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University of Alberta

Biological Variability in Risk Assessment Modeling of Industrial Gases

by

Bing Guo 

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirement for the degree of Master of Science

in

Medical Sciences – Public Health Sciences

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Dr. Steve Hrudey

Dr. David J Wilson

Dr. Warren Kindzierski

Date 6 April 2001

This thesis is dedicated to my parents

Bingcao Hu and Youhuan Guo

Abstract

The toxic load concept and probit analysis have been widely used in risk assessment of human acute exposure to industrial gases. This approach is subject to great uncertainty in estimating outcomes. Among many factors, biological variability is a major source of overall uncertainty. This thesis takes a closer look at biological variability in causation and response to toxic gases to provide better understanding for extrapolating animal toxicology data to humans. Four industrial gases, hydrogen sulfide (H_2S), chlorine (Cl_2), ammonia (NH_3), and hydrogen fluoride (HF), were chosen because of their heavy industrial use, highly toxic nature, and contribution to offensive odor. Inter-species and intra-species differences in the respiratory anatomy and physiology, as well as in response to the four gases were documented and evaluated. Human inter-individual variability in odor perception was summarized and discussed. A maximum uncertainty factor of 30 was suggested to represent human diversity in response to the lethal effects of the four gases. Appropriate expressions of inter- and intra-species variability with regard to the 3-dimension relationship between exposure concentration-time-response were presented.

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Glossary

Absorption – the transfer of a chemical from the sites of exposure, usually an external or internal body surface (e.g., skin, mucosa of the gastrointestinal and respiratory tracts) into the bloodstream.

Acute – of short duration; minutes to (max 24) hours.

Acinus – a general term describing a sac-like structure. In the case of the lungs it indicates an airway branch that connects a distal unit containing the gas-exchange surfaces of the lung in the form of respiratory bronchioles, alveoli, and alveoli ducts.

Alveolar ventilation – the tidal volume minus the volume of the dead space.

Area under blood concentration-time curve (AUC) – the concentration of a chemical in blood (plasma or serum) integrated over time.

Asphyxia – pathological changes caused by lack of oxygen in respired air, resulting in hypoxia and hypercarpnea.

Biological half-life ($T_{1/2}$) – the period of time necessary for one-half of a substance to disappear. In toxicology this may refer to such phenomena as the disappearance of a toxicant from the blood stream or from the body, or the time necessary for one-half of the total amount of a toxicant to be metabolized.

Blood-air partition coefficient – a constant of the solubility ratio of a chemical between the blood and air.

Bronchus – large airways distal to the trachea whose walls contain cartilaginous plates, smooth muscle, and submucosal glands.

Bronchiole – distal airways, smaller in caliber than bronchi, differing from the bronchi in having no cartilage plates and having cuboidal epithelial cells.

Cartilage – a specialized, fibrous connective tissue.

Cicatrisation – the formation of a scar.

Clearance – the subsequent translocation, transformation, and removal of deposited substances from the respiratory tract or from the body.

Coagulation – the process of clot formation.

Concentration – fraction of a given substance in a medium (air, water, ground, another substance).

Conjunctiva – the delicate membrane that lies the eyelids.

Deposition coefficient – a fraction of total inhaled gas or vapor that deposits on the airway wall of the respiratory tract. It is also called total intake.

Desorption – the reverse process of absorption.

Diffusion – the spontaneous movement of molecules or other particles in solution, owing to their random thermal motion, to reach a uniform concentration throughout the solvent, a process requiring no addition of energy to the system.

Diffusion coefficient – a constant of the ratio of water solubility (S) of a chemical and molecular weight (MW), as $\frac{S}{MW^{1/2}}$

Dispersion – spreading in the medium in which the substance has been released and mixing with this medium.

Distribution – a process whereby the absorbed chemical is transported by the blood stream to various organs and tissues.

Dose – 1. the total quantity of absorbed substance
2. function of concentration and exposure duration.

Dose-response relationship – a correlative relationship between the magnitude of various doses and intensity of toxic response following exposure.

Edema – the presence of abnormally large amounts of fluid in the intercellular tissue spaces of the body; usually applied to demonstrable accumulation of excessive fluid in the subcutaneous tissues.

Elimination – a process including biotransformation, exhalation and excretion.

Endothelium – the layer of epithelia cells that lines the cavities of the heart and of the blood and lymph vessels.

Epiglottis – the lidlike cartilaginous structure overhanging the entrance to the larynx and serving to prevent food from entering the larynx and trachea while swallowing.

Epithelium – the covering of internal and external surfaces of the body, including the lining of vessels and other small cavities. Epithelium is classified into types on the basis of the number of layers deep and the shape of the superficial cells.

Excretion – a process whereby a chemical is eliminated from the body by several routes, such as urine, feces and lungs.

Expiratory reserve volume (ERV) – the maximum volume of gas that can be expired from the end-expiratory level.

Geometric standard deviation (GSD) – the antilog of the standard deviation of the distribution of $\log_{10}$ parameters.

Gland – an aggregation of cells, specialized to secrete or excrete materials not related to their ordinary needs.

Inflammation – a localized protective response elicited by injury or destruction of tissues. It is characterized in the acute form by the classical signs of pain, heat, redness, swelling, and loss of function.

Inhalation – through the respiratory system

Inpiratory reserve volume (IRV) – the maximum volume of gas that can be inpired from the end-inpiratory level.

Inter-species differences – differences of one type versus another species.

Interstitial – pertaining to or situated between parts or in the inter-spaces of a tissue

Intra-species differences – differences between individuals of one and the same type (intra-species = inter-individual)

Irritant – xenobiotics that can exert their effects by direct contact with the skin or mucous membranes of the eye, respiratory or gastrointestinal tracts.

Lethal concentration – a concentration at which a given percentage of the exposed population will die during a specific time.

Linear regression – Statistical technique whereby, based on a number of observations (x_i, y_i), the parameters a and b of the function $y = a + bx$ are determined.

Liquefaction – the conversion of a material into a liquid form.

Lobe – a more or less well-defined portion of any organ, especially of the brain, lungs, and glands. Lobes are demarcated by fissures, sulci, connective tissue, and by their shapes.

Lung parenchyma – the portion of the lungs involved in gas exchange. The most prominent structure in this region is the alveolus.

Metabolism – a process whereby the absorbed substances are converted from one form to other forms by enzymatic systems in living organisms.

Minute volume – the volume of inspired or expired gas for one minute, i.e., tidal volume multiplied by the frequency of breath.

Nares – the two halves of the nasal passages; also, the external and internal openings of the nasal passages.

Necrosis – the sum of the morphological changes indicative of cell death and caused by the progressive degradative action of enzymes; it may affect groups of cells or part of a structure or an organ.

Palate – the partition separating the nasal and oral cavities.

Peak concentration (Cmax) – the measured value of highest concentration of a chemical in the blood, which is one of parameters for estimating the rate of absorption of a chemical.

Perfusion – the process that a fluid passes through the vessels of a specific organ.

Permeability – the rate of diffusion of a xenobiotic through each unit area of the barrier for a unit concentration difference.

Probit – probability unit; obtained by a statistical transformation of a percentage; very sensitive in the range around 50%, and with little sensitivity in ranges near 0 and 100%.

Probit analysis – statistical technique, with which the relationship between response and stimulus (i,e., exposure to toxic substances) is presented.

Probit of risk – a standard form for expressing the incidence of an adverse effect that basically converts an assumed gaussian distribution of individual thresholds of sensitivity into a linear index. The probit distribution has a mean of 5 and a standard deviation of 1.

Reactive airways dysfunction syndrome – the symptoms of which are the onset of cough, shortness of breath, and wheezing after a single inhalation exposure to high concentrations of toxicants. This conditions is also referred to as irritant induced asthma, which is characterized by the presence of asthma-like syndromes and non-specific bronchial hyper-responsiveness in subjects who have no previous history of asthma.

Respiratory bronchioles – the transition between conducting and respiratory regions of the lungs. These airways contain alveolar outpocketings in their walls, which are lined with squamous (type I) and cuboidal (type II) epithelial cells.

Retention – the temporal distribution of uncleared deposited materials.

Risk – a prediction of feature likelihood of an event or set of circumstances (a hazard) leading to adverse consequences over a specified time period.

Risk analysis – A detailed examination performed to understand the nature of unwanted, negative consequences to human life, health, property, or the environment; an analytical process to provide information regarding undesirable events; the process of quantification of the probabilities and expected consequences for identified risks.

Risk assessment – a process that seeks to estimate the adverse health consequence of a specified set of conditions, which includes: (1) hazard identification; (2) dose-response assessment; (3) exposure assessment; and (4) risk characterization.

Rubefaction – becoming red.

Soft palate – the posterior, fleshy part of the palate.

Species – (animal) type.

Standard deviation – measure for the spread in the observations.

Strain – a group of organisms of the same species possessing distinctive hereditary characters that distinguish them from other such groups.

Terminal bronchiole – the most distal generation of non-alveolarized conductive airways.

Tidal volume (TV) – the volume of gas inspired and expired during each respiratory cycle.

Total lung capacity (TLC)– the volume of gas in the lungs at the end of a maximum inspiration.

Toxic – Poisonous.

Toxicity – the ability of a substance to produce an adverse effect when it has reached a sufficient concentration at a certain site in the body.

Toxicodynamics – the study of action of toxicants on organisms and mechanisms of actions.

Toxicokinetics – the application of pharmacokinetics principles to animal toxicity studies to provide information on the movement of toxicants within the body, including absorption, distribution, metabolism and excretion).

Toxic load – a combination of duration of exposure to a toxic material (t) with the concentration (C) of that material, to give a measure of the toxic effects that will be produced. For most cases, the toxic load is expressed as $C^n t$ if C is constant, or $\int C^n dt$ if exposure is instantaneous, where n is a constant that is determined empirically.

Ventilation – the process of exchange of air between the lungs and the ambient air.

Ventilation/perfusion ratio (V_A/Q) – the ratio of ventilation (V_A) to blood flow (Q) remains constant although the ventilation or blood flow changes with time.

Ventilatory unit – the basic unit of ventilation consisting of the alveoli and alveoli ducts distal to the transition from one bronchiole to an alveolar duct system.

Vital capacity (VC) – the maximum volume of gas that can be expelled from the lungs by forceful effort following a maximum inspiration.

Vulnerability model – relationship between response fraction and dose for a given type of injury.

Wall – an investing part enclosing a cavity, chamber, or any anatomical unit.

List of Abbreviations

ACGIH	American Conference of Governmental Industrial Hygienists
AIHA	American Industrial Hygiene Association
ASHRAE	American Society of Hearing and Air-Conditioning Engineers
ASTM	American Society for Testing and Materials
ATSDR	Agency for Toxic Substances and Disease Registry
AUC	area under the curve
BUN	blood-urea-nitrogen
C	concentration
CCPS	Center for Chemical Process Safety
CEH	Chemical Economics Handbook
Cl_2	chlorine
Cmax	peak concentration
CNS	central nervous system
FEV_1	forced expiratory volume in the first second
EPA	Environmental Protection Agency
FRC	functional residual capacity
GM	geometric mean
GSD	geometric standard deviation
HCN	hydrogencyanic acid
HCl	hydrochloric acid
HF	hydrogen fluoride
HOCl	hypochlorous acid
H_2S	hydrogen sulfide
HSE	Health and Safety Executive
IChemE	Institution of Chemical Engineers
K	constant value
kg	kilogram
L	toxic load
LC	lethal concentration
LC_{50}	median lethal concentration
LD	lethal dose
LD_{50}	median lethal dose

m	meter
mg	milligram
min	minute
ml	milliliter
mm Hg	millimeters of mercury
n	exponent
NAS	National Academy of Sciences
NH_3	ammonia
NIOSH	National Institute for Occupational Safety and Health
NPN	non-protein nitrogen
NO_2	nitrogen dioxide
NRC	National Research Council
O_3	ozone
OSHA	Occupational Safety and Health Administration
P	probability
pKa	the negative log of the acid dissociation constant, Ka
ppb	parts per billion
ppm	parts per million
RD_{50}	concentration at which the respiratory rate is reduced by 50%
S	intensity of odor sensation
SO_2	sulfur dioxide
t	time
$T_{1/2}$	biological half-life
TLC	total lung capacity
TLV	threshold limit value
TNO	The Netherlands Organization of Applied Scientific Research
TWA	time-weighted average
μ	mean value
V	minute volume
VC	vital capacity
VT	tidal volume
W	body weight
WHO	World Health Organization
Y	probit value

Chapter 1
Introduction

1.1 Background

Some industrial gases may have a significant negative impact on human health. Experience comes mainly from occupational exposure in the workplace and more rarely from release of gases in residential areas near industrial sites. Some industrial gases are sufficiently toxic in relation to the quantities used so that they can pose a serious health risk. Four gases, namely hydrogen sulfide, chlorine, ammonia, and hydrogen fluoride, have been selected for this study because of their extensive industrial use, their acute toxicity, and the need to manage the risk of large accidental releases.

Hydrogen sulfide (H_2S) is the major chemical hazard of sour natural gas production, although it is also found in other places such as municipal sewers and sewage treatment plants, pulp and paper operations, or manure-handling operation (Guidotti 1996). Sour gas is the raw natural gas mixture containing various sulfur compounds. In Alberta, hydrogen sulfide is the predominant sulfur contaminant of sour gas with concentrations ranging from 0.01% to more than 30% (Alberta Environment 1981, Arnold et al. 1985). Hydrogen sulfide has been widely recognized as a toxic asphyxiant and irritant gas. At high concentrations, it rapidly causes unconsciousness and death, whereas at lower concentrations it may cause unpleasant odor, eye and respiratory tract irritation (Beauchamp et al. 1984). Health effects of exposure to hydrogen sulfide have become a high public concern, especially after a sour gas well near Lodgepole, Alberta blew out of control in 1982 (Alberta Health 1988).

Chlorine was the first gas used in chemical warfare during World War I. Chlorine gas has been well recognized as a potent respiratory irritant (Benjamin and Pickles 1997). It is also extremely irritating to the eyes and skin (Barrow et al. 1977). Today, industry uses millions of tons of chlorine annually in the manufacture of chemicals, plastics, and paper and for disinfection of drinking water and sewage (Anonymous 1984). In 1998, the production of chlorine was 12.3 million tons in the United States and 990,000 tons in Canada (CEH 1999). Some important scenarios of exposure to chlorine gas arise from

transportation release (Kowitz et al. 1967, Weill et al. 1969), industrial accidents affecting both workforce (Beach et al. 1969, Dixon and Drew 1968) and surrounding population (Chester et al. 1977, Kaufman and Burkons 1971), school chemistry experiments (Edwards et al. 1983), storage tank failures (Fleta et al. 1986), and swimming pool chlorination mishaps (Ploysongsang et al. 1982). Internationally, chlorine gas accounts for the largest single component of major toxic release incidents (Davis et al. 1989). The most serious incident caused by the rupture of a storage vessel in Romania in 1939 resulted in 68 deaths and 332 injuries (Health & Safety Executive, unpublished, cited by Baxter et al. 1989).

Ammonia (NH_3) has attracted scientific research because of its beneficial uses and harmful properties to humans, animals, and environments. Ammonia is widely used in a variety of industrial processes and as a fertilizer (NRC 1979a). In the US, about one-half million Americans are employed by industries in which the production of ammonia is around 20 million tons per year (WHO 1990). Historically, severe injuries and even deaths occurred after accidental release of ammonia gas during transportation from one storage or transportation tank to another. The less severe but much more common health effect of ammonia gas is its strong irritation to skin, eyes, and mucous membrane of the upper respiratory tract, and lungs (NRC 1979a). In February 2001, about 5,000 residents of Red Deer, Alberta were evacuated after a train transporting anhydrous ammonia derailed releasing ammonia. About 25 people had to be treated in hospital. One individual was found unconscious near the site of the spill and was hospitalized with serious injuries (Crowson and Dumont 2001).

Hydrogen fluoride (HF) is one of the main gaseous fluoride components in the working environment in the primary aluminum industry (Lund et al. 1997). This corrosive mineral acid is one of the most common and most deadly industrial chemicals in the world (O'Neil 1994). In 1998, the production of hydrogen fluoride was 171,000 tons in the United States (CEH 1999). Hydrogen fluoride is also used in the production of unleaded gasoline, which is the main use of hydrogen fluoride in Alberta. An accidental hydrogen fluoride release from the rupture of a storage tank, occurred in 1987 in Texas City, caused 1,037 hospitalizations with inhalation injuries (Alexeeff et al. 1993, Selcraig

1992). Hydrogen fluoride mainly produces corrosive burns of the skin. When inhaled, it also results in severe burns to the mucous membranes, bronchospasm, severe pulmonary edema, and even death (MacKinnon 1988).

In addition to these toxic effects, offensive odors emitted from these gases are important in industrial practice because of the nuisance they can cause. The significant characteristic of these odorants is that the odor thresholds are relatively low compared to many other chemicals; for instance, odor of hydrogen sulfide can be detected at a concentration as low as of 0.007 ppm (ASHRAE Handbook 1997). Both in the workplaces and residential areas near industrial sites, odor itself is a workers' concern and is an important source of public complaints; in some areas it counts for more than fifty percent of total complaints about industrial processes (Clarenburg 1987). How to evaluate and control odor pollution is an inevitable challenge in risk assessment and management of industrial gases.

Given the industrial importance and potential public health impact of these four gases, effective safety guidelines on the operation, storage, and transportation of these gases are needed to protect the population inside and outside the workplace from exposure caused by accidental releases. In order to develop such a guideline, the knowledge of acute inhalation toxicity of these gaseous chemicals is required for the purpose of risk analysis. In the past, numerous animal experimental studies have been conducted to establish dose-response relationship, and some formulae describing such relationships have been proposed.

The toxicity equations have been derived strictly by empirical curve fitting. To some extent, the models do capture the non-linear relationship between concentration, time, and response. It is obvious, however, that some difficulties might well be encountered when interpreting the empirical relationships for estimating human risk. First of all, the simplified models do not contain any physiological parameters representing the key biological processes underlying toxicity (Hilderman et al. 1999). Without comparison of any physiological parameters in animals and humans, it is difficult to adapt quantitative animal models to the human case. Another important consideration should be taken into account in the model is human variability in susceptibility. Human biological variability

is significantly greater than that among experimental animals used in toxicology studies (WHO 1999). Variability is a major source of uncertainty in estimating the consequences of hazardous release. Application of animal data to humans requires information on both inter-species and human inter-individual variability. The current models do not effectively reflect this important aspect.

1.2 Objectives and Scope

This thesis was triggered by the two problems existing in the toxic load models: lack of consideration of biological factors and biological variability. The objectives of this thesis are:

- Analytically evaluate toxicokinetics evidence on the four gases and identify the most important physiological parameters representing causal processes;
- Analytically evaluate acute toxicity studies on the four selected industrial gases and develop an understanding of underlying mechanisms of toxicity for the purposes of modeling;
- Summarize and analyze available data on inter-species variability and inter-individual variability in response to the four industrial gases, and provide an estimation of uncertainty factors representing the biological variability;
- Evaluate available data on human inter-individual variability in odor perception, and provide a basic frame to develop modeling of nuisance odors.

This thesis is dealing with acute inhalation toxicity of the four selected gases. Exposure through other routes such as via the gastrointestinal tract or skin are not considering in this thesis. The exposure duration should be from minutes to a few hours (less than 24 hours). Chronic exposure to the low levels of the four gases will not be considered in this thesis.

1.3 Structure

Detailed description and discussion of toxic load and probit analysis are provided in Chapter 2. The concepts of variability and uncertainty and their implications in the

predictive modeling are discussed in Chapter 3. Some factors involved in the causal process of toxicity of the four gases are summarized in Chapter 4. The variability in susceptibility in response to the four gases is described and discussed in Chapter 5. Because odor is a complex individualized response with its own specific characteristics, odor is discussed separately in Chapter 6. Overall discussion is provided in Chapter 7. Conclusions and recommendations for future research are provided in Chapter 8.

Chapter 2

Toxic Load Model and Probit Analysis

2.1 Haber's Law

In inhalation toxicology, the expression of "dose" of a gaseous toxicant is different from that for solid or liquid toxicants that enter body through oral or injection routes. For a gas or vapor, not only the concentration in the air, but also the exposure duration need to be considered to determine how much toxicants actually enter the respiratory tract (Doe and Milburn 1983). Therefore, the "dose" of a gas or vapor has two dimensions, i.e., exposure concentration, and exposure duration. For example, for a solid substance, LD_{50} (the lethal dose that kill 50% exposed population) can be simply expressed as 500 mg/kg. But for a gas or vapor, LC_{50} (the lethal concentration that kill 50% exposed population) should be expressed as 500 ppm for a specific time, for example 30 minutes.

Based on the extensive studies of the acute lethal toxicity of several military poison gases on experimental cats at various concentrations and exposure time, Fritz Haber, professor at Kaiser Wilhelm Institute and head of the Chemical Section of the War Ministry during the First World War, suggested that for the observed fatal response of some gases the product of concentration and time of an inhaled toxicant is constant (Haber 1924). That is,

$$C \times t = K \tag{2.1}$$

where C is the mean exposure concentration

t is the exposure duration

K is a constant value for a given level of fatalities

The smaller this product, K, the more toxic the agent. Consequently, this product, also called lethal index, was used to compare the toxicity of different chemicals. According to Haber's law, the toxicity of a toxicant equally depends on both concentration and time, as a cumulative dose. The toxicity caused by inhalation of a high concentration of gases for a short time would be equal to that by very low concentration for a relatively long time. This particularly held true for phosgene (Wachtel 1941).

On the other hand, in Haber's original paper, hydrocyanic acid (HCN) was mentioned as an exception. The toxicity of hydrocyanic acid is more dependent on the concentration, so that the lethal index, C × t, is not a constant if the concentration is below a certain limit. Below this concentration limit, the product of C × t had to be much higher for the defined fatality response. This observation indicates that for gases exposure duration is much less important than concentration. Table 2.1 shows the values of the product of C × t for some gases, including chlorine, used in warfare.

Table 2.1 Values of C × t product for several war gases

Substances	C × t (mg/m^3 min)
Bromoacetic acid ethyl ester	3000 and lower
Chloracetone	3000
Xylylbromide	6000
Chlorine	7500
Perchlormethyl mercaptan	3000 and lower
Hydrocyanic acid (HCN)*	1000
Phosgene	450
Perchloroformic acid methyl ester	500

* For HCN, the value for C × t is dependent on concentration. The value given here is based on the concentration of 1/2 ‰. At lower concentrations the value of C × t is much higher.

Adapted from Haber 1924, with translation from German into English

As shown in Table 2.1, in Haber's paper (1924), the level of fatalities was not specified, and the range of concentrations and exposure times applied in these studies was not provided. For example, in the case of chlorine, we don't know what percentages of experimental animals will be killed at the product of 7500. Later on, this product for chlorine was interpreted as the value causing 10% mortality in cats (Withers and Lees 1985a).

Consequently, Haber's law has been misunderstood and misused in the field of inhalation toxicology (Flury and Zernick 1932). One major misunderstanding is that Haber's law was thought to be able to apply to every gas and vapor. Indeed, the establishment of Haber's product law has been particularly influenced by the observation on phosgene (Flury 1921). Phosgene belongs to a class of gases that are not absorbed by the organisms and eliminated from the body; rather, phosgene only causes local damages

of the respiratory tract (Flury 1921, Flury and Zernick 1932). Absence of an elimination mechanism explains why the effect could be the same for a constant C × t even at low concentration values. Mustard gas is another good example of Haber's law (Wachtel 1941). For this type of gas responses mainly depend on the cumulative dose. However, for the gases and vapors that are first absorbed into the blood and then exert their effects, the product of C × t cannot be constant (Flury and Zernick 1932).

2.2 Toxic Load and Exponent n

Busvine (1938) investigated the toxicity of ethylene oxide to some insect pests and found that the concentration-time-mortality relationship can be well described by the formula:

$$C^n t = K \tag{2.2}$$

where C is the mean exposure concentration

t is the exposure time

n is a constant, which is usually greater than one

K is a constant value for a given level of fatalities

Later, much more extensive studies were done on the concentration-time-response relationship of a variety of toxic gases and vapors including the four gases selected in this analysis (Arts et al. 1989, Appelman et al. 1982, Bitron and Aharonson 1978, Dalbey and Bannister 1998, Kapeghian et al. 1982, Prior et al. 1988, Silver and McGrath 1948, Tansy et al. 1981, Weedon et al. 1940, Wohlslagel et al. 1976, Zwart and Woutersen 1988, Zwart et al. 1990). Mortality was chosen as the endpoint of the acute toxicity of toxic substances in many studies, while a certain degree of pulmonary damages was also employed as biological indicator of toxicity to investigate the validity of Haber's law (Gelzleichter et al. 1992). It has been found that, like hydrocyanic acid, the concentration-time-response relationship does not always obey Haber's law. Rather, the relationship is better described by the formula $C^n t = K$ as suggested by Busvin (1938). This relationship is then described by the term of toxic load, L:

$$L = C^{n} t \qquad (2.3)$$

where L is a critical value that varies for each effect level and gas

The toxic load that produces 10% or 50% lethality is expressed as LL_{10} or LL_{50}, respectively. The unit of toxic load is usually expressed as $ppm^{n}min$, or $(mg/m^3)^{n}min$, depending on the units of concentrations used. For most cases, the toxic load is expressed as $C^{n}t$ if C is relatively constant, otherwise as $C^{n}dt$ when exposure is instantaneous.

By using the method of probit analysis that is described below, ten Berge and his colleagues (1986) re-analyzed raw data from previously published studies on acute inhalation toxicity of a variety of industrial gases. About twenty gases, including the four gases investigated in this thesis, were selected for their analysis. The gases were categorized into two groups of either local irritants such as ammonia, chlorine, and hydrogen fluoride, or toxicants like hydrocyanic acid and hydrogen sulfide that act systemically. The authors suggest that the product term $C^{n} t$ predicts the mortality response to these industrial chemicals better than the product of $C \times t$ for quite a number of gases. The exponent n for these gases lies between 1 and 3.5, and the value of n of most gases is greater than 1 and between 2 and 3. For the four gases selected in this thesis, n is between 2 and 3.5 (Table 2.2).

Table 2.2 Values of the exponent n for the four gases

Gas or vapor	Exponent n	95% confidence limits
Local irritants		
Chlorine	3.5	(2.5, 4.4)
Ammonia	2.0	(1.6, 2.4)
Hydrogen fluoride	2.0	(1.2, 2.8)
Systemic action		
Hydrogen sulfide	2.2	(1.6, 2.7)

Adapted from ten Berge et al. 1986

These studies used different animal species including mice, rats, guinea pigs, cats, rabbits, dogs, monkeys, and goats. For the same gas, exponent n derived from different animal species may be different. For example, for hydrogen cyanide, n is 1.6 from rats, 1.8 from monkeys, 2.2 from goat, 2.8 from cats, 3.1 from dogs, and 4.3 rabbits. Another

noteworthy point here is that there seems to be no significant difference in the value of n between locally and systemically acting chemicals. No explanation for this was found in the literature.

