber (21) recognized the practical use of the product C.t, and this has become known as Haber's rule, or C.t = k. That is, if $C_1.t_1$ elicits some response, then $C_2.t_2$ will also produce the same response provided $C_1.t_1 = C_2.t_2$. This rule holds reasonably well if the range over which C or t is varied is limited. Deviations occur at extreme values of these variables. The plot of C.t = k is a rectangular hyperbola. In the present study, the values for 4 and 6 h exposure appear to conform to the criteria for Haber's rule, with values of 40.3/34.1 and 40.7/36.4 for 50 and 10% mortality at 4 and 6 h exposure respectively. The data from the 2 h exposure deviated markedly from these values, being approximately 40% less. This is probably because the equilibration time for the inhalation chamber is excessive for this period of exposure. That is, the equilibration time is large in comparison to the total exposure period.

TABLE III. Analysis of Covariance of Independent Variables on Weight Loss

Source of Variation	Degrees of Freedom	Sum of Squares	F-Value
Concentration	1	102	8.07[a]
Hours	2	833	32.89[a]
Sex	1	4740	374.11[a]
Strain	2	2267	89.49[a]
Hours x sex	2	380	15.03[a]
Sex x strain	2	387	15.27[a]
Hours x strain	4	309	6.12[a]
Hours x sex x strain	4	40	0.79
Residual	437	5537	

[a]Highly significant ($P < 0.01$)

Results of this study are similar to those reported by Tansy (2); and the value by Haber's rule at the LC_{50} was 35.8. In the present study, the probit equation was Y = -42.03 + 17.38X, with LC_{50}/LC_{10} values of 508/428 ppm (711/599 mg m^{-3}) and standard deviation of 0.057, for the pooled data from the Sprague-Dawley male and female rats exposed for four hours. The probit model fitted the data well as demonstrated by a nonsignificant ($P > 0.05$) χ^2 test of goodness of fit. The 95% confidence interval for LC_{50} ranged from 380 to 584 ppm (532 to 818 mg m^{-3}), and covered the value of 444 ppm (622 mg m^{-3}), reported by Tansy *et al* (14).

The severity of pulmonary edema observed in rats killed by hydrogen sulphide was extensive enough to incriminate this lesion as the most probable cause of death. Pulmonary edema has been reported as the most notable lesion in individuals killed by accidental exposure to hydrogen sulphide (20). Experimental exposure of laboratory animals to sublethal concentrations of hydrogen sulphide confirmed that this gas is edematogenic for the lung (22). The steepness of the dose-response curve (Fig. 2) suggests that once a threshold is reached pulmonary edema and death follow rapidly (22). Further work will be required to elucide the increased susceptibility of females.

An experimental rat exposure model has been established from these findings for future investigations of the toxicity of hydrogen sulphide.

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