The exponent n reflects the importance of concentrations of a toxicant for the specified toxicity. If n is equal to 1, concentration and time could be changed equally (by proportion), i.e., double concentration and halve time will yield same toxic load. When n is greater than 1, compared to the change in exposure time, a relatively smaller change in concentration is needed to keep a specified level of toxic load. Compared to Haber's law, the $C^n t$ relationship leads to more cautious values for hazardous concentrations for short time exposure (Prugh 1995). For example, for 5-minute exposure to chlorine, the LC_{50} is 1000 ppm from $C^2 t$ relationship, while a much higher concentration of 2400 ppm would be expected for the same level of effects assuming Haber's law applied.

2.3 Probit Analysis

The method of probit analysis developed originally from investigations on the effectiveness of insecticides (Bliss 1934a, b, 1935, Bliss and Broadbent 1935). It has long been noted that in many organisms, the population response, plotted against the logarithm of the concentration or logarithm of exposure time, follows a cumulative normal distribution with S-shaped curve. This distribution can be fitted to a lognormal distribution that will be further discussed in Chapter 3. The probit (probability unit) method provides a numerical transformation method to convert the S-shaped dose-response curve into a straight line (Figure 2.1).

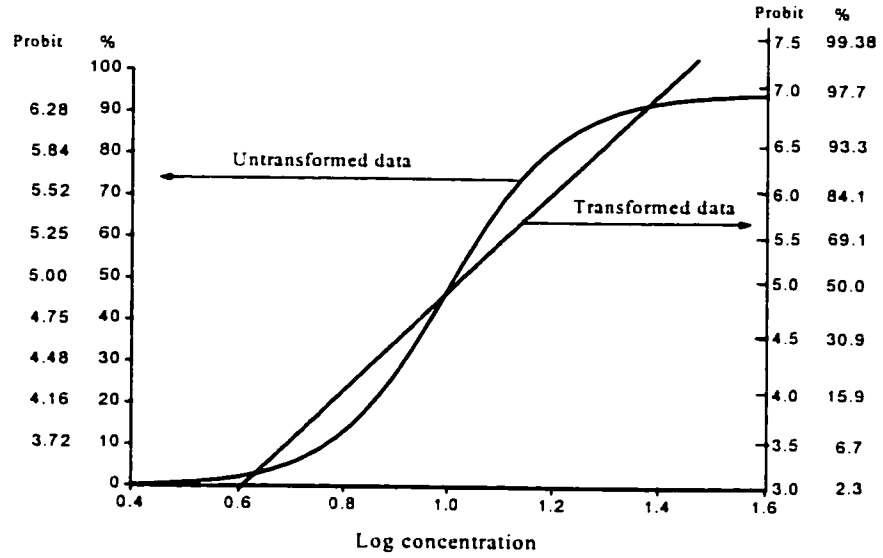


Figure 2.1 Transformation of S-shaped curve to a straight line by converting percentages to probits

Probit method is particularly designed to describe the variability in individual susceptibility to a toxic substance (Griffiths and Megson 1984). The probit variable Y is related to the probability P by (Finney 1971):

$$P = \frac{1}{\sqrt{2\pi}} \int_{-\infty}^{Y-5} \exp\left(-\frac{1}{2}u^2\right) du \qquad (2.4)$$

where P is interpreted as the percentage of population affected by a toxic exposure

u is an integration variable.

The probit variable is normally distributed, with a mean of 5 and a standard deviation of 1. The fraction of the responding population P can be also calculated from the probit value Y by using a more useful expression for spreadsheet computations:

$$P = 50\left[1 + \frac{Y-5}{|Y-5|} erf\left(\frac{|Y-5|}{\sqrt{2}}\right)\right] \qquad (2.5)$$

where "erf" is the error function. A table of converting percentages to probits is given in Table 2.3.

Table 2.3 Transformation of percentages to probits

%	0	1	2	3	4	5	6	7	8	9
0	—	2.67	2.95	3.12	3.25	3.36	3.45	3.52	3.59	3.66
10	3.72	3.77	3.82	3.87	3.92	3.96	4.01	4.05	4.08	4.12
20	4.16	4.19	4.23	4.26	4.29	4.33	4.36	4.39	4.42	4.45
30	4.48	4.50	4.53	4.56	4.59	4.61	4.64	4.67	4.69	4.72
40	4.75	4.77	4.80	4.82	4.85	4.87	4.90	4.92	4.95	4.97
50	5.00	5.03	5.05	5.08	5.10	5.13	5.15	5.18	5.20	5.23
60	5.25	5.28	5.31	5.33	5.36	5.39	5.41	5.44	5.47	5.50
70	5.52	5.55	5.58	5.61	5.64	5.67	5.71	5.74	5.77	5.81
80	5.84	5.88	5.92	5.95	5.99	6.04	6.08	6.13	6.18	6.23
90	6.28	6.34	6.41	6.48	6.55	6.64	6.75	6.88	7.05	7.33
%	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
99	7.33	7.37	7.41	7.46	7.51	7.58	7.65	7.75	7.88	8.09

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

$$Y = a + b_1 \ln C + b_2 \ln t \qquad (2.6)$$

where Y is the probit, a measure related to the percentage of an exposed population that suffer a given level of damage ranging from irritation to fatalities

a, b_1, and b_2 are regression coefficients

C is exposure concentration, and

t is exposure duration

The values of coefficients a, b_1, and b_2 for four selected gases derived by ten Berge et al. (1986) are given in Table 2.4.

Table 2.4 Regression coefficients of the concentration-time-mortality response relationships of the four gases according to the equation: $Y = a + b_1 \ln C + b_2 \ln t$

Chemical	Species, sex	Regression coefficients			Reference
		a	b_1	b_2	
Local irritant					
Chlorine	Mouse	-23.2	3.82	1.10	Bitron & Aharonson 1978
Ammonia	Male + female rat	-47.9	4.65	2.30	Appelman et al. 1982
	Male rat	-76.2	7.17	3.71	Appelman et al. 1982
	Female rat	-62.6	5.91	2.76	Appelman et al. 1982
Ammonia	Mouse	-54.5	5.95	2.89	Kapeghian et al. 1982
Hydrogen fluoride	Rabbit + guinea pig	-7.35	1.38	0.71	Machle et al. 1934
Systemic action					
Hydrogen sulfide	Cat + rabbit	-42.6	5.13	2.36	Lehmann 1892

Adapted from ten Berge et al. 1986

The general form $Y = a + b_1 \ln C + b_2 \ln t$ can lead to two different means of expressing Y, In order to emphasize the importance of concentration that varies in animal experiments, the rearranged probit equation related to toxic load is given by combining the last two terms of equation 2.6:

$$Y = a + b \ln C^n t \qquad (2.7)$$

where $n = b_1 / b_2$ from equation 2.6

b is equal to b_2 in equation 2.6

or

$$Y = a + b' \ln C t^{1/n} \qquad (2.8)$$

where $1/n = b_2/b_1$ from equation 2.6

b' is equal to b_1 in equation 2.6

The process of fitting an equation to experimental data cannot distinguish between these two possibilities, however the practice has developed to use equation 2.7 because this form is more easily integrated for concentrations varying over a period of time (Withers and Lees 1985a).

Since $C^n t$ is toxic load, equation 2.7 can be rewritten to the form:

$$Y = a + b \ln L \qquad (2.9)$$

where L is toxic load.

The concept of toxic load needs to be viewed as a means to integrate the interactive effects of exposure concentration and exposure time upon animal (or human) response. The three dimensional relationship among exposure concentration, exposure time, and response is demonstrated in Figure 2.2 for a particular toxic response, such as fatality.

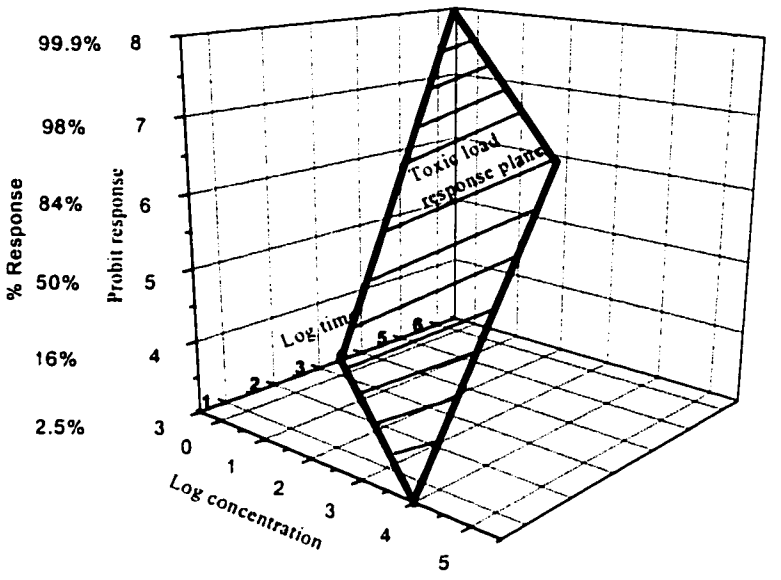
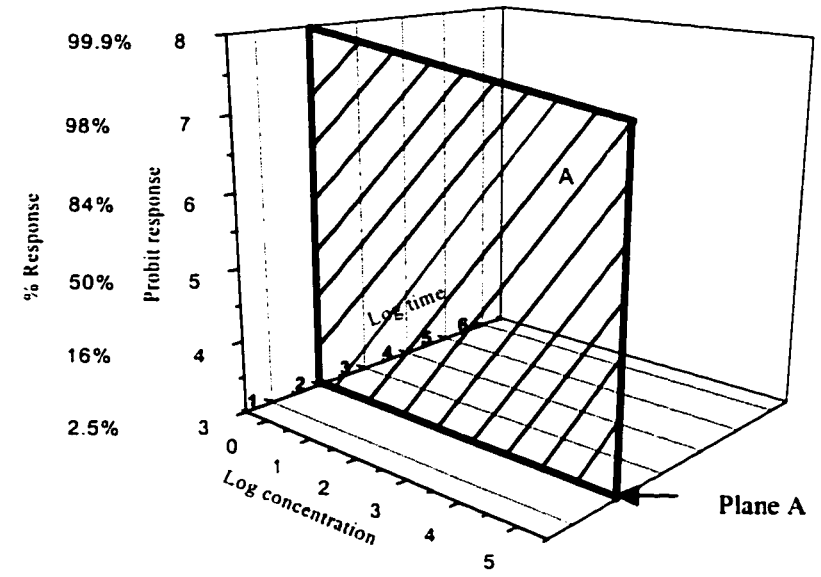
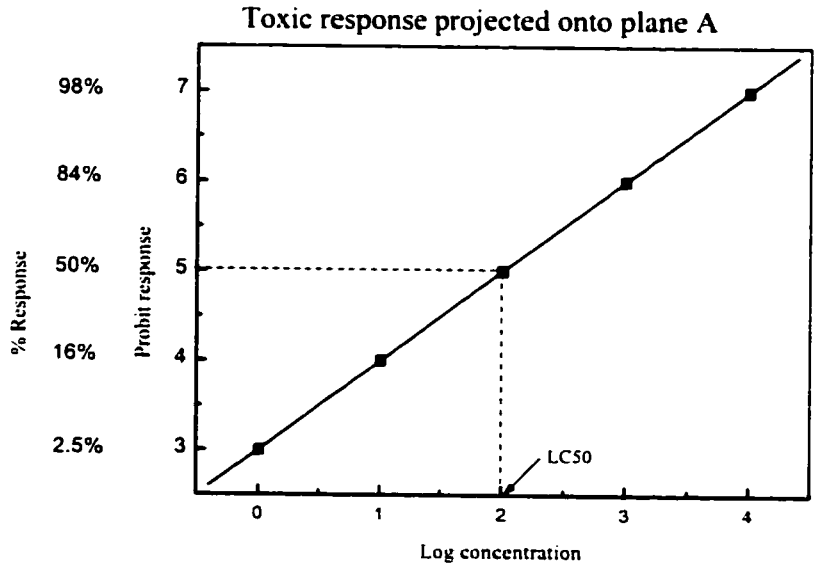


Figure 2.2 Three dimensional relationship among exposure concentration, time, and response

Typically, toxicity bioassays are performed for specified exposure duration at a range of exposure concentrations. Commonly, the dose-response curve is shown as response versus concentration or probit response versus log of concentration in an attempt to straighten out the typical sigmoid response line as discussed earlier in this chapter. Figure 2.3 a, b represents this aspect of three-dimensional relationship. At fixed exposure time (e.g., one hour), the plane "A" taken from the 3 dimensional relationship in Figure 2.3a becomes a 2 dimensional expression of concentration- response relationship in Figure 2.3b. LC_{50} is determined by looking at the intersection of the response plane with a vertical plane running through a specific time to give a 2 dimensional plot of response versus log concentration.



a

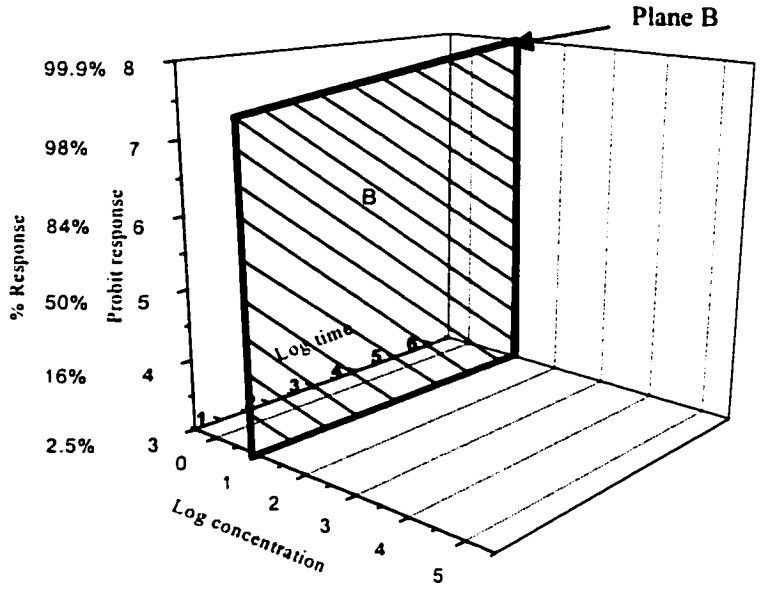


b

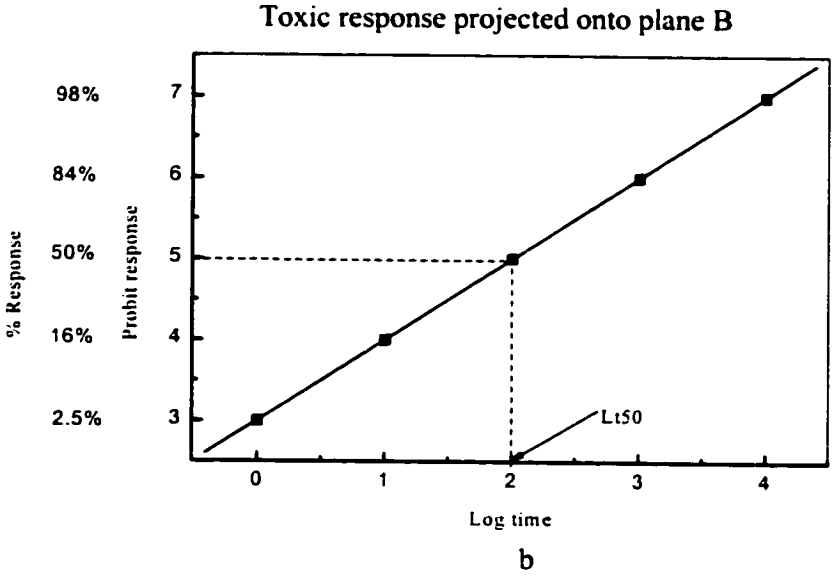
Figure 2.3 Concentration-response relationship at fixed time a. 3D expression; b. 2D expression

Alternatively, we can fix exposure concentration (say 500 ppm) and observe time-response relationship at a range of exposure durations although this approach has rarely been applied in toxicity study. As shown in Figure 2.4, the plane "B" taken from three-dimensional relationship demonstrated in Figure 2.4a becomes two-dimensional

expression of time-response relationship in Figure 2.4b. The value of Lt_{50} can be obtained from this 2 dimensional plot of response versus log time.



a

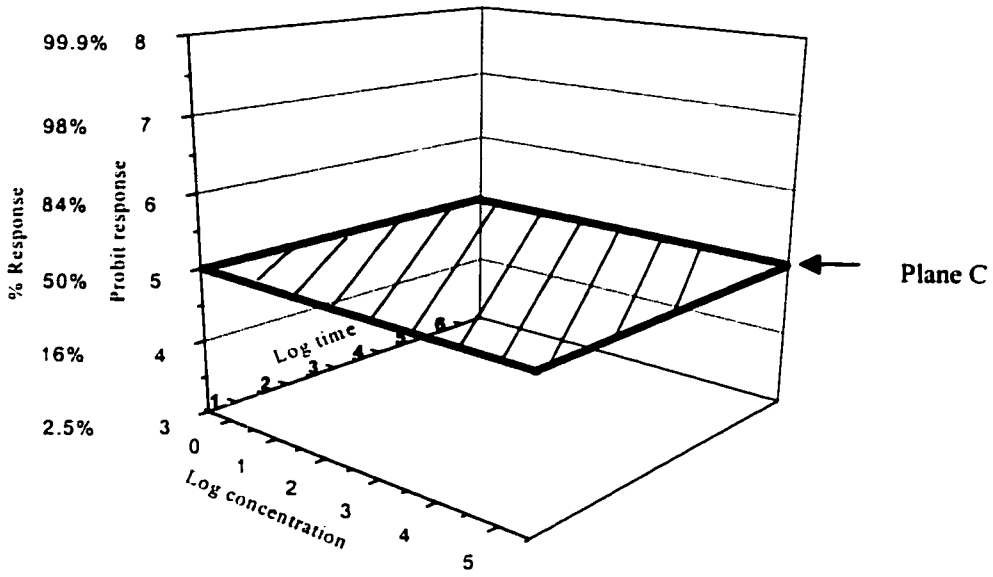


b

Figure 2.4 Time-response relationship at fixed concentration a. 3D expression; b. 2D expression

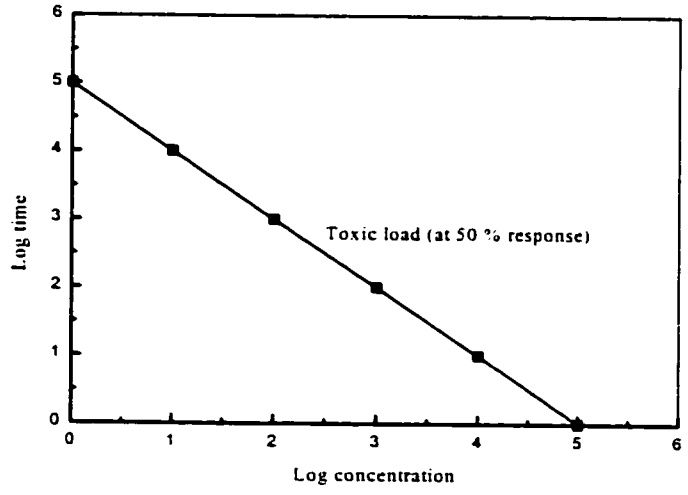
For acutely toxic gases, we also have to take note of the interaction between exposure concentration and exposure time at a specified response level. As shown in Figure 2.5, on the three dimensional response plane, for any given response percentage, e.g., 50%, the toxic load relationship could be seen as a line intersecting the horizontal plane through

50% response (Figure 2.5a) with the remaining axes of log time versus log concentration (Figure 2.5b). According to this perspective toxic load is a means of placing a vertical plane that combine the effects of exposure time and exposure concentration so that response could be plotted as a two dimensional plot of response versus toxic load (Figure 2.6).



a

Toxic response projected onto plane C



b

Figure 2.5 Concentration-time relationship at 50% response a. 3D expression; b. 2D expression

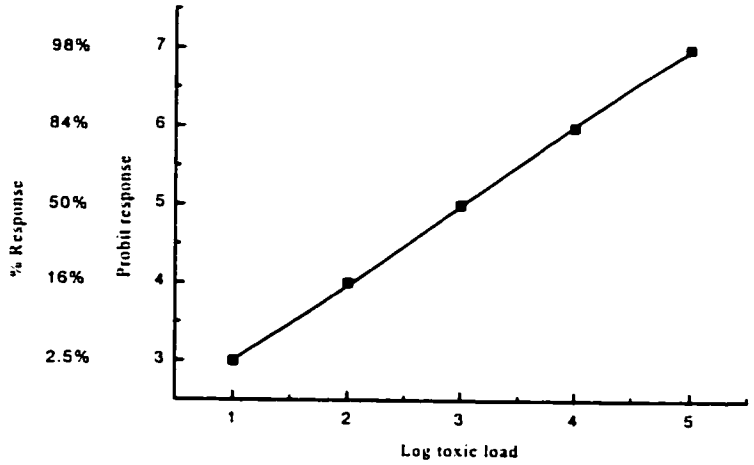


Figure 2.6 Toxic load and response relationship

The size of exponent n is determined by the slope of toxic load line, as shown in Figure 2.7. The steeper the slope, the larger the exponent n. An exponent n greater than one indicates the larger weight of exposure concentration in the concentration-time combination.

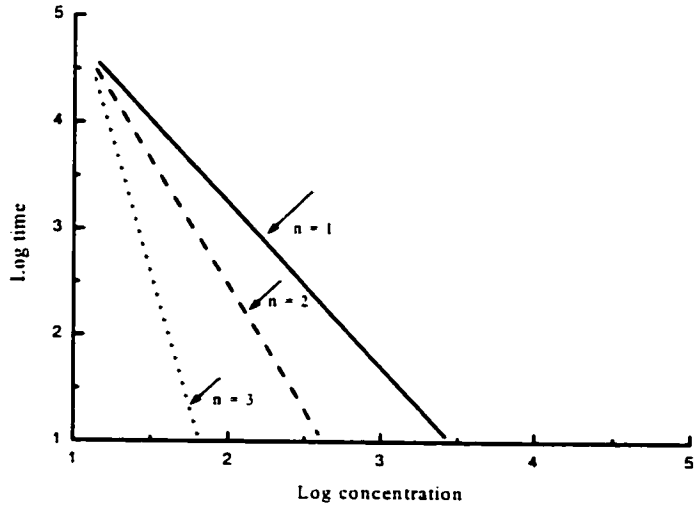


Figure 2.7 Relationship between the slope of toxic load and the size of exponent n

For a given toxic gas, if exponent n derived from different animal species is the same, toxic load line could be used to compare inter-species variability in susceptibility to this gas, as shown in Figure 2.8. The animal species whose line located on the left side of the figure (mice) is considered more sensitive than the animal species whose line located

on the right side of the figure (dogs). However, the comparison of inter-species variability in susceptibility to toxic gases would be complicated if exponent n is not equal for each species.

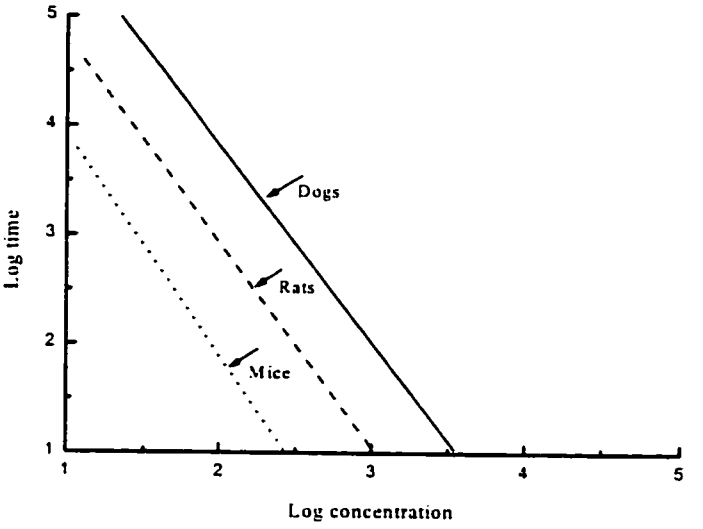


Figure 2.8 Comparison of inter-species variability in susceptibility to toxicants by using toxic load plot

2.4 Application of Toxic Load and Probit Function for Risk Analysis of Acutely Toxic Gases

The concept of toxic load and probit equation have been widely used in risk assessment of a variety of industrial chemicals including the four gases selected for this analysis. For example, in a report to U.S. Coast Guard, Eisenberg et al. (1975) proposed probit equations for chlorine and ammonia in a vulnerability model to assess human risk from marine spills of hazardous materials. The probit relationship was also employed to perform risk analysis of the six industrial installations in the Rijnmond area, Holland (Rijnmond Public Authority 1982). Withers and Lees (1985a, b, 1987) extensively reviewed and discussed various values of toxic load and probit equations for chlorine existing in the literature, and provided modified probit equations for humans to use in assessment of the hazard from chlorine release. The Center for Chemical Process Safety of the American Institute of Chemical Engineers (CCPS 1989, 1999) provided available values of probit equation constants for lethal toxicity for about 20 industrial chemicals for consequence analysis of chemical release. The Netherlands Organization of Applied

Scientific Research (TNO 1992) also provided values of probit constants for 22 chemicals. Prugh (1995) presented a compilation of probit equations for 28 chemicals, and proposed an equation to combine Haber's law and current toxic load relationship.

The available probit equations for the four gases from various sources are given in Table 2.5. Fatality is commonly chosen as the outcome of toxicity because other toxic effects are usually difficult to define and to compare from different studies. All probit equations listed in Table 2.4 are proposed for fatality. In the literature, only one study was found to provide a probit equation for non-lethal injury (hospitalization) for chlorine (Eisenberg et al. 1975).

Table 2.5 shows considerable differences in the results from different probit equations. For example, the equations from two relatively authoritative sources, the Center for Chemical Process Safety of the American Institute of Chemical Engineers (CCPS 1989) and the Netherlands Organization of Applied Scientific Research (TNO 1992), produce considerably different lethal concentrations of the four selected gases (Table 2.6). For hydrogen sulfide and chlorine, CCPS provides lower lethal concentrations than TNO, while for ammonia and hydrogen fluoride CCPS gives higher values of lethal concentrations. The differences in lethal concentrations from these two sources for hydrogen fluoride are about 7-to 10-fold; a significant difference that calls the validity of these various probit equations into question.

The process of generating toxic load and probit relationship to predict human responses is subject to great uncertainty because few data on toxic responses of humans are available to directly determine consequences following an accidental exposure. Data from controlled experiments conducted with laboratory animals have to be used for this purpose. To date, there is no standardized procedure for translating available animal data to humans. Usually, for a specific substance, the values of the probit constants used to predict human fatality were developed on a case-by-case basis. In some cases, only the qualitative aspects of differences in respiratory structures and mechanisms of action of the toxicants have been taken into consideration when applying quantitative animal data to humans (Schubach 1995). Many investigators derived values by combining data from various species without considering inter-species differences. For example, the IChemE

Table 2.5 Probit equations for fatality for the four gases

Chemical	Species	Probit equation	Lethal concentration for 30 min exposure duration			Reference
			LC_{10} (ppm)	LC_{50} (ppm)	LC_{90} (ppm)	
H_2S	Cat, rabbit	$Y = -42.6 + 2.36 \ln C^{2.2}t$	1597	2044	2615	ten Berge et al. 1986
	Human	$Y = -36.2 + 2.366 \ln C^{2.5}t$	219	272	338	Rogers 1990 (AEUB)
	Human	$Y = -31.42 + 3.008 \ln C^{1.43}t$	328	441	594	US Coast Guard 1980
	Human	$Y = -11.5 + 1.0 \ln C^{1.9}t$*	361	708	1392	TNO 1992
Cl_2	Mouse	$Y = -23.2 + 1.1 \ln C^{3.5}t$	412	574	800	ten Berge et al. 1986
	Human	$Y = -17.1 + 1.69 \ln C^{2.75}t$	26	34	44	Eisenberg et al. 1975
	Human	$Y = -36.45 + 3.13 \ln C^{2.64}t$	36	42	49	U.S. Coast Guard 1980
	Human	$Y = -11.4 + 0.82 \ln C^{2.75}t$	237	418	738	Harris & Moses 1983
	Human	$Y = -5.04 + 0.5 \ln C^{2.75}t$	170	430	1093	ten Berge & van Heemst 1983
	Regular population	$Y = -8.29 + 0.92 \ln C^{2}t$	125	250	500	Withers & Lees 1985 b
	Vulnerable population	$Y = -6.61 + 0.92 \ln C^{2}t$	50	100	200	Withers & Lees 1985 b
	Human	$Y = -5.3 + 0.5 \ln C^{2.75}t$	202	520	1320	World Bank 1988
	Human	$Y = -14.3 + 1.0 \ln C^{2.3}t$*	199	347	604	TNO 1992
NH_3	Rat	$Y = -47.9 + 2.3 \ln C^{2}t$	13653	18034	23820	ten Berge et al. 1986
	Mouse	$Y = -54.5 + 2.89 \ln C^{2}t$	4327	5400	6738	ten Berge et al. 1986
	Human	$Y = -30.57 + 1.385 \ln C^{2.75}t$	2360	3303	4623	Eisenberg et al. 1975
	Human	$Y = -35.9 + 1.85 \ln C^{2}t$	8169	11546	16318	U.S. Coast Guard 1980
	Human	$Y = -9.82 + 0.71 \ln C^{2}t$	2528	6227	15337	World Bank 1988
	Human	$Y = -15.8 + 1.0 \ln C^{2.0}t$*	4539	8608		TNO 1992
HF	Rabbit, guinea pig	$Y = -7.35 + 0.71 \ln C^{2}t$	444	1093	2693	ten Berge et al. 1986
	Human	$Y = -25.85 + 3.354 \ln Ct$	226	332	486	U.S. Coast Guard 1980
	Human	$Y = -26.4 + 3.35 \ln Ct$	268	393	575	World Bank 1988
	Human	$Y = -8.4 + 1.0 \ln C^{1.5}t$*	409	960	2254	TNO 1992

* Concentrations in mg/m^3, need to be converted to ppm when calculating LC_{10}, LC_{50}, and LC_{90}.

Table 2.6 Comparison of predicted lethality levels (30 minute LC_{50}, LC_{10}) using probit constants from CCPS and TNO

Chemical	LC_{50} (30 min) (ppm)		LC_{10} (30 min) (ppm)	
	CCPS*	TNO	CCPS*	TNO
Hydrogen sulfide	441	708	327	361
Chlorine	250	347	125	199
Ammonia	11500	8600	8160	4540
Hydrogen fluoride	6530	960	4460	409

Adapted from Schubach 1995

* CCPS data from CCPS (1989) appear to have been derived from U.S. Coast Guard 1980

Working Party (1988) found probit constants to fit the value of mixed-species average LC_{50} for ammonia and applied these data directly to humans without any quantitative consideration of species differences.

To address this problem, the Netherlands Organization of Applied Scientific Research (TNO 1992) proposed an approach to systematically develop probit constants for lethality for some toxic substances. Inter-species differences and mechanisms of toxic action have been taken into account when interpreting animal data to humans. In this approach, however, like many other studies, inter-species differences are arbitrarily represented by a safety factor of 5 for local irritants, and 10 for systemic acting substances. There was no attempt to take closer look at evidence of inter-species variability in response to toxic substances. The reason for this is because (too) little toxicity data are available for these substances (TNO 1992).

2.5 Limitations of Toxic Load Model

Although the toxic load concept and probit equations have been widely used in risk analysis, it is important to bear in mind that this is simply an empirical curve fitting relationship. The absence of underlying mechanistic understanding creates inherent limitations. Care should be taken when extrapolating information from animal studies to humans to ensure that predictions of toxic effects in humans from accidental release of industrial gases will be realistic.

2.5.1 Exposure Variables

2.5.1.1 Fluctuation of Concentration

One major problem in using the toxic load model is that almost all animal experiments use an exposure concentration that is kept constant. In reality, however, the exposure concentration usually fluctuates because of random turbulent dilution and dispersion processes. The peak concentrations can be 20 times higher than the average concentration (Hilderman et al. 1999). In some cases where toxicity depends on the total dosage, this fluctuation of concentration may not be important. However, for many toxic gases that exhibit a non-linear dose-response relationship, fluctuations of concentration will have a significant influence on toxic response. Estimates based on the mean-value will underestimate the true health risk of an accidental chemical release (Griffiths and Megson 1984, Ride 1984). Attempts have been made to address this problem; for example, by taking into account three physiological receptor factors, i.e., an uptake time constant, a recovery time constant, and a saturation concentration (Hilderman et al. 1999). This analysis will not deal with concentration fluctuations because that has been addressed by Hilderman et al. 1999. However, the evidence about the importance of concentration fluctuations for the toxicity of the four gases is reviewed.

2.5.1.2 Range of Concentration

The exponent n and probit equation for a particular gas are derived from available quantitative data of animal experiments, in which both the concentration and the exposure duration are varied. Unfortunately, there is no single standard experimental protocol available for studies that deal with the acute toxicity of various gases. Consequently, it is not surprising to find a great discrepancy in the literature for important variables, such as the exposure concentration and duration applied in animal experiments.

The concept of toxic load ($C^n t$) means that concentration will have a significant influence on the response to hazardous materials provided $n>1$. The application of different ranges of toxic gas concentration in animal experiments will demonstrate different relationships between concentration, time, and response (Gelzleichter et al. 1992). Application of a high concentration for a short time and a low concentration for a

longer period will yield considerably different dose-response relationship. Ideally, an animal study should be designed to cover a wide range of exposure concentrations in order to demonstrate a valid dose-response relationship. But in reality, one single study is usually not able to do so because of practical factors involved such as cost.

This aspect can be reflected by the information of exposure concentrations and durations extracted from the studies selected by ten Berge et al. (1986) for the four gases (Table 2.7). As shown in Table 2.7, studies of Lehmann (1892) on hydrogen sulfide and Machle et al. (1934) on hydrogen fluoride applied wide ranges of concentrations and exposure times, whereas in the study for chlorine the applied concentrations are unusually low and the range of concentrations is extremely narrow (Bitron and Aharonson 1978).

Table 2.7 Range of concentrations and exposure durations in the studies selected by ten Berge et al. 1986 to derive the exponent n of the four gases

Chemical	Species	Concentration (ppm)	Exposure time (min)	Reference
H_2S	Rabbit	130	480	Lehmann 1892
		470	375	Lehmann 1892
		750	265	Lehmann 1892
		1300	3	Lehmann 1892
		3250	2.5	Lehmann 1892
Cl_2	Mouse	170	15-160	Bitron & Aharonson 1978
		290	5-30	Bitron & Aharonson 1978
NH_3	Rat	14100-18900	60	Appelman et al. 1982
		18000-24100	40	Appelman et al. 1982
		26100-33100	20	Appelman et al. 1982
		29900-54000	10	Appelman et al. 1982
HF	Rabbit, guinea pig	29-9780	5-2460	Machle et al. 1934

There will always be a challenge in determining how to apply a relationship derived from animal study to predict the human response to an accidental release of a particular chemical, where the exposure concentration or duration are outside the range of those used in the animal experiments. To address this problem, one important aspect is to understand the physiological mechanisms behind the simplified mathematical summary of the concentration-time-response relationship. The following analysis characterizes

what is known about the biological mechanisms for the toxicity of the four chosen gases and provides a basic description of the important biological processes for development or modification of predictive models used in the risk assessment.

2.5.2 Biological Variability

The application of the toxic load model in risk assessment always has to face the difficulty that the level of uncertainty for predicting outcomes of an accidental hazardous release is not provided by the toxic load model. Biological variability, a major source of uncertainty, is extremely important when the toxicological evidence is collected from various animal species and must be extrapolated to human beings.

To some extent, the probit equation does reflect the intra-species biological variability in the response of experimental animals to toxicants. However, determination of the appropriate toxic load model for humans is somewhat arbitrary and conservative, and justification for the process is often unclear. Rules of thumb are usually used to translate evidence from animals to humans. For example, it is believed by some toxicologists that the results consistent in the studies on three different species of animals can be reasonably applied to humans (Whithers and Lees, 1985a). Yet, toxicants might react differently between small animals, large animals, and humans. The toxic load model does not efficiently reflect this difference. Selection of an animal model is extremely important for interpreting experimental data. Some times no studies on a suitable animal model are available.

Furthermore, the individual variability among human beings is much greater than among experimental animals that are bred for consistency (WHO 1999). Although some attempts have been made to address this problem by providing one probit equation for the general human population and another for the more vulnerable parts of populations (Withers and Lees 1985b), the whole issue of intra- and interspecies variability needs to be clarified in order to make more realistic and accurate predictions of outcomes for hazardous releases of industrial gases.

Chapter 3

Variability and Uncertainty

3.1 Introduction

In predictive modeling of accidental releases of hazardous chemicals, one major problem is that the degree of uncertainty for the outcomes, e.g., percentage of fatality, cannot be known. In the past, little effort has been made to provide decision-makers with adequate insight into how much or how little is known about a particular risk estimate (Finkel 1990). With regard to the toxic load and probit function, decision-makers might be satisfied with a prediction that 400 ppm chlorine for 30 minutes is expected to kill 50% of an exposed population, based on the calculation of $Pr = a + b \ln C^n t$. However, they may not appreciate how many uncertainty factors contribute to the development of the probit equation and thus how uncertain is the value of the risk estimate.

For the chain of processes that leads from chemical release to human response there are many uncertain factors that significantly influence confidence in the estimated risk. Among these factors, natural physical variability in release and dispersion of chemicals and natural biological variability in animal and human susceptibility to chemicals are most important. For the remainder of this discussion, variability will refer to these intrinsic or natural sources of true variations.

In order to reduce ignorance and better understand uncertainty in risk assessment, one option is to gain a better understanding of variability. In the past, little attention has been paid to the human inter-individual variability in susceptibility to toxic gases and vapors. Very few studies have assessed human response variability. Interest in this area has gradually increased, particularly with recent fundamental work in inhalation toxicology (Neumann and Kimmel 1999).

Historically, inter-individual variability has been treated as an unwanted factor that will complicate determination of the dose-response relationship for a toxic agent. Larger variability requires larger sample sizes to demonstrate differences between the effects of experimental and control exposures at an acceptable level of statistical confidence (Hattis 1996). Therefore, animal studies have been designed to reduce inter-individual variability

by selecting experimental animals with the same or similar characteristics such as strain, age, weight, gender, and diet. Obviously, postulating human variability from such toxicological data in experimental animals is difficult.

Information on human biological variability that is relevant to toxic gases is very limited. In the literature, studies on various therapeutic drugs may be the richest source of information on response variability. For toxic gases like hydrogen sulfide or chlorine, the opportunities for incorporating variability into risk assessment are relatively limited, because little relevant data have been reported. The question is how to deal with variability and uncertainty under this situation. Shall we just simply use universal uncertainty factors of 10 for inter-species variability and another 10 for intra-species variability? Or, should we look closer at the available data on variability for each gas? Moreover, will a close look make any difference to predictive toxicity models for human response?

The purposes of this chapter are to (1) provide a conceptual understanding of uncertainty and variability, the difference and the relationship of these two terms; (2) summarize the important sources of uncertainty and variability with emphasis on the biological variability; (3) summarize a method for analyzing variability.

3.2 Basic Concepts

To some extent, confusion and inconsistency in the use of terms uncertainty and variability exists not only between risk assessors and decision makers, but also among the risk assessment experts (Haimes et al. 1994). Uncertainty and variability are sometimes perceived as the same thing. Therefore, it is necessary to clarify what we mean by uncertainty and variability. In general, there are two types of uncertainty: type A uncertainty, which can be called natural variability, and type B uncertainty, which can be called knowledge (or true) uncertainty. The following discussion presents how these concepts will be used for the purposes of this analysis.

3.2.1 Variability

Variability, also called type A uncertainty, refers to the real difference in parameters that are measured (Hattis 1996). For example, the values of height or weight of individuals in a population differ from person to person. The ambient concentrations of certain pollutants vary from time to time and from location to location. In risk assessment of adverse effects of toxic chemicals, variability is mainly associated with difference in exposure factors, causal processes, and responses to hazardous chemicals. For example, in toxicological experiments, a group of animals are exposed to a toxicant at fixed concentrations for fixed durations. Some animals die while others do not. There is a real difference in individual response to that toxicant which is the natural variability in response.

3.2.2 Knowledge (True) Uncertainty

Knowledge or true uncertainty, also called type B uncertainty, refers to the lack of surety or certainty about parameters that are measured or estimated. In other words, knowledge uncertainty is the imprecision or inaccuracy in an estimate of a parameter caused by imperfection of our current knowledge (Bogen and Spear 1987, Finley et al. 1994). For example, before we understood the structure of DNA, we were uncertain how DNA could replicate the genetic code.

In modeling of accidental release of industrial gases, true uncertainty is our lack of confidence in predicting the degree and nature of exposure, as well as the degree and characteristics of human responses to the exposure. For example, due to technical limitations, we cannot measure the actual metabolic process of a gas or vapor after they are absorbed into the body. Thus, this whole process will be like a "black box" until we can find an appropriate approach to explain what happens.

From a statistical perspective, uncertainty about an event exists whenever the probability of that event occurring is neither 0 nor 1 (Good 1995). Uncertainty analysis may also be viewed as a statistical procedure (Smith and Ye 1995).

3.2.3 Difference and Relationship between True Uncertainty and Variability

True uncertainty and variability are different. From a risk assessment perspective, true uncertainty is a description of the imperfection in knowledge of the magnitude of risk to the individuals or whole populations (Hattis 1996) because of our inadequate understanding of processes. The degree of true uncertainty can be reduced by obtaining more accurate understanding or improving the quality of measurement and analysis (Hattis and Burmaster 1994). For the previous example, once we understood that DNA is a double helix comprised of four complementary base pairs, we can be much more confident about how DNA is able to replicate. In contrast, biological natural variability is about real differences in the biomedical parameters measured among species or among individuals caused by the differences in genetic and other host and environmental factors (NAS 1970). We can acquire a better understanding of natural variability or we can measure it more accurately, but we cannot reduce variability.

Yet, uncertainty and natural variability are closely related to each other. In risk assessment of health effects produced by toxic chemicals, variability is a major source of overall uncertainty. Thus, better understanding of variability can help reduce uncertainty in risk estimation.

3.3 Sources of Uncertainty and Variability

3.3.1 Sources of Uncertainty

There is a long list of sources of uncertainty in risk assessments of health effects for hazardous materials. These include the technical methods used for data collection, statistical variation, variability, and the comparability of animal models with human response (Grassman et al. 1999, Morgan and Henrion 1990). This analysis is focused on the possible sources of uncertainty in the prediction of human response to the four gases. The adverse effects of the four gases are considered for three different levels, that is odor, non-lethal injury, and death. Possible sources of uncertainty are summarized in Figure 3.1.

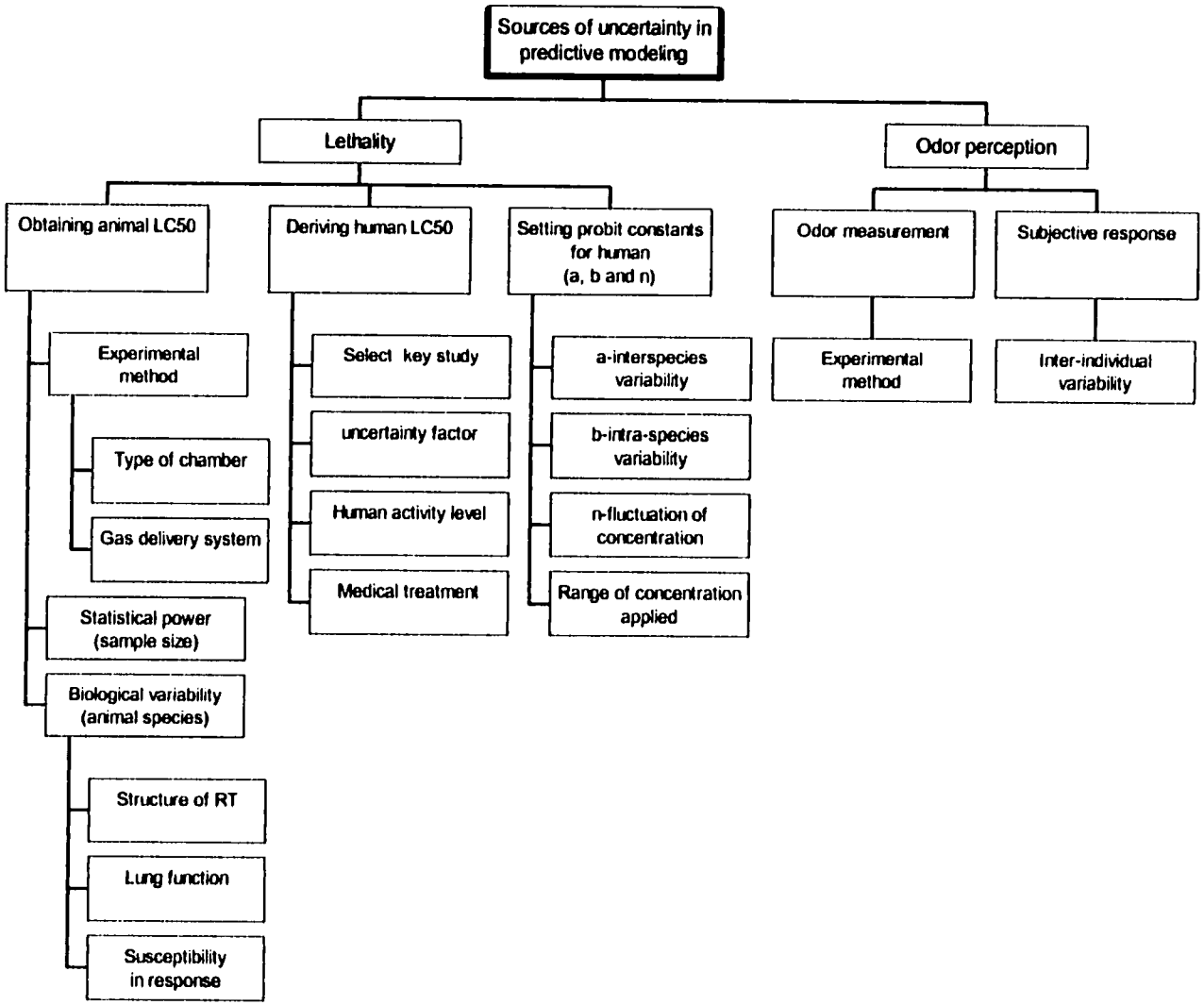


Figure 3.1 Sources of uncertainty in predictive modeling

The discussion about toxic load and probit function in chapter 2 showed three steps for the development of probit equations for humans, i.e., obtaining a value of animal LC_{50}, deriving the value of human LC_{50} based on the animal data, and setting probit constants (a, b, and n) for humans. Many uncertainty factors around these three steps will influence the confidence of these predictions of human response to the four gases. From a calculation point of view, differences of species in sensitivity influence the a-term, while intra-species variation is expressed by the steepness of the probit function, i.e., the b-term (TNO 1992). The size of exponent n is considered to be related to the influence of concentration fluctuation on the toxic effects. When exponent n is greater than one, the concentration fluctuations during an exposure will have significant effect on the toxicity outcome.

3.3.1.1 Experimental Methods

One of the most important sources of uncertainty in risk assessment of toxic gases and vapors stems from differences in experimental methods. Application of different exposure delivery systems and exposure chambers introduces substantial uncertainty in the interpretation and comparison of results from different studies. Regardless of how sophisticated an experiment may be, it is always a simulation of reality. Differences between the experimental exposure and the real exposure it seeks to simulate will inevitably contribute to uncertainty.

In inhalation toxicological experiments, there are three basic types of exposure delivery systems: (1) static, with single introduction of an intended amount of test gas into the exposure chamber; (2) recirculating, with a closed loop; and (3) dynamic, with a single-pass flow-through system (Phalen 1984). In a static exposure system, concentrations of toxicants decrease with the time because of a absorption of vapors and gases on the surfaces and deposition in the animals (McClellan and Henderson 1989). Particularly in some of the older studies, the results of longer exposures are questionable because of the inevitable rapid decline in gas concentration (Weedon et al. 1940). Barcroft (1931) found a 22 % reduction of an initial concentration of about 190 ppm of hydrocyanic acid gas in 38 minutes. In contrast, a dynamic exposure system continuously

delivers test gas, thereby maintaining stable equilibrium concentrations in the chamber during the experiments (Fraser et al. 1959).

There are two types of exposure chamber design: nose-/head-only chambers, and whole-body chambers. In the nose-/head-only chambers, primarily the head or nose is exposed to the test atmosphere, whereas in the whole-body chamber, animals are completely immersed in the exposure atmosphere. Clearly, deposition of gases or vapors on animal fur is higher in a whole-body chamber than in a nose-only experimental unit (Wolff et al. 1982).

Thus, uncertainty can arise from different methods of data collection. For example, suppose that the same species of animals were exposed to the same toxicant in two separate studies, and the reported LC_{50} for a certain equal duration (say 60 minutes) were significantly different from each other. When we compare these two results, we should keep in mind that this difference may simply be caused by the different exposure systems rather than any variability in organism response. Application of a static exposure delivery system and whole-body chamber may result in a considerable drop of exposure concentrations during experiments and thus produce a higher apparent value of LC_{50}. Interpreting that the animals showing a higher value of LC_{50} are more resistant to the toxicants would be incorrectly assigning this source of uncertainty to variability of animal response.

3.3.1.2 Statistical Power

Probit analysis is a statistical tool dealing with the distribution of a parameter that is measured; thus the sample size of experimental subjects is of great importance. Some older studies only employed one or two animals for an experiment. Obviously this very small sample size would not provide sufficient statistical power to make a meaningful conclusion.

3.3.1.3 Variability

In real exposure scenarios, natural variation in physical factors such as weather, direction and speed of wind, humidity, and atmosphere pressure will strongly affect the

dispersion of released gases and consequently influence estimates of actual exposure concentration. Biological variability between humans and different animal species is another aspect that can introduce great uncertainty in risk assessment.

3.3.1.4 Extrapolation of Animal Data to Humans

Uncertainty from extrapolation of animal dose-response relationship to humans is a major concern in risk assessment. This is also called model uncertainty (Finkel 1990). Differences in toxicokinetics and toxicodynamics among species and individuals make the calculated results considerably uncertain. For example, rodents are commonly used as surrogates for humans, but their responses to any given chemical might be quite different from human beings. With regard to the extrapolation of inter-individual variability, although data from animal experiments may provide limited information on inter-individual variability within the test species, human variability is believed to be greater due to greater genetic and acquired diversity among the human population compared with the experimental animal population that is selected to be as homogenous as possible (WHO 1999).

In addition, when deriving the value of LC_{50} for humans based on the animal data, some special aspects should be taken into consideration. For example, while experimental animals are passively exposed to the toxic materials, humans may take some actions to escape exposure. Also, humans may receive medical treatment after they are injured. These factors can effectively reduce the degree of injury of humans compared to experimental animals. This may introduce an additional layer of safety for humans, provided the actions taken are beneficial. Normal practice in risk assessment would be not to provide any credit for reduced risk from appropriate human responses. For the purpose of modeling toxic effects of the four gases on humans, how to choose animal models and how to assess inter- and intra-species differences are crucial to the determination of the value of LC_{50} (or EC_{50}) and probit constants for humans.

3.3.2 Source of Variability

In risk assessment of health effects after exposure to environmental contaminants, the source of total variability may include exposure variability, biological variability, methodological variability, and variability from stochastic processes (Grassman et al. 1999).

3.3.2.1 Exposure Variability

Exposure refers to the contact of an organism with a chemical or physical agent (EPA 1992). In the context of accidental release of toxic gaseous chemicals, exposure variability may be due to a different location or distance from release center, non-uniform distribution of plume, or a different breathing rate among individuals. Breathing rate is an important determinant of exposure to the four chemicals investigated in this study because inhalation is the major route of exposure and breathing rate will govern the rate at which the chemical agents are taken into the respiratory system.

3.3.2.2 Biological Variability

Biological variability refers to the differences in parameters representing biological processes among species (inter-species variability), among individuals (inter-individual or intra-species variability), and within an individual over time (intra-individual variability). For inhalation toxicology, these differences can be divided into two parts: differences in toxicokinetics, and differences in toxicodynamics. Variability in toxicokinetics involve differences in the process of absorption, distribution, metabolism, and excretion of a chemical within the body of an organism, while variability in toxicodynamics is associated with differences in the degree and mechanism of action of a chemical upon an organism. Action, pathway of metabolism, and rate of elimination are the greatest sources of variation among species and individuals (Gillette 1976). Parameters such as elimination half-lives ($T_{1/2}$), area under the curve (AUC), and peak concentration (Cmax) can be used to describe the variability in the causal process (Hattis and Silver 1994). However, data on these measurements for our four industrial gases are very limited.

Response variability is the variation in the type or magnitude of biological effect due to genetic or acquired differences between individuals under identical conditions of exposure. Response variability involves two aspects: qualitative and quantitative variability. Qualitative variability refers to the variety of responses produced by an exposure. For example, smoking may cause different diseases including chronic obstructive pulmonary disease, lung cancer, or cardiovascular diseases (Doll et al. 1994).

Many factors such as genetics, gender, ethnicity, life-style, and preexisting disease may contribute to the qualitative variability in response (Grassman et al. 1999). Quantitative variability indicates the differences in the magnitude of a given response encountered among individuals within an exposed population. For example, the severity of respiratory irritation may be different among individuals after an equivalent exposure to toxic gases.

3.3.2.3 Methodological Variability

Methodological variability is associated with the acquisition of information describing the outcome and includes variability associated with the statistical analytical methods. This aspect is beyond the scope of present study.

3.3.2.4 Stochastic Processes

Stochastic processes are events that occur randomly or by chance (Grassman et al. 1999). Development of cancer is generally believed to involve stochastic processes. If a response is caused by stochastic process, the population dose-response relationship may not represent inter-individual variability in susceptibility. Rather, the dose-response represents the distribution of chance occurrence of response as a function of dose. However, cancer development is recognized to also involve a number of deterministic processes in addition (Albert 1997). Consequently, the overall dose-response curve will be a combination of effects from both stochastic and deterministic processes.

3.4 Sources of Information on Variability

3.4.1 Human Data

Human dose-response data can be obtained from case reports, epidemiological studies, and controlled exposure studies (Grassman et al. 1999).

Accident case reports contain important information for risk assessment. They are records of real human exposure and outcomes. In other words, they provide the best estimates of what actually happened. Usually accident case reports document the most serious outcomes, for example, hospitalization or death. They may contain individual information such as age, gender, and health status that may provide an indication of individual variability to toxic agents. The major problem with this kind of data is that accurate information on exposure concentration and duration is almost always lacking or incomplete. These deficiencies make difficult the subsequent efforts to quantitatively analyze the dose-effect relationship.

Epidemiological studies such as case control or cohort studies may contain useful dose-response data related to environmental or occupational exposure to airborne contaminants. Many studies deal with mild or subtle effects, or chronic effects. Parameters such as changes of pulmonary function are usually chosen in this type of studies. The advantage is that this type of study can involve a large number of exposed subjects in a population, which can be statistically sufficient to estimate inter-individual variability in the responses. However, this type of data does not permit any precise dose-response relationship to be deduced, and interpretation of data is made difficult by the presence of many confounders (Bates 1988)

Controlled exposure studies usually apply to low concentration levels for short periods. The effects observed are generally reversible short-term effects. This type of study can provide accurate information of actual exposure levels and onset or degree of adverse effects. Sometimes the sample size is large enough for the meaningful statistical analysis.

3.4.2 Animal Data

Since the four industrial gases addressed in this thesis are highly toxic, most information of their toxicity and toxic load models comes from animal studies. Thus, animal data inevitably have to be used in the present study to provide detailed exposure-response relationships for each gas and to estimate inter-species and intra-species variability.

In general, exposure concentrations used in animal toxicity studies are higher than exposure levels commonly experienced in the real world. This is one of the major problems when converting animal results to humans. However, the present study deals with accidental releases of chemical gases that cause serious health effects, therefore the real concentrations of such accidental releases are more likely to be close to the concentration applied in the animal studies.

Another problem is the difference in mechanisms of toxicity between animals and humans. Generally, the major difference of effects caused by an exposure to a chemical lies in the different metabolic processes between animals and humans. However, the four gases selected in this study are irritants although hydrogen sulfide also acts systemically. They react with mucous membrane of the respiratory tract before they are absorbed into the blood. Thus, in this case, animal data are of greater value for the predictive modeling of health effects than in other cases where chemical metabolism is a major issue. At least for irritants, the focus can be on how differently the animal model response is to this specific mode of action rather than to the entire range of anatomical and physiological difference that may be relevant for systemic toxicants. In regards to human individual variability in response, animal data may provide little information because of the reasons mentioned earlier in this chapter.

3.5 Analysis of Variability

3.5.1 Distribution Form of Data

3.5.1.1 Lognormal and Normal Distributions

In order to quantitatively analyze variability, it is important to know the distribution of parameters. Normal and lognormal distribution are two common forms of distribution.

Normal distribution, also called Gaussian distribution, is a bell-shaped, symmetric distribution. The shape of the normal curve is determined entirely by the mean response, μ, and standard deviation, SD. The area under the curve represents the percentage of organisms affected for a specified response interval. The response interval within one standard deviation of the mean represents 68% of the individual organisms. Two standard deviations represent 95.5% of the total individuals. The total area under the curve has an area of 1, representing 100% of the individuals.

Lognormal distribution refers to a distribution in which the logarithms of a parameter have a normal Gaussian distribution. That is, if x is lognormally distributed, then $y = \ln x$ is normally distributed (Morgan and Henrion 1990). It has been found that many biological parameters follow or approximate lognormal distributions. On a linear scale, this type of distribution is usually distinctly skewed, and the range of variation among the values can be very large. One well-known example is the response of insects to insecticidal fumigation. Some insects are extremely resistant and produce an extended "tail", thus the distribution of threshold response concentrations of insecticides is asymmetric. The mechanism behind log-normality is that if there are many factors contributing to the differences among individuals and each factor exerts a small but multiplicative influence on the overall parameter measured, the distribution is log normal (Hattis 1999). In contrast, in a normal distribution, the factors involved exert an additive influence on the parameter measured (Morgan and Henrion 1990).

3.5.1.2 Probability (Z-score) Plot and Probit Plot

Probability plotting is generally used for distribution data in the form of a set of actual parameter values, e g., blood concentration of PCB. Conventionally, the logarithm of the values of the parameter is plotted on the y-axis, and z-score on the x-axis. Z-score is a number that is assigned to represent the cumulative percentage of response in a population at a specific dose. Z-score is in units of standard deviation. For example, a z score of -2 is assigned if 2.5% of people show a response because this point is 2 standard deviations below the midpoint (median) of lognormal distribution. Similarly, a z score of 0 represents 50% response, and z score of +2 represents 97.5% (2 standard deviations

above the midpoint of lognormal distribution) response (Figure 3.2). If the data are perfectly lognormal distributed, the z-score plot should be a straight line (Figure 3.3). In a regression line calculated for this type of plot, the intercept (z = 0) is an estimate of the median, and the slope is an estimate of standard deviation (Hattis 1996).

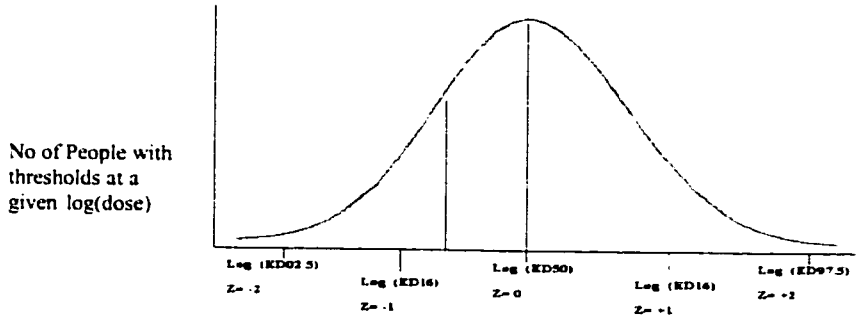


Figure 3.2 Relationship of Log (Threshold Dose) and Z-Score (Adapted from Hattis 1996)

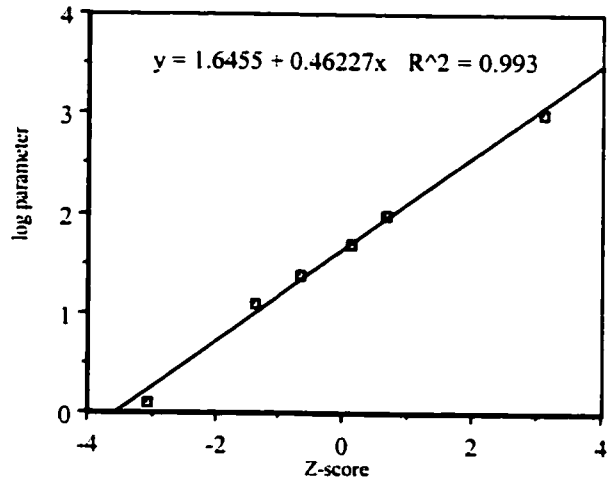


Figure 3.3 Example of a Z-score plot for experimental data

A probit plot is commonly used for distributions of threshold-type effect (Hattis 1996). As described in chapter 2, probit analysis is a way of converting an assumed lognormal distribution of individual thresholds into a straight line. The probit is simply the z-score plus 5 (5 was added to allow toxicologists to avoid having to deal with negative numbers in the days before electronic calculators were available) (Finney 1971) and thus probits have units of standard deviations. Usually the logarithm of the values of the parameters are plotted on the x-axis, and probit values on the y-axis (Figure 3.4).

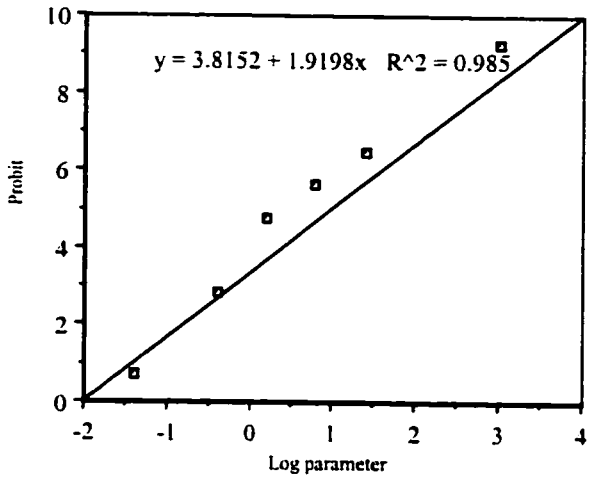


Figure 3.4 Example of a probit plot for experimental data

In this analysis, the probit plot has been chosen as the method to look at the distribution form of toxicological data for the four gases.

3.5.2 Quantal or Continuous Effect Parameters

Quantitative response variability can be described by quantal or continuous data. Quantal responses refer to the presence or absence of adverse effects (all or nothing), for example, the presence of cancer, or death. The mechanism behind quantal data is the assumption that an observed response is produced when some underlying continuous parameter exceeds some critical threshold (Hattis and Silver 1994). Quantal health effects are usually expressed as a cumulative distribution of the exposure concentrations or time required to produce adverse effects. Individual dose-response curves are not possible for quantal responses because the quantal parameter is the onset (yes or no) of the adverse effects (Grassman et al. 1999). The response variability in a population can be expressed as the range of exposures that cause the onset of an adverse effect (Grassman et al. 1999), as indicated by the arrow in Figure 3.5.

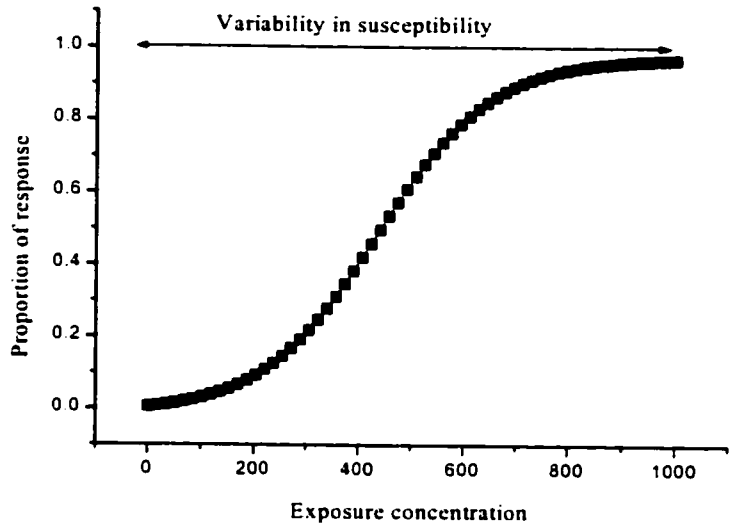


Figure 3.5 Response variability of quantal data (Adapted from Grassman et al. 1999)

Continuous effect parameters are measurements of a function parameter that can take on any value within some continuous range (Hattis and Silver 1994). For example, forced expiratory volume in the first second (FEV_1) is a continuous parameter. When a continuous outcome is measured in a population, the magnitude or intensity of response is given as a function of the level of exposure. The response variability can be described by the range of responses at a given level of exposure (Grassman et al. 1999). The variability in response may change as the magnitude of the exposure changes. As indicated by the arrow in Figure 3.6, at a hypothetical exposure level of 60, the inter-individual variability is shown to be the greatest.

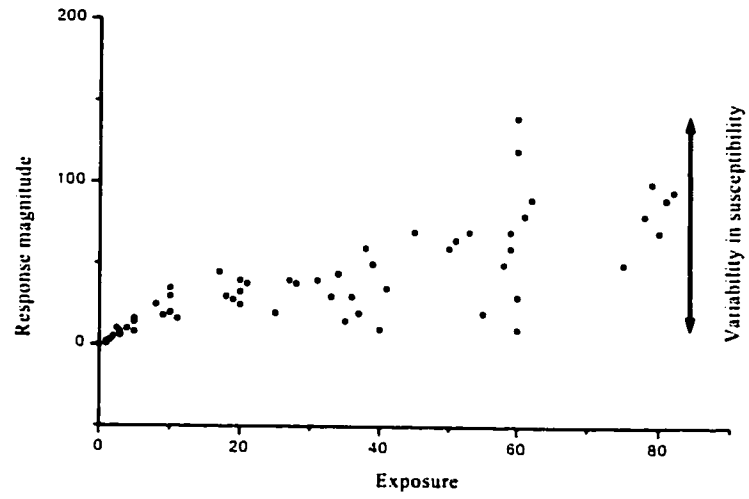


Figure 3.6 Response variability of continuous data (adapted from Grassman et al. 1999)

3.5.3 Stochastic or Deterministic Causal Process

A causal process of a toxic effect should be distinguished to the extent possible as being stochastic or deterministic. Stochastic process occurs by random chance. For example, carcinogenesis and mutagenisis are generally recognized to be caused at least partly by stochastic processes. For effects caused by stochastic process, the population dose-response relationship may not reflect individual susceptibility. In contrast, if the causal process is strictly deterministic on an individual basis, then the population dose-response relationship does reflect variability in individual susceptibility and will be a direct measure of the population distribution of individual thresholds (Hattis 1999). In this analysis, the causal processes of the toxicity of the four gases are considered to be deterministic.

3.5.4 One-step and Multi-step Models

To assess inter-individual variability, one-step models or multi-step models can be applied depending on the characteristics of available data. One-step modeling is a simple input-output approach that allows direct fitting of dose versus response. This type of modeling can be used where the direct observation of effects within a certain range of exposure dose and duration is possible (Hattis 1999). Multi-step modeling approaches

require multiple source of information and involve several steps revealing the relationship between exposure, a series of intermediate parameters, and end effects (Hattis 1999). This approach can provide more detailed information along the processes from exposure to endpoints.

3.5.5 Assessment of Variability

For lognormal distribution, if we have a set of individual parameter values, variability can be expressed by the $\log_{10}$ geometric standard deviation. Geometric standard deviation (GSD) is the antilog of the standard deviation of the logarithms of parameters that are measured. If original data sets are not available but data are presented in the form of a histogram, for example, probit analysis can be used to estimate variability. The slope of the regression line in a probit plot is an estimate of the number of SD of the population distribution of thresholds (Hattis 1997). The probit slope (the coefficient of the x term) is simply the reciprocal of the log (geometric standard deviation) of the population distribution of threshold doses. Larger values of the probit slope correspond to smaller degrees of inter-individual variability as characterized by the 5 to 95% or 1 to 99% range (Table 3.1). The standard deviation or log (GSD) calculated from the raw data do not only reflect "true" inter-individual variability but also include variability resulted from measurement or dose estimate error (Hattis 1997). Therefore the true inter-individual variation is somewhat less than that reflected in the observed SD or log (GSD). If data are available, the true inter-individual variability can be obtained by subtracting measurement error from total variability (Hattis 1997).

Table 3.1 A scale for understanding lognormal variability - fold differences between particular percentiles of lognormal distribution

Probit slope ($1/\log_{10}(GSD)$)	$\log_{10}(GSD)$	Geometric standard deviation	5-95% Range (3.3 SD)	1-99% Range (4.6 SD)
10	0.1	1.26	2.1-fold	2.9-fold
5	0.2	1.58	4.5-fold	8.5-fold
3.33	0.3	2.0	10-fold	25-fold
2.5	0.4	2.5	21-fold	73-fold
2	0.5	3.2	44-fold	210-fold
1.67	0.6	4.0	94-fold	620-fold
1.43	0.7	5.0	200-fold	1800-fold
1.25	0.8	6.3	430-fold	5300-fold
1.11	0.9	7.9	910-fold	15000fold
1.0	1	10.0	1900-fold	45000-fold
0.91	1.1	12.6	4200-fold	130000-fold
0.83	1.2	15.8	8900-fold	380000-fold

From: Hattis 1996

3.6 Summary

Risk assessment of adverse health effects following accidental exposure to the four toxic gases is inevitably subject to uncertainty because of significant natural physical variability, biological variability, and lack of basically required toxicity data. Many factors contribute to the total variability and overall uncertainty. Although biological variability is the emphasis of this thesis, some other factors also need to be taken into account in the probit analysis. According to Figure 3.1, three aspects are considered to be the major sources of uncertainty involved in the predictive modeling:

- Variability in exposure to, causal process of and response to the four gases;
- Differences in experimental methods applied in animal studies;
- Human actions during and after exposures.

These three aspects will be discussed in the following chapters. Some factors such as natural physical variability are also important but beyond the scope of this thesis.

Chapter 4

Factors That Contribute to the Variability in the Causal Processes

4.1 Introduction

The toxic effects of chemicals on humans are the results of interactions of biological systems and toxicants. For ethical reasons, laboratory animals such as rats, mice, guinea pigs, rabbits, dogs, and monkeys are commonly used in inhalation toxicology research as surrogates for human exposures (Table 4.1). Since inhalation exposure is the most common and important route for gaseous chemicals, this interaction will mainly involve the biology of the respiratory tract.

Table 4.1 Animal species used in the toxicological studies of the four gases

Gas	Canary	Rat	Mouse	Guinea pig	Rabbit	Cat	Dog	Goat	Cow*	Monkey
H_2S	+	+	+	+	+	+	+	+	+	+
Cl_2		+	+	+	+	+	+			+
NH_3		+	+	+	+	+	+			
HF		+	+	+	+	+	+	+		+

* Cows were not used as surrogate for humans but because of concerns for H_2S effects on livestock

The toxicity of an inhaled gas depends partially on the location and extent of disposition in the respiratory tract. Deposition of a gas, in turn, depends on its physicochemical properties, physical characteristics of the airways, and kinetics of the airflow in the airways. Differences in deposition characteristics between animals and humans will influence interpretation of toxicity results from experimental animals. Thus, a comparison of the anatomy of the respiratory tract and pulmonary function of different species can provide basic understanding of what is different and why it is different in the causal processes.

When extrapolating animal data to humans, some factors such as inhalation rate, body weight, and lung surface area should be taken into consideration. Usually the comparison of the absolute value of a variable among different species is less useful because of the significant difference in body mass. Allometric scaling, in which a system variable is related to some measure of that system's size, is commonly used for interspecies comparison (Schubach 1995). For example, a comparison of minute volume (the volume of inspired gas per minute) can be made by either minute volume per unit body weight or by that per unit of animal surface area. Use of different scaling for comparison may produce different quantitative results.

The objectives of this chapter are to:

- Compare the anatomy of the respiratory tract (Section 4.2) and pulmonary function (Section 4.3) among different species, to provide a basic understanding of the biological characteristics of the respiratory tract as a portal to various toxicants;
- Summarize and discuss some important physical and chemical properties (Section 4.4) of the four gases to indicate the underlying mechanism of the fate of these gases after they enter the animal or human body;
- Summarize and discuss the characteristics of toxicokinetics (Section 4.5) of the four gases to provide a rationale of the important factors in the causal process with regard to the modification of the toxic load model and possible explanation of variability in response to the four gases.

4.2 Anatomy of the Respiratory Tract

4.2.1.Overview

The respiratory system is a very complex system and is the most important portal of entry for a variety of airborne toxic substances because of its huge surface area. For inhalation toxicology, the structure of the respiratory tract is usually divided into three distinct regions (Figure 4.1):

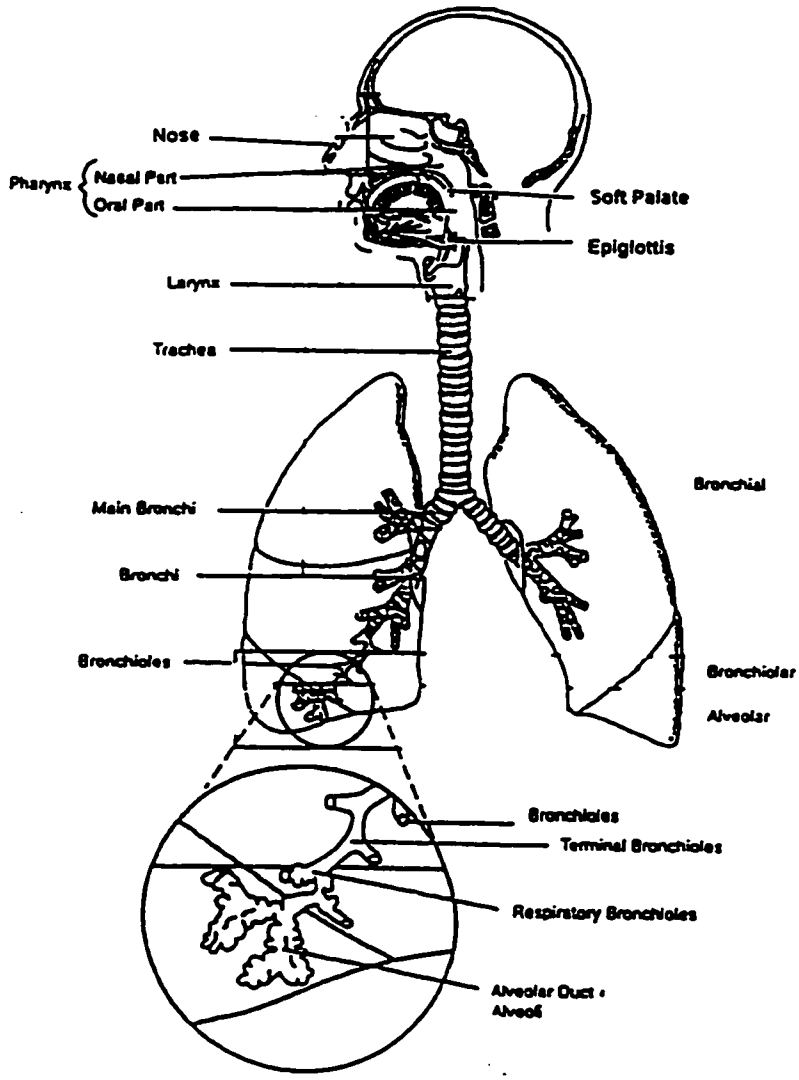


Figure 4.1 Three compartments of the respiratory tract (adapted from Raabe 1999)

- the upper airways, including the nose, nasopharynx, oral cavity, pharynx, and larynx; this part serves to warm, moisten, and filter the inspired air before it reaches the respiratory region of the lung.
- the conducting airways, including the trachea, bronchi and bronchioles; the anatomy of this region significantly influences the distribution of inhaled substances.
- the pulmonary region, including the respiratory bronchioles, alveolar ducts, alveolar sacs, and alveoli; the principal function of the pulmonary region is gas exchange (Costa and Schelegle 1999, Haschek and Witschi 1991).

These three compartments were originally proposed by the Task Group on Lung Dynamics of the International Commission on Radiological Protection (1966) based on anatomical features and on particle deposition and clearance phenomena that occur within each compartment. They are widely used in the toxicological studies of inhaled substances. A variety of studies on the anatomy of the respiratory tract mainly contain information of these three regions in different animal species. Therefore, the comparison of the structure of the respiratory tract among species discussed below will be based on this division of three compartments, since this division also represents the locational differences of toxic effects caused by the four gases.

4.2.2 The Upper Airways

4.2.2.1 Basic Structure

The upper airways begin at the anterior nares and include the respiratory airway down to the level of the larynx. The nasal cavity is bilateral and is separated by a central septum. The primary filtration network in the nasal cavity consists of a series of turbinates. The nasal cavity is lined with four types of epithelium: squamous, ciliated respiratory, non-ciliated columnar, and olfactory epithelium (Harkema 1992). The epithelium contains a rich vasculature. In submucosa, there is a very complicated arrangement of blood vessels. Because of these rich blood vessels, the main nasal airways in nasal cavity can widen or narrow (Proctor 1982).

4.2.2.2 Inter-species Comparison

Although the basic structure of the upper airways is similar among species, there are significant differences in the macroscopic and microscopic anatomy of the nasopharyngeal region, as well as in the distribution of the different types of epithelia and in the types of cells within specific regions.

In many species, the nose is the major route through which air and airborne substances enter the respiratory system. But it is the only route of entry in some species such as rats, which are called obligate nose breathers. In most animal species there is a close anatomical approximation between the epiglottis and the soft palate (See Figure 4.1). Thus, normally there is no direct communication between the mouth and the nasal air passages. When the mouth is open, the inspired air continues to pass through the nose. In man and higher primates, however, there is a wide gap between the epiglottis and the soft palate. When the mouth is open there is a direct communication between the mouth and nasal airways, resulting in oral respiration. Interestingly, in infants, the structure between the epiglottis and the soft palate is similar to rats, with infants being obligate nose breathers (Proctor 1982). Most adults usually breathe through the nose, but when the pulmonary requirements become sufficiently high, oral or oronasal (combined nose and mouth) breathing will take place.

The most significant difference in anatomy of the upper airways among species is the structure of the turbinate region (See Table 4.2). This difference can affect deposition of particles and distribution of inhaled gases in the nasal cavity. The structure of nasal turbinate is relatively simple in humans and the higher primates, whereas it is complex in rodents and extremely complex in dogs. Because of this structural difference, in rodents and dogs inhaled air follows a path through the upper half of the nasal cavity, but in humans and primates airflow is confined to the lower two-thirds of the nasal cavity (Proctor and Chang 1983). The bend in the nasopharynx of man and monkey is much sharper, and the flow rates through the nasal cavities are much higher.

The nasal surface epithelium also has significant variation. It is evident that the nasal surface epithelium varies in (1) the types of cells present in various intranasal locations in the same species; (2) the types of cells in different species in the same relative intranasal

location; and (3) the abundance and distribution of stored secretory product in different intranasal regions and in different species (Harkema 1992). The major difference is that rodents and dogs have higher percentage of the nasal airway covered by olfactory epithelium than humans and monkeys.

Table 4.2 Interspecies comparisons of nasal cavity characteristics

	Rat	Guinea pig	Beagle dog	Rhesus monkey	Man
Body weight (kg)	0.25	0.6	10	7	~70
Naris cross-section (mm^2)	0.7	2.5	16.7	22.9	140
Bend in naris (°)	40	40	30	30	n.a.
Length (cm)	2.3	3.4	10	5.3	7-8
Greatest vertical diameter (mm)	9.6	12.8	23	27	40-45
Surface area (both sides of nasal cavity) (cm^2) *	10.4	27.4	220.7	61.6	181
Volume (both sides) (cm^3) *	0.4	0.9	20	8	16-19
Bend in nasopharynx (°)	15	30	30	80	~90
Turbinate complexity	Complex scroll	Complex scroll	Very complex membranous	Simple scroll	Simple Scroll

Adapted from Schreider 1986 (* Includes sinuses for rat, guinea pig, beagle dog, and rhesus monkey. Does not include sinuses for man).

4.2.2.3 Importance of the Upper Airways in Inhalation Toxicology

The upper airways serve as the first line of defense for the respiratory tract and thus encounter the highest concentration of airborne toxic gases. Because the upper airways have large surface area and are covered with a mucosa rich in blood capillaries, gas molecules can be retained by the upper airways if they are very water-soluble or react with cell surface components. Thus, the upper airways act as a "scrubber" for water-soluble and highly reactive gases and vapors to protect the lung from injury (Klaassen 1996). High doses of airborne contaminants can injure the nasal tissue (Harkema 1991), especially for the nose-breathing animals such as the rat. Epithelial cells lining the luminal surface of the nasal cavity are the first cellular targets of inhaled toxicants.

In the case of very water-soluble gases, under normal breathing conditions there may be no major species differences in absorption (Proctor and Chang 1983). For less soluble gases, higher absorption may occur in those species with lower flow rates, more complex turbinate, greater relative surface areas, and smaller airway dimensions. In other words,

rodents and dogs with latter characteristics may absorb more toxic gases with low water solubility than humans. However, this situation is also complicated by species differences in the response to irritants in which the minute volume is decreased (by apnea) with a resulting decrease in absorption (Chang et al. 1981).

The differences in the abundance and distribution of the secretory cell types in the nasal airway suggest functional differences in this area among species. These differences may affect the amount of mucus available on the epithelia surface for mucociliary clearance. Mucociliary clearance of the respiratory system is an important protective mechanism in which inhaled toxicants are trapped or dissolved in the mucous layer and then removed from the site by cilia-generated mucous flow.

4.2.3 The Conducting Airways (Tracheobronchial Region)

4.2.3.1 Basic Structure

In humans and other mammals, the trachea, a flexible tube with U-shaped cartilages setting in its wall, divides into two main branches, principal bronchi, which enter the right and left lung. These two principal bronchi divide further with decrease in the diameter to the level of bronchioles. The bronchioles branch continuously into the terminal bronchioles, which divide further into well-developed respiratory bronchioles in some species such as humans, dogs, and cats, and then end at the alveolar ducts. In rodents, cattle, sheep, and pigs the terminal bronchiole directly ends in alveolar ducts with no respiratory bronchioles. Generally, tracheal dimensions can be predicted from body size. The ratio of tracheal diameter to tracheal length is relatively constant among species, approximating 1:8 (McBride 1992).

The tracheobronchial region is lined by respiratory epithelium similar to that of the nose and nasopharynx. It contains several types of cells including ciliated epithelia cells, mucous and serous epithelia cells, nonciliated bronchiolar epithelia (Clara) cells, neuroendocrine cells, and other cells. *Ciliated cells* are the most numerous epithelial cells in the conducting airways. The proportion of ciliated cells, number of cilia per cell, and length of cilia decrease with the diameter of the airway. Respiratory cilia are able to move viscoelastic mucus at the air-fluid interface. Ciliated cells also have a secretory function,

releasing macromolecules from their lumeral surface. *Mucous cell and serous epithelia cells* contribute to the secretion of airway mucus. Hypersecretion and mucus cell hyperplasia occur commonly in response to inhaled irritants. *Nonciliated bronchiolar epithelia cells* (Clara cells) are present in large numbers in the bronchioles of mammals, but not in birds. Clara cells have three major functions: (1) synthesize, store, and secret protein components of the extracellular lining of the bronchioles; (2) may transform into mucus-secreting cells after continued airway irritation; (3) play an important role in the metabolism of xenobiotics by the lung (Haschek and Witschi 1991)

4.2.3.2 Inter-species Comparison

The organization and composition of the tracheobronchial airway tree exhibits a significant amount of interspecies diversity in mammals. The average distance through this tree from a proximal reference point, generally the larynx, to the gas-exchange area, is highly variable. The size of daughter branches and the angle of branching is also a species-specific characteristic. Compared to the common laboratory animals, human lungs have more symmetric tracheobronchial airway branches (Haschek and Witschi 1991).

There is variation in the number of glands (an aggregation of cells, specialized to secrete or excrete materials not related to their ordinary needs) in the tracheal and bronchial submucosa and the distance that cartilage extends down the airways among species (Table 4.3). Rodents generally have few tracheal glands, whereas cat, sheep, pig, dog, cow, monkey, and human have a large number of well-developed submucosal tracheal glands. Bronchial submucosal glands are abundant in the cat but not in the dog. In the mouse, cartilaginous rings are present only in trachea, while in the rat they are found in the trachea and extrapulmonary bronchi. In humans, cartilage extends for varying distances along the intrapulmonary bronchi but not in the bronchioles.

Table 4.3 Inter-species comparison of pulmonary anatomy

Anatomical Characteristics	Mouse, rat, hamster	Dog, cat, Rhesus monkey	Cow, pig	Human
Pleura	Thin	Thin	Thick	Thick
Secondary lobulation	Absent	Absent	Present	Incomplete
Cartilage and submucosal glauds in intrapulmonary bronchi	Absent	Present	Present	Present
Respiratory bronchioles	Essentially absent	Present	Essentially absent	Present

Adapted from Haschek and Witschi 1991

The basic structure of the respiratory epithelium is the same in all mammals; however, the location of the various cell types within the epithelia is different among species (Table 4.4). The differences exist (1) at the same airway level in different species; (2) at different airway level in the same species; (3) in the types of cells present in different airway generations; and (4) in the overall abundance of secretory product and the number of cells in different airways (Haschek and Witschi 1991). Table 4.4 indicates that dogs resemble humans quite closely, whereas mice are very different.

4.2.3.3 Importance of the Conducting Airways in Inhalation Toxicology

The importance of this region in inhalation toxicology is that the dimensions and geometry of the conducting airways to a large extent determine particle penetration and deposition of inhaled particles. These airways contribute a major portion of the overall resistance to airflow below larynx (Pedley et al. 1970). Generally, the conducting airways are of less importance for the deposition of toxic gases or vapors. However, the evidence for the four gases is very limited.

The epithelia lining of respiratory tract is a major site for pulmonary injury resulting from exposure to toxic chemicals. Ciliated cells are extremely sensitive to injury caused by inhaled toxic agents such as nitrogen dioxide (NO_2), sulfur dioxide (SO_2), ozone (O_3), and cigarette smoke (Haschek and Witschi 1991).

Table 4.4 Inter-species comparison of respiratory epithelia

Epethelia types	Mouse	Rat	European hamster	Syrian hamster	Dog	Human
Pseudostratified ciliated, with goblet cells and basa cells	Trachea	Trachea; main bronchi; segmental bronchi	Trachea; main bronchi; lobar bronchi	Trachea; main bronchi	Trachea; main bronchi; lobar bronchi; segmental bronchi	Trachea; main bronchi; lobar bronchi; segmental bronchi; subsegmental bronchi
Simple columnar ciliated, with Clara cells	Main bronchi; lobar bronchi; segmental bronchi	Subsegmental bronchi; bronchioles; terminal bronchioles	Segmental bronchi; subsegmental bronchi; bronchioles; terminal bronchioles	Lobar bronchi; segmental bronchi; subsegmental bronchi	Peripheral bronchioles; terminal bronchioles	Peripheral bronchioles; terminal bronchioles
Simple cuboidal, mainly Clara cells, occasionally ciliated cells	Subsegmental bronchi; bronchioles; terminal bronchioles	Respiratory bronchioles	Respiratory bronchioles	Bronchioles; terminal bronchioles; respiratory bronchioles	Respiratory bronchioles	Respiratory bronchioles

From: Reznik-Schuller and Reznik 1979

4.2.4 The Pulmonary Region

4.2.4.1 Basic Structure

The respiratory bronchioles are tubular structures with diameters of about 0.5 mm and lengths of about 1.0 mm (Costa and Schelegle 1999). The parenchyma of the lung includes alveolar ducts, alveolar sacs, alveoli, alveolar capillaries, and pulmonary lymphatics. The term acinus refers to the functional unit of the lung, which consists of the terminal bronchiole and all of the air spaces supplied by it (Naigaishi 1972, Schreider and Raabe 1981, Weibel 1983). The adult human lung contains 200 to 600 million alveoli, depending on the individual's height (Angus and Thurlbeck 1972) with a total alveolar surface area of about 70 to 80 m^2 (Horsfield 1974). Because of this massive increase in cross-sectional area extending from the terminal bronchiole to the alveoli, the inhaled gas transport mechanism changes from mass flow to molecular diffusion.

The epithelium of this region is made up primarily of two types of cells, type I and type II. Type I cells cover over 95% of the alveolar surface area (Overton and Miller 1988). The major functions of type I cells are to provide a surface for gas exchange and serve as a permeability barrier (Simon 1992). Type II cells have metabolic and secretory functions as well as being involved in the repair of damage (Overton and Miller 1988). The important role of type II cells is to maintain alveolar stability and pulmonary function. In the pulmonary region, endothelial cells of the alveolar capillaries form a barrier to prevent leakage of excess water and macromolecules into the pulmonary interstitium. Intercellular junctions between endothelial cells are less tight than the epithelial junctions, thus the major permeability barrier in the lungs is the alveolar epithelium (Haschek and Witschi 1991).

4.2.4.2 Inter-species Comparison

The biological significance of the species differences in this region relates to the differences in the acinus. In animals without respiratory bronchioles such as rodents, the terminal bronchiole ends by forming several alveolar ducts, resulting in a relatively simple acinus that may have a relatively small volume. In humans and animals that have respiratory bronchioles such as cats and dogs, the acinus is more complex and probably

larger because of the presence of respiratory bronchioles. The complex acini of species with respiratory bronchioles have airway cell types associated with respiratory bronchioles, in addition to the alveolar cell types that are common to both simple and complex acini (Tyler and Julian 1992). In addition, the size and total number of alveoli varies considerably from species to species, with age in a given species, and even from individual to individual. Over the range from mouse to human lung, there is an approximately 50-fold increase in acinar number, a 170-fold increase in acinar volume, and a 7-fold increase in acinar diameter (Mercer and Crapo 1992).

4.2.4.3 Importance of the Pulmonary Region in Inhalation Toxicology

The species difference in the presence or absence of the respiratory bronchioles is of critical importance for inhalation toxicology. The junction of the bronchioles with the alveolar ducts is the most vulnerable part of the lung to injury caused by many inhaled toxicants including both gases and particles at low to moderate concentrations (Schwartz et al. 1976, Hyde et al. 1978, 1985, 1988). Irritants that reach this area are likely to be completely absorbed. The larger volume of acini with respiratory bronchioles may result in a larger dose of inhaled toxicants to the sensitive cells of the area of junction between the conducting airways and the pulmonary region, in humans and in animals with respiratory bronchioles.

The alveolar epithelial type I cells are highly susceptible to injury by a number of toxic agents because they have a large surface area. In addition, they are relatively unable to repair damage. Endothelial cells of the alveolar capillaries are susceptible to injury by a variety of toxic agents. The injury may affect intercellular junctions or result in necrosis and may contribute to the development of pulmonary edema (Haschek and Witschi 1991).

In summary, there are significant differences in the anatomy of three regions of the respiratory tract among animals and humans. The respiratory structures of dogs and monkeys are closer to humans compared to other small animals such as rodents. The differences in the upper airways and pulmonary region may be more important than the conducting airways in the deposition of toxic gases and vapors.

4.3 Pulmonary Function

The principal function of the lungs is gas-exchange, i.e., to supply the organism with oxygen and to remove carbon dioxide. This process involves ventilation, diffusion and perfusion. Because ventilation may have the most significant influence on the toxicity of the four gases, comparison of pulmonary function among species will mainly focus on the ventilation.

4.3.1 Ventilation under Normal Breathing

Capacities and volumes of the human lungs at the rest are listed in Table 4.5.

Table 4.5 Capacities and volumes of the human lung at the rest

Lung volumes and capacities	Definition	Values (ml)
Tidal volume (TV)	The volume of gas inspired or expired during each respiratory cycle. Also the depth of breathing	500
Inspiratory reserve volume (IRV)	The maximum volume of gas that can be inspired from the end-inspiratory level	3100
Expiratory reserve volume (ERV)	The maximum volume of gas that can be expired from the end-expiratory level	1300
Total lung capacity (TLC)	The volume of gas in the lungs at the end of a maximum inspiration (the maximum inspiratory level)	6000
Vital capacity (VC)	The maximum volume of gas that can be expelled from the lungs by forceful effort following a maximum inspiration	4800
Minute volume	The volume of gas inspired or expired for one minute, i.e., tidal volume multiplied by the frequency of breath	6000 (12 rpm)

Adapted from Phalen 1984

The comparison of body weight and several respiratory parameters among species is given in Table 4.6.

Table 4.6 Mean volumes for selected respiratory parameters in mammalian species

Species	Body wt (kg)	Tidal vol (ml)	Minute vol (ml/min)	Frequency (min^{-1})	Lung volume (ml)
Mouse	0.023	0.18	24	109	0.74
Rat	0.14	1.4	84	115	6.3
Rabbit	3.6	23.9	1040	37	79
Monkey	3.7	20	694	33	184
Dog	22.8	193	2920	19	1500
Human	75	500	6000	12	7000

Data from Altman & Dittmar 1974, Boyd & Mangos 1981, Crosfill & Widdicombe 1961, Phalen 1984.

Based on the data given in Table 4.6, the values of minute volume per unit body weight for each species were calculated and compared (Figure 4.2). This figure shows that rodents (mice and rats) have largest minute volume per unit body weight compared to other species, while humans have the smallest minute volume per unit body weight. The implication of this comparison is that rats and mice may inhale more toxicants when exposed to toxic gases through inhalation than other species, compared on a body mass basis, although some other factors also influence the amount of toxicants entering the respiratory tract.

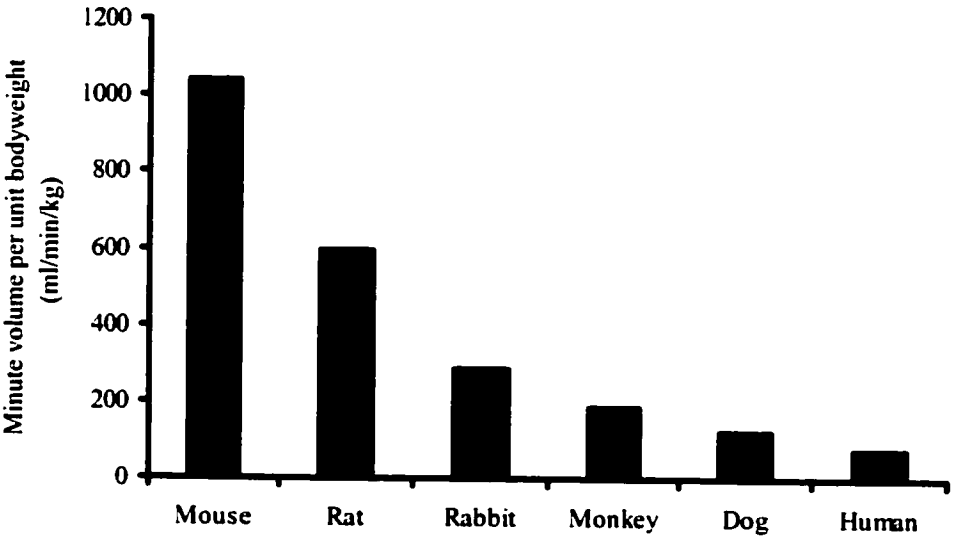


Figure 4.2 Comparison of minute volume per unit bodyweight among species

4.3.2 Ventilation during Exercise

The effects of exercise during an inhalation exposure on the magnitude of the response to inhaled toxicants are very important in inhalation studies. From a predictive modeling perspective, response to a toxicant during exercise resembles the situation of accidental release of large amount industrial gases where unprepared workers may panic and try to escape dangers by running away. During exercise, an increased volume of air breathed may lead to greater exposure to a toxicant. For example, compared to sitting, the minute volume can be increased 2 to 3 times by walking, and more than tenfold during maximal exertion (Henderson and Haggard 1943, Table 4.7). In exercise, there is often a

shift from nasal to oronasal breathing, and this shift decreases the resistance of the upper airway to air flow (Phalen 1984). However, the portion of air that enters the mouth does not receive the benefit of nasal scrubbing of larger particles and toxic gases. Therefore, exercise tends to increase the biological impact of an inhaled toxicant.

Table 4.7 Inhalation and oxygen consumption rate for various levels of enhanced activity for man

Activity	Inhalation rate (l/min)[a]	Oxygen consumption rate (l/min)[b]
Rest in bed, fasting	6	0.24
Sitting	7	0.30
Standing	8	0.36
Walking, 2 mile/h	14	0.65
Walking, 4 mile/h	26	1.20
Slow run	43	2.00
Maximum exertion	65-100	3.00-4.00

From Henderson and Haggard 1943
[a] Measured at 0°C and 760 mmHg
[b] Measured at 20°C

There are some other factors in exercise which may change the subjects response to a toxicant: (1) the potential overriding of the protective reflex breathing pattern changes (e.g., a shift to more shallow breathing); (2) the widening of the larynx in response to exercise; (3) changes in tissue metabolism which may alter local tissue sensitivity; and (4) the possibility of decreased scrubbing efficiencies in the upper airways (Phalen 1984).

4.3.3 Reflex Actions

A series of reflex actions can be evoked by the sensory irritation on the respiratory tract including coughing, sneezing, bronchial constriction, reductions in the volumes and rates of ventilation, and excess secretion of mucus (Phalen 1984). Generally, these reflex actions serve as protective mechanisms to limit or prevent toxicants from entering the respiratory tract.

Different species may react differently to inhaled irritants because of differences in neuro- and muscular anatomy. In humans, the most common reflex is the sneeze, which

expels the inhaled substances in a forceful expiratory effort (Proctor and Chang 1983). It has been observed that tracheobronchial airway resistance increased following inhalation of sulfur dioxide due to bronchoconstriction (NAS 1978). Lower concentrations of SO_2 can cause shallow and rapid breathing, while stronger irritants have been shown to depress breathing (Proctor and Chang 1983).

In animals, the most frequently observed reflex is apnea, a phenomena involving a temporary stop in breathing. Apnea has been observed in rodents, rabbits, cats, pigs, and dogs (Widdicombe 1977). A study on the sensory irritant, formaldehyde, has shown that the apnea reflex induced a greater degree of minute volume reduction and hence more flow limitation in animals than in humans (Chang et al. 1981).

4.3.4 Pulmonary Function Test

Tests of pulmonary function are very useful tools for evaluating the toxicology of inhaled toxic chemicals. An understanding of the fundamentals of respiratory and cardiovascular physiology is necessary for understanding pulmonary function tests. Pulmonary function tests are a nondestructive means of assessing the functional impact of alterations of lung structure. The tests provide information on presence (whether or not function is impaired), nature (type of impairment), and extent (magnitude of impairment) of function loss. The tests are used in inhalation toxicology as an indicator of toxic response, to characterize the pathogenesis of lung disease, and to extrapolate the impact of toxicant-induced lung disease from laboratory animals to humans. Inhalation toxicology studies and studies using specific experimentally induced lung diseases in animals have shown that functional responses of man and animals to different type of lung injury are similar (Mauderly et al. 1983, Mauderly 1984, 1987). Based on this foundation, pulmonary function tests can be used to estimate the impact in man of responses to inhaled materials studied in laboratory animals (O'Neil and Raub 1983).

In summary, the minute volume per unit body weight is different among species, which indicates that small animals such as rodents may receive more gases than large animals and humans. However, the strong reflex action caused by irritants in rodents may substantially reduce the minute volume and thus prevent toxic gases from entering the

respiratory tract. In other words, the value of the minute volume per unit body weight alone cannot determine the amount of toxic gases entering the respiratory tract.

4.4 Physical and Chemical Properties of Gases

The dispersion of gases and vapors in the air after an accidental release and the behavior of inhaled gases in the biological system largely depend on some important physical and chemical properties of the gases such as their molecular weight, specific gravity, solubility in different media, vapor pressure, and reactivity.

Gases that are highly soluble in water will be largely absorbed into the wet lining layer of the upper airways. Gases not trapped in the upper airways will move further down into the small airways and diffuse deep into the lung (Phalen 1984). This diffusion is driven by concentration gradients. If the atmospheric partial pressure of a gas in inhaled air is greater than that in the alveoli, the gas will undergo net movement into the alveolar region. In alveoli, the gas will move toward equilibration with the lung surface.

Dissolved gas will distribute within tissue, including the blood, and will partition among aqueous and non-aqueous (e.g., fat or lipid) compartments. Gas dissolved in the blood will distribute throughout the body and deposit in various organs in accordance with their relative blood flow and composition (e.g., percentages of water and lipid).

According to Henderson and Haggard (1943), gases and vapors can be classified into four groups: (1) Irritants; (2) Asphyxiants; (3) Volatile drugs and drug-like substances; (4) Inorganic and organometallic gases. Irritants can be further divided into *primary* or *secondary* irritants. *Primary* irritants have little or no systemic effect in the concentration that causes death, whereas the *secondary* irritants produce systemic toxic effects in addition to surface irritation. This classification of gases and vapors can help understanding the similarity and differences in actions of different gases upon inhalation.

4.4.1 Hydrogen Sulfide

Hydrogen sulfide is a colorless gas under normal condition with a characteristic odor of rotten eggs. It is a secondary irritant (Henderson and Haggard 1943) and asphyxiant gas. Hydrogen sulfide is heavier than air and generally stable when properly stored in

cylinders at room temperature. However, in the air, it is flammable and explosive. Hydrogen sulfide is water-soluble. An aqueous solution of hydrogen sulfide exhibits two acid dissociation constants. The first dissociation occurs with ionization of first proton and yields a hydrosulfide anion (Equation 4.1). A second proton may dissociate from the resultant hydrosulfide anion to give the sulfide ion (Equation 4.2).

$$H_2S \rightleftharpoons H^+ + HS^- \quad (4.1)$$

$$HS^- \rightleftharpoons H^+ + S^= \quad (4.2)$$

The dissociation constant (pKa) for the first and second dissociation steps of H_2S are 7.04 and 11.96, respectively; therefore under physiological conditions hydrogen sulfide exists primarily in the undissociated form (H_2S) and as the hydrosulfide anion (HS^-) (Beauchamp et al. 1984). Some important physical and chemical properties are summarized in Table 4.8.

Table 4.8 Physical and chemical properties of hydrogen sulfide

Property	Value	Reference
Molecular weight	34.08	Budavari et al. 1996
Boiling point at 1 atm	- 60.33°C	Budavari et al. 1996
Freezing point at 1 atm	- 85.49°C	Budavari et al. 1996
Specific gravity	1.192	ACGIH 1991
Vapor pressure at 21.9°C	1929 kPa, 14,469 mmHg	Lide & Frederikse 1993
Density at 0°C, 760 mmHg	1.5392 g/l	Budavari et al. 1996
Solubility in water at 20°C	4.13 g/l	Budavari et al. 1996
Conversion factor at 25°C, 1 atm	1 ppm = 1.40 mg/m^3	NIOSH 1994

4.4.2 Chlorine

Chlorine (Cl_2) is a greenish-yellow, non-combustible gas at room temperature and atmospheric pressure with a pungent odor (Das and Blanc 1993). It is heavier than air with a specific gravity 2.49 times that of air (Charan et al. 1985). Chlorine is a very reactive element that readily combines with a variety of organic compounds and radicals. It is a primary irritant (Henderson and Haggard 1943). For industrial use, it is stored or

transported in liquefied form under pressure. Chlorine is slightly soluble in water and hydrolyzes to form hypochlorous (HOCl) and hydrochloric (HCl) acids, which are both highly soluble in water. Under biological conditions of pH 7.4 and 37°C, chlorine has the following reaction with water:

$$Cl_2 + H_2O \rightleftharpoons Cl^- + HOCl + H^+ \quad (4.3)$$

This reaction has an equilibrium constant at 25°C (Whitney and Vivian 1941) of:

$$10^{-pH}[Cl^-]\,[HOCl] / [Cl_2] = 5 \times 10^{-4}\ (mol/l)^2 \quad (4.4)$$

where $[Cl^-]$ and [HOCl] indicate molar concentrations of the chloride ion and hypochlorous acid in the aqueous phase, respectively, and $[Cl_2]$ is the concentration of Cl_2 gas in dissolved form. For mucus that has a $[Cl^-]$ of ~ 0.16 mol/ l (Matthews et al. 1963) and a pH of ~ 6.6 (Bodem et al. 1983), Eq. 4.4 indicates that the concentration of dissolved Cl_2 in hydrolized form ([HOCl]) is 120,000 times $[Cl_2]$. In other words, the effective solubility of Cl_2 between the respired gas and mucus phase is five orders of magnitude larger than the physical solubility (without hydrolysis) of chlorine in water because of the hydrolysis (Nodelman and Ultman 1999a). HOCl carries all of the oxidizing capacity of the Cl_2 gas once the hydrolysis to HOCl and HCl has occurred. Some important physical and chemical properties of chlorine are listed in Table 4.9.

Table 4.9 Physical and chemical properties of chlorine

Property	Value	Reference
Molecular weight	79.906	Turner &Fairhurst 1990a
Boiling point at 1 atm	- 34.04°C	CRC 2000
Melting point at 1 atm	- 101.5°C	CRC 2000
Specific gravity	1.56	CRC 2000
Vapor pressure at 20°C	3.65×10^5 Pa	Turner &Fairhurst 1990a
Density at 0°C, 760 mmHg	3.214 g/l	CRC 2000
Solubility in water at 20°C	14.6 g/l	Turner & Fairhurst 1990a
Conversion factor at 25°C, 1 atm	1 ppm = 2.90 mg/m^3	Turner & Fairhurst 1990a

4.4.3 Ammonia

Ammonia (NH_3) is a colorless gas at ambient temperature and pressure with a characteristic pungent smell. It is lighter than air and can be stored and transported as a liquid at a pressure of 10 atm at 25°C (WHO 1986). Ammonia is categorized as a primary irritant (Henderson and Haggard 1943).

Ammonia has high water solubility and dissolves readily in water where it ionized to form the ammonium ion (NH_4^+). Ammonia and ammonium reach an equilibrium in water as following:

$$NH_3 + H_2O \rightleftharpoons NH_4^+ + OH^- \qquad (4.5)$$

The water solubility of ammonia increases with decreasing pH. At pH 9.25, half of the ammonia will be un-ionized (NH_3) and half will be ionized (NH_4^+). At pH 7.25, 99% of the ammonia will be ionized (ATSDR 1990). Therefore, at the pH of most biological systems, ammonia exists predominantly in the ionized form (WHO 1986). Some important properties of ammonia gas are listed in Table 4.10.

Table 4.10 Physical and chemical properties of ammonia

Property	Value	Reference
Molecular weight	17.03	Budavari et al. 1996
Boiling point at 1 atm	- 33.42°C	Payne et al. 1990
Freezing point at 1 atm	- 77.74°C	Payne et al. 1990
Specific gravity	0.5967	Budavari et al. 1996
Vapor pressure at 25°C	10 atm	Payne et al. 1990
Density at 0°C, 1 atm	760 g/ m^3	Payne et al. 1990
Solubility in water, 101kPa		WHO 1990
at 0°C	895 g/l	
20°C	529 g/l	
40°C	316 g/l	
60°C	168 g/l	
Conversion factor at 25°C, 1 atm	1 ppm = 0.70 mg/m^3	Payne et al. 1990

4.4.4 Hydrogen Fluoride

Hydrogen fluoride is a colorless liquid or gas with a pungent odor at room temperature. It is heavier than air and is completely soluble in water below 20°C (WHO

1984). Being a weak acid hydrogen fluoride ionizes at physiologic pH (Morris and Smith 1982). Anhydrous hydrogen fluoride is one of the most acidic substances known. Although many different fluoride compounds widely exist in air, water, and food, hydrogen fluoride is the most important fluoride manufactured in the industrial setting (WHO 1984). Hydrogen fluoride is classified as a primary irritant (Henderson and Haggard 1943). Fluoride ions can be released and absorbed contributing to fluoride effects. Some important physical and chemical properties of hydrogen fluoride are listed in Table 4.11.

Table 4.11 Physical and chemical properties of hydrogen fluoride

Property	Value	Reference
Molecular weight	20.01	Turner & Fairhurst 1990b
Boiling point at 1 atm	19.54°C	WHO 1984
Freezing point at 1 atm	- 83°C	WHO 1984
Specific gravity	1.858	Environmental Protection Service 1984
Vapor pressure at 20°C	103.42 kPa	Environmental Protection Service 1984
Density at 25 °C	2.201 g/l	Environmental Protection Service 1984
Solubility in water at 20°C	infinite	Turner & Fairhurst 1990b
Conversion factor at 25°C, 1 atm	1 ppm = 0.83 mg/m^3	Turner & Fairhurst 1990b

In Summary, hydrogen sulfide, chlorine, ammonia, and hydrogen fluoride have similarities and differences in their physical and chemical properties as summarized in Table 4.12. They all have relatively low molecular weight ranging from 17.03 to 34.08 except for chlorine with a moderate molecular weight of 70.906.

Table 4.12 Comparison of physicochemical properties of the four gases

Chemical	Molecular weight	Specific gravity	Water solubility (g/l, at 20°C)	Reactivity	Classification
H_2S	34.08	1.192	4.13	high (reducing agent)	Secondary* irritant
Cl_2	70.906	1.56	14.6	high (oxidizing agent)	Primary* irritant
NH_3	17.03	0.5967	529	high (base)	Primary* irritant
HF	20.01	1.858	infinite	high (acid)	Primary* irritant

* See Section 4.4 introduction for explanation

Hydrogen sulfide, chlorine, and hydrogen fluoride are heavier than air while ammonia is lighter than air. When released at ground level, the first three gases will form a dense plume near the ground, whereas ammonia will form a buoyant plume that will disperse vertically in the atmosphere.

Ammonia and hydrogen fluoride are highly water soluble and reactive as a base and acid, respectively. Hydrogen sulfide and chlorine are less water soluble. Chlorine is also very reactive as an oxidizing agent while hydrogen sulfide is a reducing agent. One volume of water dissolves two volumes of hydrogen sulfide while one volume of water dissolve 400 volumes of ammonia. Chlorine is much more soluble in mucus than in water which has an important implications for the absorption of chlorine by the respiratory tract. Gaseous chemicals possessing both high water solubility and reactivity such as ammonia and hydrogen fluoride tend to largely deposit and react in the upper respiratory tract after being inhaled. Gases that are less water soluble such as hydrogen sulfide and chlorine will be expected to extend their action throughout the entire length of the respiratory tract and the lower respiratory tract will suffer the greatest damage. Recognizing these differences based on the physical and chemical properties of gases can help to understand the different location and extent of toxic actions exerted by different gases.

4.5 Toxicokinetics of the Four Gases

4.5.1 Overview

Toxicokinetics, a term originated from pharmacokinetics, refers to the process of the absorption, distribution, metabolism, and excretion (ADME) of xenobiotics. In the context of inhalation toxicology of the four gases investigated in this study, toxicokinetics mainly involves absorption or uptake of gases by the respiratory tract, deposition of gases within the respiratory tract and throughout the whole body via the bloodstream, transformation from original form to the more or less toxic metabolites, and finally, elimination through urine or exhaled air.

Definitions: in inhalation toxicokinetics, *absorption* or *uptake* refers to the transfer of a toxic gas from the site of exposure (usually the respiratory tract) to the surrounding tissues and the systemic circulation; *Desorption* is the reverse process. *Penetration* is the

airborne entry of a gas into the distal portions of the respiratory tract beyond the upper airways (Morgan and Frank 1977). *Deposition* refers to the fractions of inhaled airborne substances, particularly particles, which are never exhaled, but ultimately deposited on surfaces in the respiratory tract. *Clearance* refers to the subsequent translocation, transformation, and removal of deposited substances from the respiratory tract or from the body. The temporal distribution of uncleared deposited materials is called *retention.* For a brief exposure, the relationship of these three concepts may be described by the equation:

Retention (t) = Deposition - clearance (t)

where (t) refers a function of time after deposition occurs (Raabe 1982).

Inhaled gases and vapors, even those with relatively high volatility and low water solubility, may be largely absorbed in the nasal cavity rather than in the lungs, although lung uptake also may be substantial (Stott and McKenna 1984). The removal of soluble vapors by the nose increases with respiratory airflow rate (Aharonson et al. 1974). For inhalation with many irritant gases and vapors, the upper airways, especially the nose, are the most common site to suffer toxic effects (Buckley et al. 1984). The action of most of the irritant gases on the respiratory tract results in their destruction or neutralization; therefore, they are not absorbed into the body in their original form (Haggard 1924).

Primary factors that affect the uptake of a reactive gas in the respiratory tract include (Overton and Miller 1988):

- the morphology and anatomy of the respiratory tract,
- the route of breathing (nasal, oral, or oronasal),
- the depth and rate of breathing, the physical and chemical properties of the gas,
- the processes of gas transport, and
- the physicochemical properties of the liquid lining of the lung, lung tissue, and capillary blood.

The transfer of gas molecules from inhaled air to the epithelium surfaces of the airways depends on convective diffusion, which, in turn, depends on breathing rates and volumes and on molecular diffusivity. Not all molecules that hit the surface of the airways can be taken up into the tissues. Some absorbed gas and vapor molecules may be

released back into the air and exhaled. The uptake process is affected by the factors controlling the rate of transfer of these molecules through the liquid-gas interface and epithelial membranes including the air - to- tissue and air- to -blood partition coefficients, which depend on solubility in body fluids.

Distribution refers to the process through which toxic gases transfer to the distal organs and tissues via the bloodstream after absorption by the respiratory tract. Gases may distribute to distal target organs and tissues to be metabolized to more or less toxic forms, and exert their toxic effects or be detoxified. Excretion refers to the process of removal of toxic gases or their metabolites from the blood and return to the external environment. For toxic gases, the major routes for excretion include urine and exhalation. Excretion through other pathways, e.g., feces, is relatively minimal.

For a gas with high solubility in water, deposition of the inhaled gas in the upper respiratory tract is very important with respect to its toxicity. The process of deposition involves dissolution of inhaled gas molecules in the fluid lining layer of the upper respiratory tract. Once in solution, diffusion away from the lining layer and perhaps removal via the bloodstream, chemical reaction with water or tissue components, or resorption back into the gaseous phase may occur (Morris and Smith, 1982). Removal of gaseous solutes via the bloodstream or via chemical reaction will push the equilibrium toward the liquid phase by reducing the partial pressure of the free gas in solution. Thus, highly soluble and/or reactive gases would be deposited efficiently in the upper respiratory tract.

4.5.2 Hydrogen Sulfide

4.5.2.1 Absorption

Human

Inhalation is the most important route of hydrogen sulfide exposure, although hydrogen sulfide can also be absorbed through skin and the gastrointestinal tract (Laug and Draize 1942, Wetterau et al. 1964). Quantitative data on the absorption of hydrogen sulfide by humans through inhalation is very limited. Knowledge comes mainly from case reports of acute hydrogen sulfide poisoning (Adelson and Sunshine 1966, Breysse 1961,

Deng and Chang 1987, Hagley and South 1983, Kimura et al. 1994, Osbern and Crapo 1981, Parra et al. 1991). At lethal concentrations, hydrogen sulfide is rapidly absorbed through the lungs with toxic effects occurring within seconds to minutes. Most of accident case reports provide little information on accurate exposure concentrations and duration. Measurements of hydrogen sulfide or its metabolites in the victim are also rare, which makes it difficult to estimate either the rate or the extent of absorption.

Only one human experiment on absorption of hydrogen sulfide has been located in the literature. Lehmann (1893) applied different concentrations of hydrogen sulfide ranging from 150 to 540 ppm to human volunteers (the number and ages of volunteers were unknown) and observed significant absorption ranging from 85.9% to 99~100%. The absorption of hydrogen sulfide was more complete at lower concentrations than at higher concentrations.

Kimura et al (1994) measured sulfide concentrations in the blood and other body tissues of three male adults after they died from exposure to hydrogen sulfide. The results confirmed that hydrogen sulfide is absorbed through the lungs and sulfide concentrations in the brain and the lungs are clear indicators of hydrogen sulfide exposure. Winek et al (1968) also reported sulfide concentration in the blood and other body tissues in a man killed by exposure to a hydrogen sulfide concentration range of 1900 to 6000 ppm.

Animal

Animal studies on absorption of hydrogen sulfide through inhalation are surprisingly limited. Some studies (Beck et al. 1979, Kage et al. 1992, Khan et al. 1990, Lopez et al. 1989, Nagata et al. 1990, Prior et al. 1988, 1990, Smith and Gosselin 1964, Tansy et al. 1981) have indicated that absorption of hydrogen sulfide through the lungs is rapid. Nagata et al (1990) observed postmortem changes in sulfide concentrations in body tissues of autopsied rats exposed to hydrogen sulfide concentration of 550 to 650 ppm and in non-exposed rats. The sulfide concentration in the blood analyzed immediately after death averaged 0.48 μg/g, while no sulfide was found in the controls. However, no studies were located that provide quantitative information on the rate and the extent of absorption of hydrogen sulfide.

Comparison

Limited information has qualitatively indicated that hydrogen sulfide is rapidly (a few seconds to minutes) absorbed in humans and animals through inhalation at high concentrations. No quantitative comparison can be made with respect to the location, rate or extent of absorption of hydrogen sulfide between animals and humans because of the lack of data.

4.5.2.2 Distribution

Human

In a fatal case study, sulfide was detected in the blood, brain, lung, liver, kidney, and spleen after death of three male adults who were exposed to hydrogen sulfide concentrations estimated at 550-650 ppm (Kimura et al. 1994). The sulfide levels in lung and brain were suggested to represent tissue levels at the time of death. In another report (Winek et al. 1968), sulfide was detectable in the blood, brain, kidney, and liver at autopsy in a male adult who died after exposure to 1900-6000 ppm hydrogen sulfide. These data suggest that sulfide was widely distributed throughout the body after absorption into the blood in humans.

Animal

Rats, guinea pigs and rabbits have been used to study the distribution of hydrogen sulfide. Adult male rats exposed to 550 - 650 ppm hydrogen sulfide until death were examined for sulfide concentration in body tissues (Nagata et al. 1990). Sulfide was detected in the blood, lung, brain, and muscle in the exposed rate immediately after death, but not in the control animals. Sulfide can be found in liver and kidney in both exposed and unexposed rats because of the tissue decomposition after death. Hydrogen sulfide appears to be widely distributed throughout the whole body after inhalation exposure. Sulfide concentrations in the brain and lung can be used as indicators of hydrogen sulfide exposure. Warenycia et al. (1989) found that accumulation of rat brain sulfide was linearly proportional to the applied dose of NaHS, and was strongly correlated with mortality data.

A study using a histochemical method to identify hydrogen sulfide after inhalation exposure in rats and guinea pigs found that hydrogen sulfide was distributed to the brain, liver, kidneys, pancreas, and small intestine (Voigt and Muller 1955).

A study with Japanese white rabbits has shown that thiosulfate, a major metabolite of sulfide, was detectable in the blood, lung and brain but not in liver, kidney, and muscle after the rabbits were exposed to hydrogen sulfide at a lethal concentration of 500-1000 ppm for 60 minutes (Kage et al. 1992).

Comparison

Distribution of hydrogen sulfide is quite similar in rats, guinea pigs, rabbits, and humans although the concentrations of tissue hydrogen sulfide are different among species (Table 4.13). Available data suggest that hydrogen sulfide is rapidly distributed throughout the body after absorption from the respiratory tract in experimental animals and humans. However, sulfide can also be produced in significant quantities in the liver and kidneys after death due to tissue decomposition.

Table 4.13 Comparison of distribution of hydrogen sulfide in different species

Species	Air conc. of H_2S (ppm)	Time (min)	Tissue concentrations of sulfide					Reference
			Blood (μg/g)	Lung (μg/g)	Brain (μg/g)	Liver (μg/g)	Kidney (μg/g)	
Rat	550-650	until death	0.48	600	310	1670	1450	Nagata et al. 1990
Rat	75	20-60	10 *	20	25	20	30	Kohno et al. 1991
Rabbit	500-1000	60	2.41*	2.86	0.69			Kage et al. 1992
Human	550-650	unknown	0.13	0.37	0.55	1.42	0.96	Kimura et al. 1994
Human	1900-6000	5	0.92		1.06	0.38	0.34	Winek et al. 1968

* Reported as μg/ml which is approximately μg/g

In most studies cited in Table 4.13, sulfide or sulfate levels in the blood and tissues have been measured after animals or humans died. Sulfide in the lungs and brain has been suggested to be a better indicator of inhalation hydrogen sulfide exposure (Nagata et al. 1990).

Very limited data are available to reflect human variability in exposure to hydrogen sulfide. Table 4.14 lists the tissue sulfide level of three male adults after they died from exposure to hydrogen sulfide. Although the sample size is too small to make any statistically meaningful comparison among individuals and detailed individual information was lacking in this study, the data presented below does, to some extent, indicate that sulfide levels in most tissues were very similar in three victims except for the lung and brain in the case of victim C. As a result, the ratio of lung sulfide concentrations to brain sulfide concentrations is quite different among these three victims, ranging from 0.3 to 5. The reason for this difference is not clear. The fact that 62-year-old victim B has higher ratio of 5 compared to other two younger victims suggests that the relative lower brain sulfide level may be due to the decrease in brain blood supply in old people. The processes of exposure to hydrogen sulfide were expected to be similar among these healthy male individuals although much more information is necessary for comparison of other subpopulations.

Table 4.14 Comparison of sulfide concentrations in human tissues

Human	Age (years)	Air conc. of H_2S	Tissue concentrations of sulfide					
			Blood (μg/g)	Lung (μg/g)	Brain (μg/g)	Liver (μg/g)	Kidney (μg/g)	Spleen (μg/g)
Victim A	40	550-650	0.10	0.20	0.68	1.56	0.90	0.32
Victim B	62	550-650	0.20	1.06	0.21	1.39	1.50	0.64
Victim C	30	550-650	0.08	0.40	0.23	1.30	0.47	0.45

Data from Kimura et al. 1994

4.5.2.3 Metabolism

According to a number of studies, hydrogen sulfide is metabolized through three distinct pathways: (1) oxidation to sulfate; (2) methylation; and (3) reaction with metallo- or disulfide-containing proteins (Beauchamp et al. 1984). The first two pathways are considered as detoxification mechanisms while the third one is believed to be responsible for toxic action of hydrogen sulfide.

Experiments on isolated rat liver, lungs and kidney demonstrated that sulfide ion is mainly oxidized to thiosulfate in the liver, and then converted to sulfate which is excreted

in urine (Bartholomew et al. 1980). This is the principal detoxification route of hydrogen sulfide. Because hydrogen sulfide is rapidly metabolized and excreted, it is not accumulated in the body. Methylation of hydrogen sulfide is also considered as a detoxification mechanism since the mono- and dimethylated products are believed to be significantly less toxic than hydrogen sulfide (Ljunggren and Norberg 1943, Weisiger et al. 1980).

Reaction of hydrogen sulfide with proteins present in vital metalloenzymes such as cytochrome oxidase represents the most likely toxic mechanism of hydrogen sulfide. The biochemical mechanism of hydrogen sulfide is believed to be involve the inhibition of mitrochondrial electron transport (NRC 1979b, Smith and Gosselin 1979). Hydrogen sulfide has a higher potency than hydrogen cyanide to inhibit cytochrome oxidase, the final enzyme in the respiratory chain (Nicholls 1975, Nicholls et al. 1976, Peterson 1977, Wever et al. 1975). Inhibition of cytochrome oxidase prevents oxygen from acting as the final electron acceptor thereby causing blockage of oxidative metabolism by inhibiting the electron transport chain. This blockage reaction is considered to be largely responsible for the lethal effects of hydrogen sulfide (NRC 1979b).

Human

In a human experimental study, urinary thiosulfate levels were measured in volunteers exposed to 8 ppm, 18 ppm and 30 ppm hydrogen sulfide for 30-45 minutes (Kangas and Savolainen 1987). The urinary thiosulfate concentration changed linearly with the product of exposure concentration and duration. The concentration reached the highest level at 15 hours after exposure and decreased to control levels by 17 hours after exposure, which indicates that a major portion of inhaled hydrogen sulfide was metabolized within 15 hours (Kangas and Savolainen 1987). However, this study did not report the number of volunteers nor sufficient individual information to allow analysis and comparison.

Animal

In animal studies, Japanese white rabbits exposed to 500 - 1000 ppm (the lethal concentration) for 14-30 minutes had thiosulfate detected in blood, lung and brain (Kage et al. 1992). When rabbits were exposed to 100-200 ppm hydrogen sulfide for 60 minutes, thiosulfate concentrations were highest 1-2 hours after exposure and could still be tested in urine 24 hours after exposure (Kage et al. 1992). Thiosulfate level in blood was highest right after exposure and could not be detected after 4 hours (Kage et al. 1992).

Comparison

From human and animal studies mentioned above, thiosulfate, the product of the oxidation of hydrogen sulfide, can be measured in the urine of animals and humans. This finding indicates that the major detoxification pathway, i. e., oxidation of hydrogen sulfide to thiosulfate, is similar in both animals and humans.

4.5.2.4 Excretion

Human

Hydrogen sulfide is mainly oxidized in the liver to thiosulfate, and then to sulfate, with both being excreted in the urine (Beauchamp et al. 1984). In human volunteers, thiosulfate can be measured in the urine (Kangas and Savolainen 1987). There is no information on the excretion of hydrogen sulfide through exhalation. Since hydrogen sulfide is rapidly metabolized to sulfate and then excreted in the urine, urinary excretion appears to be the primary excretion route for hydrogen sulfide exposure.

Animal

In rabbits exposed to 100-200 ppm hydrogen sulfide for 60 minutes, urinary thiosulfate concentration was highest 1-2 hours after exposure and could still be detected 24 hours after exposure (Kage et al. 1992).

Comparison

The time that thiosulfate reached peak concentration in urine is much longer in humans than in rabbits when comparing the two studies mentioned above. However, in the human study, the interval of urine sampling was 10 hours between the peak concentration and previous one after exposure, thus the true time for thiosulfate to peak is likely to be less than 15 hours.

In summary, although the toxicity of hydrogen sulfide has been recognized for more than a century, knowledge on its toxicokinetics is still surprisingly limited. Qualitative information on the absorption, distribution, metabolism, and excretion of hydrogen sulfide summarized above have indicated that the basic process is similar in some species of animals (rats, guinea pig, and rabbits) and humans. Hydrogen sulfide is rapidly and significantly absorbed through inhalation and then distributed throughout the body. It is mainly oxidized in the liver to thiosulfate and then converted to sulfate, which is excreted in the urine. At high concentrations, body detoxification capacity will be exceeded and hydrogen sulfide may inhibit cytochrome oxidase through reaction with proteins present in cytochrome oxidase. This mechanism is considered to be responsible for the toxic effects of hydrogen sulfide at high concentrations. No physiologically based pharmacokinetics (PBPK) models have been developed for hydrogen sulfide (ATSDR 1998). This deficiency makes the risk management modeling of toxic effects of hydrogen sulfide much more uncertain.

4.5.3 Chlorine

Information on toxicokinetics of chlorine is extremely limited in the literature. This is reflected by the fact that there is no section regarding the toxicokinetics of chlorine in "Chlorine and hydrogen chloride", one of a series "Environmental Health Criteria" published by World Health Organization (WHO 1982). The contact reactivity of chlorine is likely responsible for the absence of toxicokinetic information on such a prevalent toxic gas.

Lehmann (1893) exposed human volunteers to 1.8 ~2 ppm chlorine and found 100 % absorption. Two recent studies have been found in the literature regarding the absorption

of chlorine gas through inhalation. Absorption of chlorine in humans through inhalation has been investigated by using the noninvasive bolus inhalation method (Nodelman and Ultman 1999 a, b). Five healthy male and five healthy female nonsmokers were exposed to 0.5-3.0 ppm chlorine during both nasal and oral breathing at a quiet respiratory flows of 250 ml/s. More than 90% of the chlorine inhaled during quiet breathing is absorbed in the upper airways regardless of nasal or oral breathing. In addition, changing chlorine concentration from 0.5 ppm to 3 ppm had no effect on the airway absorption during nasal breathing. The author concluded that the dissolution, diffusion, and chemical reactions governing chlorine uptake from respired gas to the nasal mucosa are all linear processes (Nodelman and Ultman 1999 a).

Comparing these two recent studies to Lehmann's study that was conducted 100 years ago, the extent of absorption of chlorine in humans at the similar range of concentrations applied was quite similar.

4.5.4 Ammonia

4.5.4.1 Absorption

Human

Studies dealing with absorption of ammonia by humans are quite scarce in the literature. Landah and Herrmann (1950) conducted an experiment with volunteers by applying ammonia gas in concentrations ranging from 40 to 350 mg/m^3 (57-500 ppm) for up to two minutes exposure. They found that about 92% of the gas was retained in the respiratory system (mouth, lung). Differences in concentrations of ammonia exposures did not affect retention values. This indicates that the concentration of gas may not play a major role in determining the percentage retained in either the nose or the lung during the first few minutes of respiration. Gas retained in the nasopharynx was about 83% at a flow rate of 18 l/min, and only 63-71% was retained when the flow rate increased to 54 l/min. In this study, it was noted that the variability of nasal retention from time to time is greater than that of pulmonary retention. But overall, the variability among subjects was suggested to be small, although the sample size in this study was too small and there is no information on subjects.

In another study, seven male volunteers were exposed to ammonia gas at concentrations of 350 mg/m^3 (500 ppm) for 30 minutes (Silverman et al. 1949). The initial retention on one subject was about 75% but was not reported for 6 other subjects. After 10-27 minutes, retention decreased progressively to 4-30 % at equilibrium. Irritation in nose and throat has been observed in some subjects. This suggests that at this concentration ammonia is primarily absorbed in the upper respiratory tract. There were no changes in levels of blood-urea-nitrogen (BUN), non-protein nitrogen (NPN), urinary-urea, and urinary-ammonia which indicated only small amounts of ammonia are absorbed into the systemic circulation at this concentration; most of the quantities retained in the upper respiratory tract are excreted in expired air within 30 minutes.

An early study revealed that absorption of ammonia in humans ranged from 77.1% to 90% at concentrations from 200 ppm to 310 ppm (Lehmann 1893).

Animal

Animal species used to conduct experiments on absorption of ammonia gas include rats (Schaerdel et al. 1983), rabbits (Dalhman 1963), and dogs (Egle 1973).

Schaerdel et al (1983) measured the blood ammonia concentration in rats after exposure to ammonia at concentrations of 15, 32, 310, and 1157 ppm for 24 hours. The results showed that at low concentrations of 15 and 32 ppm the blood ammonia did not change. At high concentration of 310 and 1157 ppm the blood ammonia concentrations significantly increased after 8 hours exposure and then steadily declined after 12 and 24 hours exposure. This suggests that ammonia is absorbed through the respiratory tract and the body increases ammonia metabolism to compensate for the increasing blood ammonia level.

Egle (1973) carried out an experiment on dogs to investigate the uptake of ammonia gas by the entire respiratory tract and by upper and lower portions under varying conditions of ventilatory rate, tidal volume, and concentration inhaled. 10-37 dogs per study were exposed to ammonia at concentrations between 150-500 mg/m^3 (214-714 ppm). Retention by the total respiratory tract was about 78% on average, and the same percentage was found for lower-tract and upper-tract retention. However, with

unidirectional perfusion that simulated a complete ventilating cycle of normal ventilation, retention of the upper respiratory tract with ammonia gas increased to about 89%. This study demonstrated that the retention of ammonia tended to be higher in the upper respiratory tract than that of lower tract. Tidal volume ranging from 100-200 ml had no effect on retention of ammonia, but higher tidal volumes resulted in an increase in ammonia retention. In addition, retention of ammonia was not affected by concentration, which is consistent with the findings of Landahl and Herrmann (1950) in the human studies. Regarding the variation in retention, the author stated that retention values for individuals are quite similar.

The study by Dalhamn and Sjöholm (1963) also showed high resorption in the nasal cavity of rabbits about 95% with high concentrations of 2000-3000 ppm for 45 minutes.

Comparison

Animal and human data have demonstrated that ammonia is rapidly and extensively taken up in the respiratory tract, particularly by the upper airways. In humans and dogs, change of ammonia concentration had no effect on the retention values (Table 4.15). These data suggest that the process of absorption of ammonia through inhalation is similar in animals, especially dogs, and humans.

Table 4.15 Comparison of absorption of ammonia in different species

Species	Conc in air (ppm)	Time	Retention through different locations			Blood ammonia	Reference
			Upper-tract	Lower-tract	Total-tract		
Rat	15, 32 310, 1157	8-24 hr 8 hr				No change Significantly increased	Schaerdel et al. 1983
Rabbit	2000-3000	45 min	95%				Dalham & Sjöholm 1963
Dog	214-714		89%	78%	78%		Egle 1973
Human	57-500	> 2 min	63-83%		92%		Landah & Hermann 1950
Human	500	30 min	75%(initial) 4-30% (after 10-27 min)			No change in BUN*, NPN *	Silverman et al. 1949
Human	200-310	5-10 min			77.1~90%		Lehmann 1893

* BUN: blood-urea-nitrogen; NPN: non-protein nitrogen

4.5.4.2 Distribution

Ammonia exists normally in all tissues in the body occurring as a dynamic pool. Information on the distribution of endogenously produced ammonia suggests that any ammonia absorbed through inhalation would join the body pool of ammonia and rapidly distribute to all body compartments via the blood, where it would be used in protein synthesis or as a buffer. The distribution of total ammonia between compartments is influenced by pH. The lower the pH of a compartment, the greater its total ammonia content (WHO 1986, 1990, NRC 1979a).

Human

In human beings, inhalation of 500 ppm of ammonia for 30 minutes had no effect on blood-nitrogen levels (Silverman et al. 1949). This finding indicated that at this concentration only small amounts of ammonia are absorbed into the systemic circulation. No human data are available in regard with the fate of ammonia in human body following inhalation exposure.

Animals

Distribution of ammonia depends on exposure route. Through ingestion, ammonium ions are mainly transformed by the liver to urea, and subsequently excreted in the urine. In contrast, intravenously administrated ammonium salts are more available as non-essential nitrogen for protein synthesis (Furst et al. 1969). The fate of ammonia absorbed through inhalation should be similar to that of intravenous administration. A study on rats has demonstrated that after an intravenous injection of ^{15}N-ammonium lactate, its major metabolites, glutamine and urea, were quickly distributed throughout the body (Duda and Handler 1958). The labeled glutamine and urea was found in liver, kidney, heart, brain, spleen, carcass, and testes.

4.5.4.3 Metabolism

Most organisms have mechanisms for conjugating ammonia into non-toxic compounds for excretion (WHO 1986). The metabolic fate of exogenous ammonia

depends on the route of administration. After intravenous administration, exogenous ammonia is metabolized to glutamine as the major early product (Duda and Handler 1958). The ammonia fixed in glutamine may eventually end up in amino acids, purines, pyrimidines, or other nitrogen-containing compounds (WHO 1986). Ammonia does not react with body components in the manner of alkylating agents or compounds that modify hemoglobin (WHO 1986).

4.5.4.4 Excretion

Human

In human beings, ammonia can be measured in the expired air (Larson et al. 1977). Ammonia is also excreted through the kidney. After intravenous administration of ammonium lactate to human healthy volunteers, urinary -ammonia increased 8-fold and urea excretion decreased by one-half (Gay et al. 1969).

Animal

Ammonia can be found in the expired air in dogs after intravenous administration of ammonium acetate (Robin et al. 1959) and in rodents (Barrow and Dodd 1979, Barrow and Steinhagen 1980). In mammals, urea is one form of excretion through the kidneys. Mammals also directly secrete ammonia into the urine (WHO 1986).

In summary, ammonia is taken up rapidly and significantly through the upper respiratory tract in animals and humans. Ammonia appears very often in the literature because of its reputation for being highly water soluble and mainly absorbed in the upper airways. Information on the distribution, metabolism, and excretion following inhalation is very limited. Data from other sources of exposure indicate that once ammonia is absorbed into the blood, it will join the body ammonia pool and be distributed throughout the body. The final product of metabolism of ammonia in mammals is urea, which is excreted through the kidney. These processes seem to be similar in animals and humans.

4.5.5 Hydrogen Fluoride

4.5.5.1 Absorption

The current knowledge about fluoride absorption is based on numerous animal experiments and observations in humans that primarily focus on the absorption through the gastrointestinal tract and these are of little value for judging airborne exposure. Only a few studies exist in the literature regarding absorption through inhalation.

Human

Largent (1961) made the first published observations on human subjects with experimentally controlled concentration of hydrogen fluoride in air. Five volunteers were exposed to 0.9 - 8.1 ppm hydrogen fluoride for 6 hours/day. The elimination of fluoride significantly increased through urine and feces indicating absorption of a relatively large amount fluoride through inhalation (Largent 1961). There are some other similar human studies in which very small numbers of human volunteers (usually two) were exposed to the low concentrations of hydrogen fluoride and urinary fluoride was found to be increased (Collings et al. 1951, 1952). There was no marked difference between these subjects regarding the absorption process although the sample size was too small for statistical significance (Collings et al. 1951).

Animal

In animal studies, regional deposition and absorption of inhaled hydrogen fluoride was investigated in anesthetized rats (Morris and Smith 1982). At concentrations of 43, 116, and 212 ppm (36, 96, 176 mg F/m^3) more than 99.7-99.9% of the hydrogen fluoride drawn into the upper respiratory tract was removed from the air stream during passage through that area. Plasma fluoride concentration significantly increased in rats exposed to 212 ppm (176 mg F/m^3) hydrogen fluoride compared to controls (Morris and Smith 1982). Plasma fluoride levels correlated closely with the concentration of hydrogen fluoride in the air passed through the surgically isolated upper respiratory tract (Morris and Smith 1982). Other authors also reported that almost 100% of inhaled hydrogen

fluoride is absorbed which was indicated by the amounts of fluoride found in the bones of exposed animals (Machle and Largent 1943).

Comparison

Limited information summarized above shows that hydrogen fluoride is rapidly absorbed in humans and animals following acute inhalation exposure. Hydrogen fluoride is nearly completely absorbed by rats through the upper respiratory tract while the extent of absorption in humans has not been determined. However, combined with the observations that fluoride appeared in the urine within at least two hours in humans and at least 40 minutes after intermittent exposure in rats, it can be estimated that hydrogen fluoride is likely also completely absorbed by humans through the respiratory tract.

4.5.5.2 Distribution

Human

There are no data regarding the distribution of fluoride in humans after inhalation of hydrogen fluoride. Information of occupational exposure to hydrogen fluoride and fluoride dust in humans suggests that fluoride distributes to bone and accumulates there (Boivin et al. 1988, Chan-Yeung et al. 1983, Czerwinski et al. 1988).

Animal

Studies on animals have shown that fluoride is widely available through the blood. Whole-body exposure of male rats to 13 to 140 ppm (11 to 116 mg/fluoride/m^3) hydrogen fluoride for 6 hours resulted in a dose-dependent increase in lung and plasma fluoride concentrations (Morris and Smith 1983). Animal studies also indicated that intermittent high-level exposure might result in greater accumulation of fluoride in bones and teeth than continuous exposure (Stokinger et al. 1950).

4.5.5.3 Metabolism

Once fluoride is absorbed, blood-borne fluoride is rapidly distributed in the extra-cellular body fluid. Rates at which fluoride leaves the blood are about 30% to 40% per

minute (Hodge 1961). The principal distribution and metabolism pattern of fluoride is that it is either deposited in the bone or excreted via the kidney. The kidney holds fluoride temporarily on its way to excretion in the urine while bone storage is a prolonged process. Only bone and calcified tissues serve as storage sites (Hodge 1961). Storage in bone and excretion via kidney are two major means by which the body prevents circulation of toxic amounts of fluoride ion (Hodge 1961). Urinary excretion of fluoride is significantly decreased by diminished renal function (Kono et al. 1984).

The fluoride ion carried in human blood serum exists in two forms, inorganic ion F^- and in combination with organic molecules (Halton et al. 1984). A portion of the circulating inorganic fluoride may act as enzyme inhibitor since it forms a metal-fluoride-phosphate complex that interferes with the activity of those enzymes requiring a metal ion cofactor. Fluoride may also interact directly with the enzyme or the substrate. The effect of this action on human health remains unclear.

4.5.5.4 Excretion

Human

Fluoride rapidly appears in the urine following absorption. Fluoride excretion varies from person to person among aluminum smelter workers and from time to time within an individual (Dinman et al. 1976a, b, c, d). In five volunteers exposed to 0.9-8.1 ppm hydrogen fluoride through inhalation for 6 hours a day, total 10-50 days, urinary and fecal excretion of fluoride was significantly increased (Largent 1961). In another study, two volunteers were exposed to 4.57 to 6.75 ppm (3.8 to 5.6 mg/m^3) hydrogen fluoride for an 8-hour work shift. Fluoride was excreted during the exposure and reached peak 2-4 hours after cessation of exposure, that is, about 10 hours after beginning of exposure (Collings et al. 1951).

Animal

Overnight urinary fluoride excretion in dogs and rabbits exposed to 8 ppm (7 mg/m^3) hydrogen fluoride for 6 hours/day, 6 days/week for 30 days was about 1.5 times that of controls (Stokinger 1949).

Comparison

Biomonitoring data has shown that in both humans and animals the urinary metabolite of hydrogen fluoride is fluoride ion (Brondeau et al. 1999).

In summary, hydrogen fluoride is rapidly and completely absorbed in the upper respiratory tract in humans and animals because of its high water solubility. After absorption, fluoride distributes throughout the body, then either stores in the bone or excretes in the urine. When concentrations of fluoride increase, excretion via the kidney also increases. The capacity of excretion in the urine decreases with renal function failure. Therefore, people with chronic renal diseases may face more risk when exposed to hydrogen fluoride.

4.6 Summary

There is considerable variation in the structure of the respiratory tract and pulmonary function among animals and humans. Rodents, being obligate nose breathers, efficiently prevent toxic substances from entering deeper structure of the lungs. Small animals such as rodents may receive higher toxicant loading based on the calculation of minute volume/body weight. On the other hand, differences in protective reflex actions evoked by the irritants may also affect the amount of toxicants entering the body. Predicting toxic effects in humans based on the animal data is complicated by various factors involved in the causal process.

For the purpose of toxicological modeling, the four gases selected in this study can be divided into two groups based on their physicochemical properties and toxic actions. Group I includes chlorine, ammonia, and hydrogen fluoride, which are primarily local irritants, and group II includes hydrogen sulfide that involves both local irritation and systemic effects.

Gases of group I are site-of-contact toxicants. They are rapidly and almost completely taken up by the respiratory tract following inhalation and they exert their toxic effects directly on the tissues of the respiratory tract that they contact. Ammonia and hydrogen fluoride are mostly absorbed in the upper respiratory tract due to their high water solubility, while chlorine tends to penetrate into the deeper airways; this difference

determines the location of actions of different chemicals. The severity of toxic effects caused by these chemicals is mainly dependent on how much gets into the airways, and into contact with tissues of the respiratory tract. The more mass that enters the airways, the deeper into the respiratory tract the tissues will receive injury. These chemicals are highly reactive, and the tissues will not become saturated with gases before they are irreparably damaged. Consequently, metabolism and elimination of these chemicals appears to be relatively unimportant regarding their toxicity. From this perspective, the amount of toxic gases entering the respiratory tract within a certain short time (minutes to hours) should be the most important determinant of the toxic effects. As discussed above, minute volume per unit body weight and reflex action are two major factors controlling the amount of air into the respiratory tract. Therefore, taking all these factors into account, the relationship between exposure and response for these three chemicals can be expressed in the following equations:

For exposure that occurs in residential areas (e.g., leak of gases from pipelines) when most people are assumed to be breathing normally.

$$L \propto [(V/W)_u - (V/W)_r]^m \times C^n t \qquad (4.6)$$

where L = toxic effect;

V = minute volume;

W = body weight;

m = exponent;

C = exposure concentration;

n = exponent that is specific for the different gases;

t = exposure duration;

$(V/W)_u$ = minute volume per unit body weight under normal breathing;

$(V/W)_r$ = decrease in minute volume per unit body weight due to protective reflex.

For exposure in workplaces (e.g., leak of gases from storage tanks) when workers are working:

$$L \propto [(V/W)_e - (V/W)_r]^m \times C^n t \qquad (4.7)$$

where L = toxic effect;

V = minute volume;

W = body weight;

m = exponent;

C = exposure concentration;

n = exponent that is specific for the different gases;

t = exposure duration;

$(V/W)_e$ = minute volume per unit body weight during exercise which simulates the situation of working;

$(V/W)_r$ = decrease in minute volume per unit body weight due to protective reflex.

$[(V/W)_e - (V/W)_r]$ is the final volume of air containing toxic gases that enters the respiratory tract.

From the discussion in chapter 2, in the toxic load model $L = C^n t$, the dose-response relationship is only represented by the external dose, $C^n t$. In equation (4.6) and (4.7), $[(V/W)_{u \text{ or } e} - (V/W)_r]^m \times C^n t$ can be viewed as an internal dose. Furthermore, considering the fact that these three chemicals are almost completely absorbed in the respiratory tract, this internal dose is expected to be very close to the effective dose. Also, this relationship takes variability in the minute volume and reflex action among species into consideration, thus it might be more realistic than classical toxic load model.

The action of hydrogen sulfide is different from the other three chemicals. Hydrogen sulfide is less water soluble so that it is not completely removed on contact with the respiratory tract, and thus can be significantly absorbed into the blood. It is promptly oxidized in the liver in animals and humans, and then the less toxic metabolites are excreted in the urine. This oxidation process is considered to be the major detoxification pathway for hydrogen sulfide. At high concentrations (>700 ppm) where the body capacity of detoxification is exceeded, hydrogen sulfide will inhibit cytochrome oxidase

and thus exert systemic toxic effects with the major target site being the neurological system. Therefore, there are two distinct mechanisms involved in the toxicity of hydrogen sulfide at different concentrations. In the toxicological modeling for hydrogen sulfide it is necessary to take the detoxification process into consideration. However, due to lack of basic quantitative data on this process, it is difficult to estimate a meaningful value for a parameter (e.g., a recovery constant) representing this process.

Chapter 5

Assessment of Variability and Uncertainty of Biological Response to the Selected Industrial Gases

5.1 Introduction

In order to interpret results of animal studies regarding toxic effects of chemicals it is important to choose appropriate biological endpoints or effects. Generally, the biological endpoints can be any response or functional change evoked by the interaction of foreign substances and the organisms. They may occur at different levels such as organs, tissues, cells, and molecules. Functional changes are often based on the change of structure. However, some times structural change cannot be easily found while functional changes are already apparent.

For the purpose of predictive modeling and risk management for major facilities, the regulatory authorities focus on toxic effects that are significant, serious, and possibly irreversible to assess the risk of accidental release of chemical. Effects of a strictly transient nature are a possible source of nuisance but they do not provide a basis for risk management to reduce rare exposure events. According to their properties discussed in the previous chapter, all four gases are irritants. Irritation is their most important action on the organisms. In some cases, exposures result in serious consequences that may threaten animal or human life, and later may cause long-term effects.

Extrapolation of responses observed in animals to humans is difficult and very uncertain, particularly when the metabolic processes involved in the response differ between animals and humans. There are two general principles in regard with the extrapolation between species: (1) the toxic effect is likely to be similar only if the target organ is the same in the two species; (2) the amount of the toxicants which reaches the target organ is more important for the toxic effects than the total amount that enters the body (Lees 1996). For the inhalation toxicity of four irritant gases, the principal target organs are in the respiratory system and the amount of toxic gas reaching the target organ can be well estimated. For these cases the toxicity data obtained from animals can generally be directly applied to humans (Lees 1996). As an exception, hydrogen sulfide

also affects the neurological system. Thus, extrapolating animal data to humans in this aspect involves much more uncertainty.

In this analysis, the discussion of toxicity of the four gases is focused on the three distinct levels of toxic effects, that is, odor, non-lethal injury, and death. Since odor is a subjective response and has special methods of measurements, it will be discussed separately in Chapter 6.

The purposes of this chapter are:

- summarize and compare the principal toxic effects of the four gases on animals and humans after acute exposure;
- compare susceptibility in response of different species to the four gases;
- provide evidence of long-term after effects following acute exposure to the four toxic gases

5.2 Irritants and Irritation

It has been estimated that 60% of all threshold limit values (TLVs) have been established based on their potency to cause irritation in humans (Anger 1984). The four gases selected in this study are irritants of the respiratory tract and the eyes. Their toxicity is largely based on their irritation effects, which range from mild discomfort to death. Knowledge of the nature and characteristics of irritation can provide basic understanding of toxic effects caused by these four irritant gases.

5.2.1 Mechanisms of Action

An irritant gas or vapor can induce the phenomena of inflammation when it directly contacts tissues. Inflammation is induced by the coagulation, liquefaction, dehydration, or other disturbance of the normal state of balance in living protoplasm caused by irritants (Haggard 1924). Irritants act directly on the surface tissues such as skin, and respiratory membranes. The severity of the effects is primarily determined by the extent of penetration of the surfaces, which is usually dependent on their moisture. Thus, the epithelium and the mucous membrane at the respiratory passages and the conjunctiva are the most sensitive sites of action for irritants.

According to Alarie (1973a), irritants can be classified into four groups (Table 5.1):

Table 5.1 Classification of irritant gases

Classification	Definition and example
Sensory irritant	Gases which when inhaled via the nose will stimulate trigeminal nerve endings, evoke a burning sensation of the nasal passages, and inhibit respiration. Also, most will induce coughing from laryngeal stimulation. Ammonia and hydrogen fluoride are typical sensory irritants.
Pulmonary irritant	Gases which when inhaled will stimulate sensory receptors within the lung and increase respiratory rate with a decrease in tidal volume resulting in rapid shallow breathing.
Bronchoconstrictor	Gases which when inhaled will induce an increase in resistance to airflow within the conducting airways of the lung. The action can be via direct effect on smooth muscles of the conducting airways by axonal reflex, vago-vagal, or trigeminal-vagal reflexes following stimulation of nerve endings belonging to these systems or by liberation of histamine. Ammonia is also a bronchoconstrictor.
Respiratory irritant	Gases which when inhaled can act as a sensory irritant, bronchoconstrictor, and pulmonary irritant. These chemicals are capable of all three actions and there is little difference between the concentration at which they are sensory irritant and pulmonary irritant. Chlorine and hydrogen sulfide belong to this class.

Adapted from Alarie 1973a

5.2.2 Location of Action

Characteristics of irritation effects on any part of the respiratory tract are eventually the same. Only the degree of injury is different (Haggard, 1924). Different types of irritant gases may cause different symptoms and pathology that mainly depend on the location of action. Some irritants such as ammonia and hydrogen fluoride mainly cause congestion of the upper respiratory tract and immediate death from laryngeal spasm or edema, while others such as phosgene may cause little irritation of the upper respiratory tract, but induce lung edema or pneumonia through their action on the alveoli. The actions of some irritants such as chlorine and hydrogen sulfide are between these two extremes and they may exert their effects on the entire respiratory tract.

The selective actions by different irritants mainly relate to their physical properties, particularly water solubility. A gas that is very soluble in water and is readily diffusible in its solution is taken out of the inspired air rapidly by contact with the first moist tissue. Thus, the upper respiratory tract is mostly affected, while the lungs are relatively less

affected because the concentrations of the irritants that reach them are greatly reduced. In the case of a gas with very low water solubility, the upper respiratory passage suffers relatively little, because only a small amount is absorbed, and the main damage occurs deep in the lungs.

The symptoms and pathology of irritation occurring in the different parts of the respiratory tract are summarized in Table 5.2. The symptoms are clearly dependent on the location of irritation. Injuries in the region of the respiratory bronchioles can be categorized into three conditions: (1) the irreversible loss of these airways, (2) the potentially reversible accumulation of mucus, and (3) irreversible fibrosis with narrowing of airway (Ranga and Kleinerman 1978, Thurlbeck 1973). The respiratory bronchioles region is commonly injured by various toxicants. The reason for this is not clear. It may be related to an excessive vulnerability of these transitional tissues, or to an unusually high dose of toxicants delivered to this region (Phalen 1984).

In general, damage to the upper respiratory tract and tracheobronchi region may be efficiently repaired and no permanent damage persist, while severe irritation on the lower respiratory tract may cause severe pulmonary edema and pneumonia which, if not fatal, may cause chronic inflammation (Table 5.2). Individuals with this chronic inflammation condition may appear normal at rest, but some symptoms will occur when doing mild to moderate exercise. In addition, this abnormality may not be detected by chest x-ray examination or lung function tests.

5.2.3 Protective Reflexes

In general, the immediate physiological response to the inhalation of an irritant gas is more significant in the upper than in the lower portions of the respiratory tract. The irritation of the upper respiratory tract will elicit a series of reflexes, including coughing, constriction of the larynx and bronchi, closure of the glottis, and inhibition of respiration, to prevent the penetration of the irritant to the deeper respiratory structures (Haggard 1924).

Table 5.2 Comparison of irritation in different portions of the respiratory tract

Location	Symptoms	Pathology	Consequence
URT*	Acute pharyngitis, acute laryngitis	Rubefaction, necrosis of the tissues	Little permanent damage
	Laryngeal edema (severe case)	Edematous swelling of larynx, functional spasm of the glottis, opening into trachea occluded	Rapidly fatal due to asphyxia
Bronchi	Tracheitis, bronchitis	Efficient repair of large bronchi,	Little permanent damage
		Inefficient repair of small tube	Permanent thickening of the walls
Lungs	Pulmonary edema	Accumulation of fluid interstitially, lymphatics are engorged, fluid escapes from capillaries	Fatal in severe case due to asphyxia; if not fatal, may result in chronic inflammation condition
	Pneumonia	Lobal or bronchial or mixed	Mortality is high, with death occurring from 4 days to 2 weeks; if not fatal, may leave focal infections persisting for a long time

*URT: Upper respiratory tract

In animals, a decrease in respiratory rate is a consistent characteristic response during exposure of the upper respiratory tract to irritating airborne chemicals such that it is used as a quantitative measure of the degree of sensory irritation. This response is an upper respiratory tract phenomenon, mediated via the trigeminal nerve endings located in the nasal mucosa, and is termed sensory irritation (Alarie 1973a). The characteristic decrease in respiratory rate of mice during exposure to airborne chemicals has been found to be an excellent predictor of how humans will react when exposed to similar concentrations of the same chemicals (Alarie 1973a). A concentration capable of inducing a 50% decrease in respiratory rate (RD_{50}) in mice would induce intolerable sensory irritation and would be incapacitating to humans; that at 1/10 RD_{50} humans would report some slight stinging or burning sensation of the eye, nose, and the throat, but such exposures would be tolerable; and that at 1/100 RD_{50} very slight or no sensory irritation would occur (Alarie 1973a, Alarie et al. 1975).

In summary, the term "irritation" indicates the action following direct contact of irritants with surface tissues. Different symptoms and pathological changes caused by different irritants are primarily due to the different location of action, which in turn, is mainly determined by the water solubility of irritants. Irritants with high water solubility

mainly affect the upper respiratory tract which may allow relatively complete repair of damage if doses are not excessive. Irritants with lower water solubility primarily damage the deeper pulmonary structures, which may induce permanent pathological and functional changes. This distinction in the action of different irritants is important for understanding the acute phase and possibly chronic phase of the inhalation toxicology of the four gases.

5.3 Variability in Response to Hydrogen Sulfide

Unlike the other three chemicals selected in this study, the toxicity of hydrogen sulfide involves two distinct types of toxic effects: (1) local irritation, and (2) systemic poisoning (Milby 1962, Mitchell and Yant 1925, Vannatta 1982). Hydrogen sulfide can directly react with moist tissues causing local inflammation of the eyes and the respiratory tract. Free un-oxidized gas presenting in the blood stream can cause severe systemic effects that often obscure symptoms of local irritation (Ahlborg 1951, Haggard 1925, Milby 1962). The acute systemic poisoning primarily affects the nervous system resulting in complete respiratory paralysis, while irritation acts on the respiratory tract resulting in pulmonary edema. Death from acute systemic poisoning is caused by paralysis of the respiratory center, whereas death from local irritation is mainly associated with pulmonary edema (Mitchell and Yant 1925).

5.3.1 Irritation

Hydrogen sulfide can cause irritation of eyes, which has been called "gas eyes", and the respiratory tract. Because of its relatively low water solubility, hydrogen sulfide extends its action throughout the entire respiratory tract, and the deeper pulmonary structures such as the alveoli suffer the greatest damage. Edema of the lungs is a consequence of inflammation of these structures (Haggard 1925).

5.3.1.1 Human

Two human experiments on the effects of hydrogen sulfide applying concentrations higher than threshold limit values (10 ppm) have been found in the literature (Lehmann

1892, Mitchell and Yant 1925). In a series of experiments, Lehmann (1892) exposed five healthy volunteers including himself to various concentrations of hydrogen sulfide ranging from 20 ppm to 575 ppm for a few minutes to 4 hours. Exposure to 20-40 ppm hydrogen sulfide for several hours had no effects and 70-90 ppm caused slight irritation, while exposure to concentrations higher than 100 ppm caused noticeable irritation of eyes, the nose, larynx, and trachea, as well as headache, and decrease in sight. One volunteer was exposed to different concentrations for various periods. After exposure to 575 ppm for 3 hours and 19 minutes it is not surprising that he could not tolerate any more. No physical and x-ray examination had been done after the experiments. One problem in this study is that the exposure concentrations of hydrogen sulfide were not continuously monitored and sometimes were not constant. Concentrations of air contaminants may decrease during the experiments, especially for longer periods of exposure. Thus, the concentration of 575 ppm mentioned above might be questionable.

Mitchell and Yant (1925) found that exposure to 50-200 ppm hydrogen sulfide for several hours or 500-600 ppm for 5-10 minutes caused conjunctivitis, pharyngitis, or bronchitis. 100 ppm hydrogen sulfide definitely caused irritation in humans but no details were provided.

Most data of acute intoxication of hydrogen sulfide in humans come from reports of accidental exposures. Early investigations on accidental exposure to hydrogen sulfide of refinery workers have revealed that local irritation can be caused at low concentrations (50-200 ppm) for several hours or high concentration (500-600 ppm) for 5 to 10 minutes (Mitchell and Yant 1925). Symptoms included conjunctivitis, pharyngitis, or bronchitis. Pulmonary edema, hemorrhage bronchitis, or pneumonia develop in the severe exposure cases (Arnold et al. 1985, Burnett et al. 1977, Deng and Chang 1987, Parra et al. 1991)

5.3.1.2 Animal

Canaries, rats, mice, guinea pigs, rabbits, cats, dogs, and goats were used to study acute toxicity of hydrogen sulfide (Lehman 1892, Mitchell and Yant 1925). The responses to hydrogen sulfide seemed to be similar among different species (Mitchell and Yant 1925). At concentrations ranging from 35 ppm to 300 ppm for a few hours, animals

developed symptoms of irritation of eyes and the respiratory tract. In severe cases pulmonary edema also developed (Mitchell and Yant 1925).

Relatively recent animal studies suggest that hydrogen sulfide induced pulmonary edema has a threshold-type of response (Bitterman et al. 1986, Lopez et al. 1987, 1988). Fischer-344 rats exposed to 400 ppm of hydrogen sulfide for 4 hours developed severe but transient pulmonary edema. Below this concentration there were no significant biological and cytological changes.

5.3.1.3 Inter-species Variability

Studies on irritation effects revealed that canaries were most sensitive and goats were most resistant to hydrogen sulfide. Susceptibility to hydrogen sulfide was not definitely associated with the body size of animals (Mitchell and Yant 1925). For example, dogs were less sensitive than rats but more susceptible than guinea pigs. The inter-species difference can be seen from Table 5.3.

Table 5.3 Comparison of susceptibility of species to H_2S

Species	Approximate concentrations of H_2S for irritation (ppm)
Canary	50-200
White rat	50-550
Dog	50-650
Guinea pig	50-750
Goat	50-900
Human	70-555

Data from Lehmann 1892, Mitchell and Yant 1925

Experimental animal and human data indicate that humans react to hydrogen sulfide in a manner similar to experimental animals. The symptoms described in human experiments as well as in accidental case reports are similar to that observed in the animal experiments. As shown in Table 5.3, the susceptibility of humans to irritation effects of hydrogen sulfide is similar to rats and dogs. Lehmann (1892) also suggested that the

susceptibility of humans to non-lethal effects of hydrogen sulfide is closest to that of dogs.

5.3.1.4 Intra-species Variability

Within the same species, response differs from one animal to another. Lehmann (1892) observed that two apparently similar dogs were exposed to hydrogen sulfide under the same condition, one dog appeared very sick after one hour exposure and died 9 hours later, while the other one came out of his chamber after 5 hours exposure without significant effects.

On the other hand, at relatively lower concentrations, the reaction of three volunteers to hydrogen sulfide appeared to be similar (Lehmann 1892). The author concluded that human inter-individual variability in local irritation from hydrogen sulfide was small for this very small sample.

5.3.2 Neurological Effects

5.3.2.1 Human

Numerous reports of accidents have found that exposure to high concentration of hydrogen sulfide causes immediate loss of consciousness (Burnett et al. 1977, Mitchell and Yant 1925, Tvedt et al. 1991a, b, Wang et al. 1989). When victims are moved away from the toxic atmosphere, they may regain consciousness. The reversible unconsciousness is called "knockdown". Most of these reports did not provide information on exposure concentrations, but exposure time was usually estimated ranging from a few minutes to 30 minutes (Arnold et al. 1985, Hoidal et al. 1986, Hurwitz and Taylor 1954). Other signs and symptoms of exposure include altered behavior pattern, confusion, vertigo, agitation or somnolence, headache, and nausea (Arnold et al. 1985, Burnett et al. 1977).

Two reports of 221 and 250 cases of acute hydrogen sulfide intoxication showed high incidence (54.6% and 74%) of loss of consciousness indicating the central nervous system is a major target site of hydrogen sulfide toxicity (Arnold et al. 1985, Burnett et al. 1977). Most victims (98%) were healthy male workers, and their age ranged from 20 to

60 years old with the majority within 20-30 years. Clinical data showed no association between the severity of poisoning and age. Little information is available in the literature regarding the acute poisoning of hydrogen sulfide in other subpopulations outside male workers.

5.3.2.2 Animal

At high concentrations hydrogen sulfide can cause unconsciousness and death within a few minutes in various animals. It has been reported that concentrations ranging from 750-1000 ppm cause such effects in different species including rats, mice, guinea pigs, rabbits, dogs, and goats (Beck et al. 1979, Lehmann 1892, Lopez et al. 1989, Mitchell and Yant 1925, Weedon et al. 1940). Loss of righting reflex, indicated by animal's failure to right themselves after having been placed on their back, was used to measure unconsciousness of mice at different concentrations and exposure times (Clanachan 1979). The results showed that acute toxicity of hydrogen sulfide is time-dependent. The concentrations of H_2S required to produce unconsciousness and death were greater at the shorter exposure durations (<15 minutes) as would be expected from the toxic load concept.

5.3.2.3 Inter-species Variability

Rapid loss of consciousness at high concentrations of hydrogen sulfide has been observed in both animals and humans. This indicates that the major target site of acute systemic poisoning of hydrogen sulfide is the nervous system in both animals and humans.

5.3.2.4 Intra-species Variability

In a survey of accidental exposure to hydrogen sulfide of 158 workers from different countries including Americans, Irish, French, German, Polish, Scottish, Hungarian, Italian, Austrian, Russian, Spanish, and Dutch, it has been found that there was no special race resistance to the acute hydrogen sulfide poisoning (Mitchell and Yant 1925). In addition, the age of reported cases ranged from 18 to 60 years with the majority being

below 40 years; the length of employment at refineries ranged from two months to 33 years. Based on this information, the authors concluded that humans have been poisoned by hydrogen sulfide regardless of nationality, age, and length of service (Mitchell and Yant 1925), although detailed individual information and the comparison of severity of intoxication among different groups was not provided.

Kilburn (1997) investigated neurobehavioral impairment in 16 subjects following acute (11 subjects) and chronic (5 subjects) exposure to reduced sulfur gases including hydrogen sulfide. Among many parameters tested, simple reaction time was chosen to show the inter-individual variability in response to hydrogen sulfide (Figure 5.1).

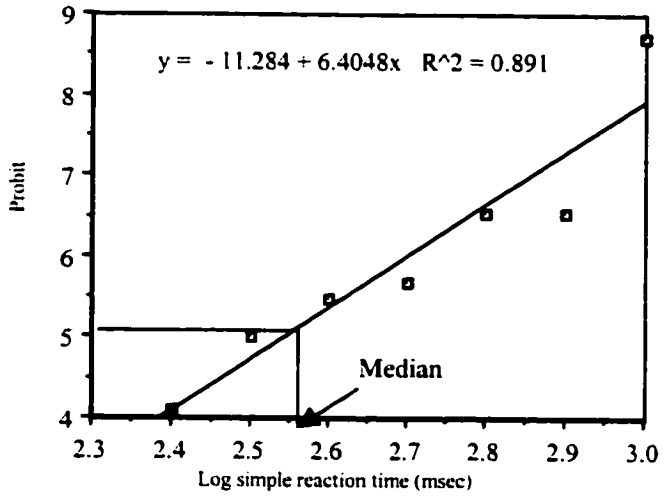


Figure 5.1 Log probit of simple reaction time following exposure to reduced sulfur gases (Data from Kilburn 1997)

As shown in Figure 5.1, reaction time follows a lognormal distribution over a small range (2.4 to 3.0 msec).

5.3.3 Long-term Effects after Acute Exposure to Hydrogen Sulfide

Long-term effects after acute exposure to hydrogen sulfide remain controversial. Ahlborg (1951) conducted a two-month to three-year follow up of fourteen workers after acute hydrogen sulfide poisoning and found no evidence of permanent damage. According to the retrospective studies on more than 200 cases of acute hydrogen sulfide intoxication that occurred in the oil and gas industries in Alberta, it appears no or few

long-term adverse effects were reported following acute inhalation exposure to hydrogen sulfide (Arnold et al. 1985, Burnett et al. 1977). However, lack of follow-up in these two studies leaves this conclusion open to question.

Some other investigators provided supportive evidence regarding long-term effects. One study reported that abnormal pulmonary function showing a mild restrictive disease persisted for 5 months following acute exposure to hydrogen sulfide in a healthy male worker (Parra et al. 1991). This patient was diagnosed with pneumonitis caused by inhalation of toxic gas. Based on a postmortem examination of a victim who died at the same exposure, it has been suggested that the development of a mild fibrosis resulting from the toxic exposure could explain the persistence of exertion dyspnoea and decrease in lung function (Parra et al. 1991).

Tvedt et al (1991a, 1991b) reported 5 years follow-up of six patients with unconsciousness after acute exposure to hydrogen sulfide. They found that longer duration (> 5 minutes) of unconsciousness in high concentration hydrogen sulfide atmosphere can cause permanent brain damage with memory and motor function being most affected. Duration of exposure is of extreme importance in this case because of the critical limit of 4-5 minutes for brain damage caused by hypoxia following hydrogen sulfide poisoning. The exposure duration in other reports of permanent brain damage caused by hydrogen sulfide varied from 5-10 minutes (Arnold et al. 1985), 20 minutes (Hoidal et al. 1986), to 30 minutes (Hurwitz and Taylor 1954). Another recent report also provided similar evidence for long-term effects with persistent cognitive and motor deficits after acute hydrogen sulfide poisoning (Schneider et al. 1998).

Kilburn (1997) conducted neurobehavioral function tests in 16 subjects who were exposed to reduced sulfur gases including hydrogen sulfide 1.7-6.3 years previously. Permanent neurobehavioral impairment was apparent in all 16 subjects. Five men exposed to high concentrations with knockdown were found to have more severe after effects compared to low doses exposure. Some other studies also found persistent neurological impairment in individuals after exposure to hydrogen sulfide (Kilburn 1993, Kilburn and Warshaw 1995, Wasch et al. 1989).

From the information reviewed above, it is evident that acute hydrogen sulfide poisoning causes two major types of serious effects, loss of consciousness and pulmonary edema followed by pneumonia. The mortality rate of these two effects is high and hospitalization and immediate treatment are required. According to the concentration and duration of exposure, these two types of injuries may progress into chronic damage of the brain or the respiratory tract resulting in permanent disability. Therefore, for the purpose of risk assessment of an industrial facility, "loss of consciousness (especially longer than 5 minutes)" and "pulmonary edema and secondary pneumonia" qualify as "serious and irreversible" toxic effects of acute hydrogen sulfide poisoning.

5.3.4 Death

5.3.4.1 Overview

A number of accident reports indicated that exposure to high concentrations of hydrogen sulfide can be rapidly fatal (Adelson and Sunshine 1966, Allyn 1931, Breysse 1961, Deng and Chang 1987, Freireich 1946, Hagley and South 1983, Kage et al. 1998, Kimura et al. 1994, Mitchell and Yant 1925, Osbern and Crapo 1981, Parra et al. 1991, Wang et al. 1989). Almost all case reports did not give actual exposure concentrations. Some reports gave an estimate made by measuring hydrogen sulfide concentrations in air after the accidents, e.g., 429 ppm by Hsu et al (1987), 1900 –6000 ppm by Winek et al. (1968), or by estimating from a related animal study, e.g., 550-650 ppm by Kimura et al. (1994). The lethal exposure time was estimated from a few seconds to minutes. The fatality rate of exposed workers was reported to be 6.0% (Burnett et al. 1977) to 2.8% (Arnold et al. 1985) in oil industry in Alberta. Because accidents have usually occurred in the workplace, most victims were healthy adult males.

Canaries, rats, mice, guinea pigs, cats, rabbits, dogs, and goats, monkeys have been used to study lethal effects of hydrogen sulfide (Arts et al. 1989, Lehmann 1892, Lopez et al. 1989, Lund and Wieland 1966, MacEwen and Vernot 1972, Mitchell and Yant 1925, Prior et al. 1988, Tansy et al. 1981, Zwart et al. 1990). The exposure-response curve for lethality is extremely steep for hydrogen sulfide (Guidotti 1994, 1996).

5.3.4.2 Inter-species Variability

LC_{50}

LC_{50} is the calculated concentration of a gas or vapor expected to kill 50% of a population exposed to that gas or vapor for a specified time, e.g., 10 minutes or one hour. The values of LC_{50} for different species are commonly used to compare the sensitivity of species to certain toxicants. Comparison of LC_{50} values of different species must be made for the same exposure duration. Generally, species with smaller values of LC_{50} are considered to be more sensitive to toxicants.

According to available data on hydrogen sulfide, an inter-species comparison is only possible between rats and mice (Figure 5.2). As shown in Figure 5.2, LC_{50} values for rats and mice are very close although LC_{50} is slightly higher in mice than rats for 10 minute exposures.

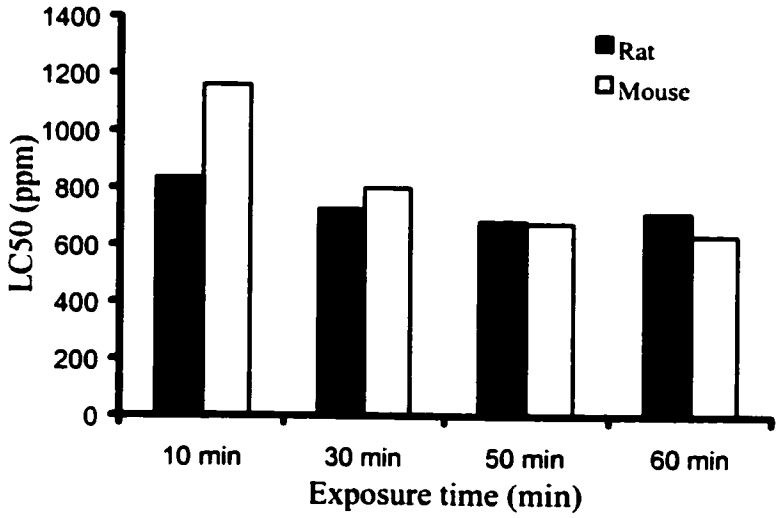


Figure 5.2 Comparison of LC_{50} for hydrogen sulfide in animals
(Data from Arts et al. 1989, Zwart et al. 1990, MacEwen & Vernot 1972)

Lethal Toxic Load

Figure 5.3 to 5.5 provide graphic summaries of the lethal doses of hydrogen sulfide in different species. In these figures, the concentrations of hydrogen sulfide in air are plotted against exposure time on a log-log scale. The combination of these two variables, that is toxic load, produces different levels of fatality. Figure 5.3 shows the toxic load for

10% fatality (ranging from 1% to 20%), Figure 5.4 for 50% fatality (ranging from 40% to 60%) and Figure 5.5 for 90% fatality (ranging from 80% to 100%). All data points on the graphs are from animal experiments or human accidental reports existing in the literature (See Table A in Appendix).

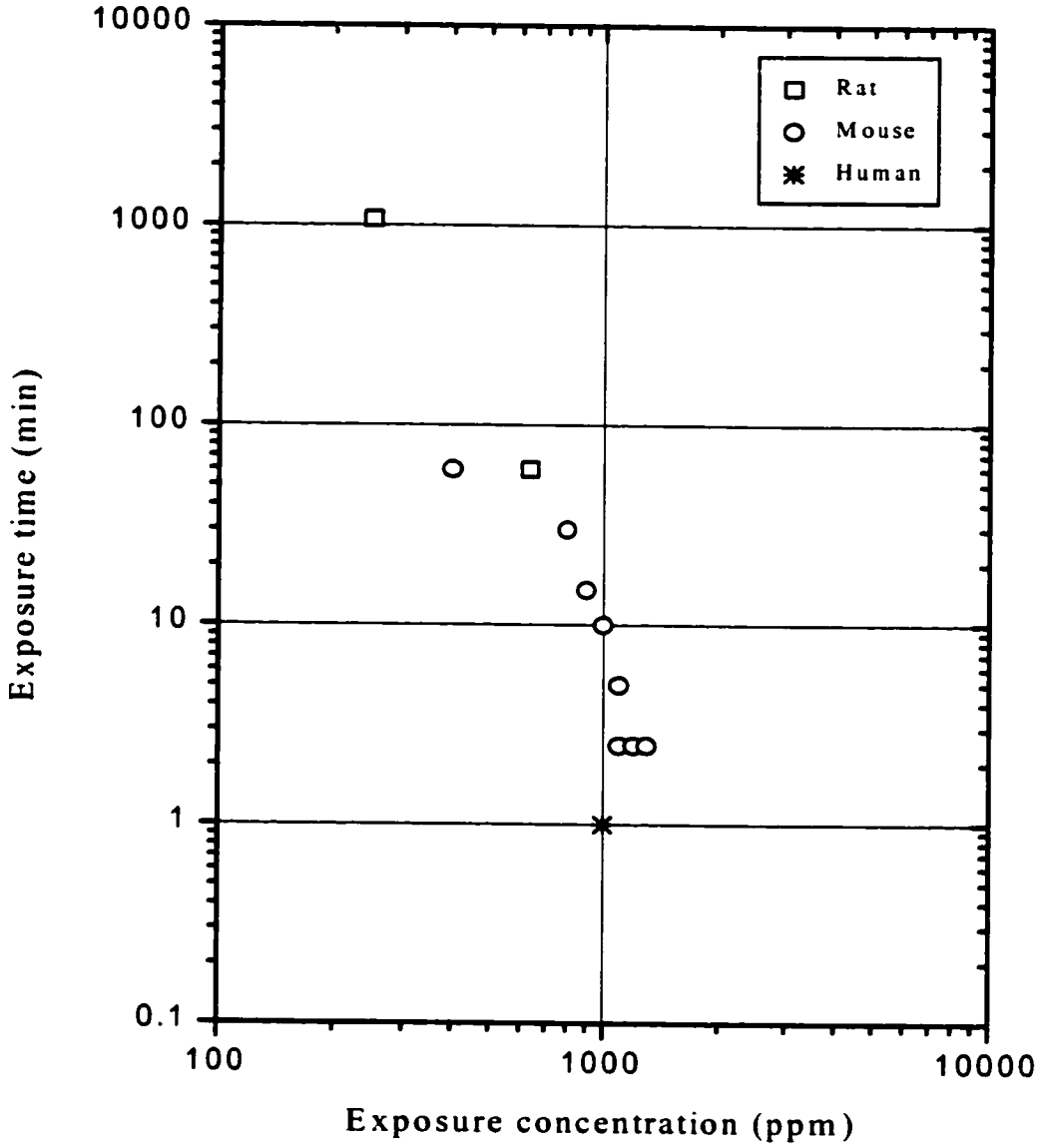


Figure 5.3 Lethal effect (10% fatality) of hydrogen sulfide

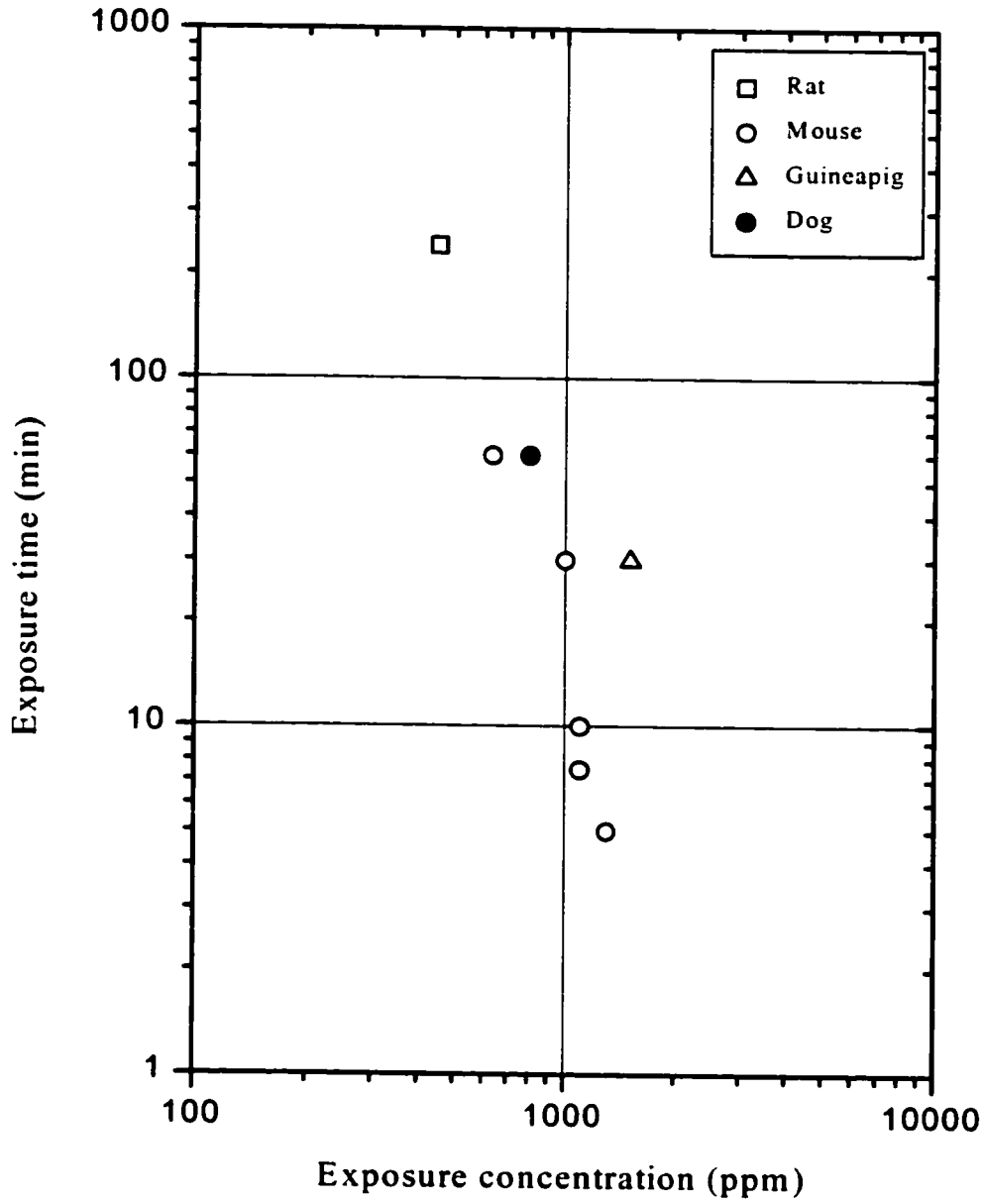


Figure 5.4 Lethal effect (50% fatality) of hydrogen sulfide

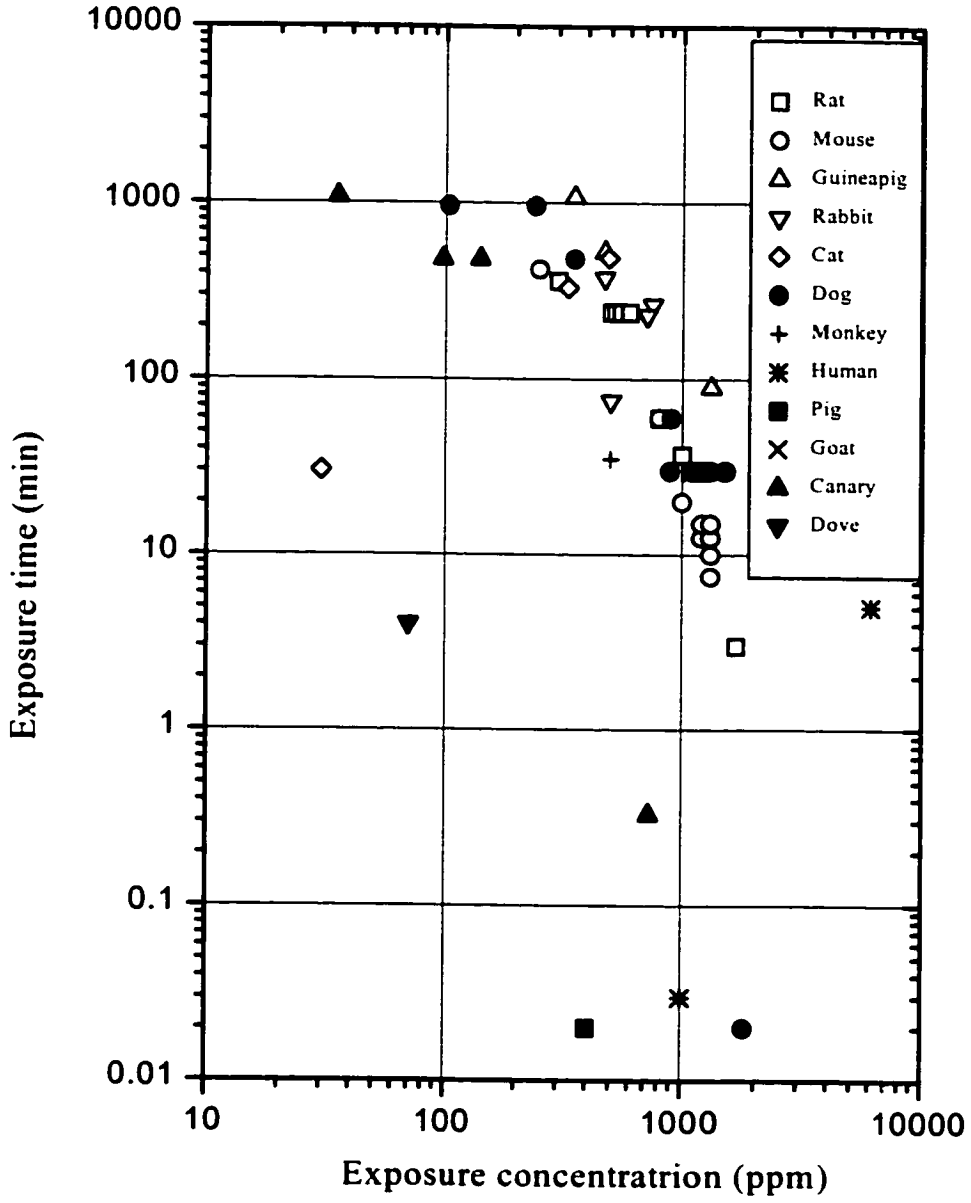


Figure 5.5 Lethal effect (90% fatality) of hydrogen sulfide

Figure 5.3 shows that at 10% fatality level, mice appear to be slightly more sensitive to hydrogen sulfide than rats. Based on a single observation, humans appear to be more sensitive than mice. Figure 5.4 indicates that at 50% fatality level, mice are more sensitive than dogs and guinea pigs based on single observation for the latter. Figure 5.5 contains data points for 90% fatality for twelve species, thus provides a relatively

complete picture of inter-species variability in response to hydrogen sulfide. Data points located in the right upper corner of the figure indicate lower sensitivity, while data points located near the left bottom indicate higher sensitivity. As indicated in Figure 5.5, pig is most sensitive followed by dove and human. Data for cats are suspected because data points distribute both to the right upper corner and to the left. Other species are less sensitive. Overall, there is a considerable inter-species difference in sensitivity to the acute lethal effects of hydrogen sulfide.

Lehmann (1892) observed that symptoms of human volunteers exposed to 100-500 ppm hydrogen sulfide were similar to those noted in dogs exposed to the same concentration of hydrogen sulfide. Thus, he postulated that the reaction of humans to higher concentrations would be comparable to that of dogs. Mitchell and Yant (1925) compiled Lehman's observations and their own findings, and tabulated acute toxicity of hydrogen sulfide on human (Table 5.4). As the symbol * shows in this table, the exposure parameters (concentration and time) for the lethal effects are actually from the observations on dogs. Later on, some authors used these data as if they were human data.

In order to compare different probit equations proposed for humans, three toxic load equations for 90% lethality of hydrogen sulfide proposed by Rogers (1990), TNO (1992), and U.S. Coast Guard (1980, cited in CCPS 1999) are plotted in Figure 5.6 on the background of Figure 5.5. The purpose of this plot is to provide a visual measurement of a toxic load equation by comparing it with the actual lethal data mostly from animal studies. An equation line above all data points is considered to be not conservative, while an equation line far below all data points indicates a great caution. The reason for choosing 90% fatality level for this plot is that at this fatality level much more data points for different species are available than for other fatality levels (See Figure 5.3 to 5.5).

As shown in Figure 5.6, the equation line for Roger (1990) locates right in the lower fringe of data points, while the lines for TNO (1992) and U.S. Coast Guard (1980) are more close to the center of most data points. This indicates that the toxic load equation proposed by Roger (1990) is slightly more conservative than TNO (1992) and U.S. Coast Guard (1980) but they are all within the range of data points.

Table 5.4 Acute toxicity of hydrogen sulfide on humans

H_2S (ppm)	0 –2 min	2-15 min	15-30 min	30–60 min	1-4hr	4-8 hr	8-48 hr
100-150		Coughing, irritation to eyes; loss of sense of smell	Disturbed respiration; pain in eyes; sleepiness	Throat irritations	Salivation and mucous discharge; sharp pain in eyes; coughing	Increased symptoms*	Hemorrhage and death*
150-200		Loss sense of smell	Trachea and eye irritation	Eye and trachea irritation	Difficult breathing; in-distinct vision; light shy	Serious irritating effects	Hemorrhage and death*
250-350		Irritation of eyes; loss of sense of smell	Irritation of eyes	Painful secretion of tears; weariness.	Light shy; nasal catarrh; pain in eyes; difficult breathing; pain in head; injection of conjunctiva.	Hemorrhage and death*	
350-450		Irritation to eyes; loss of sense of smell	Difficult respiration; coughing; irritation of eyes.	Increased irritation to eyes and nasal tract; dull pain in head; weariness; light shy	Dizziness; weakness; increased signs of irritation; and death*	Death*	
500-600	Coughing, collapse,and unconsciousness*	Respiratory disturbances; irritation to eyes; difficulty of respiration; collapse*	Serious eye irritation; light shy; palpitation of heart; few cases of death*	Severe pain in eyes and head dizziness; trembling of extremities; great weakness and death*			
600-700	Collapse* unconsciousness*	Collapse*; unconscious-ness;* death.*					
700-800	Collapse* unconsciousness*	Collapse*; unconscious-ness;* death.*					
800-1000	Collapse* unconsciousness*	Collapse*; unconscious-ness;* death.*					
1000-1500	Collapse* unconsciousness*	Collapse*; unconscious-ness;* death.*					

Adapted from Mitchell and Yant 1925 (Table 18). * Data secured from experiments on dogs, which have susceptibility similar to men, according to Lehmann (1892).

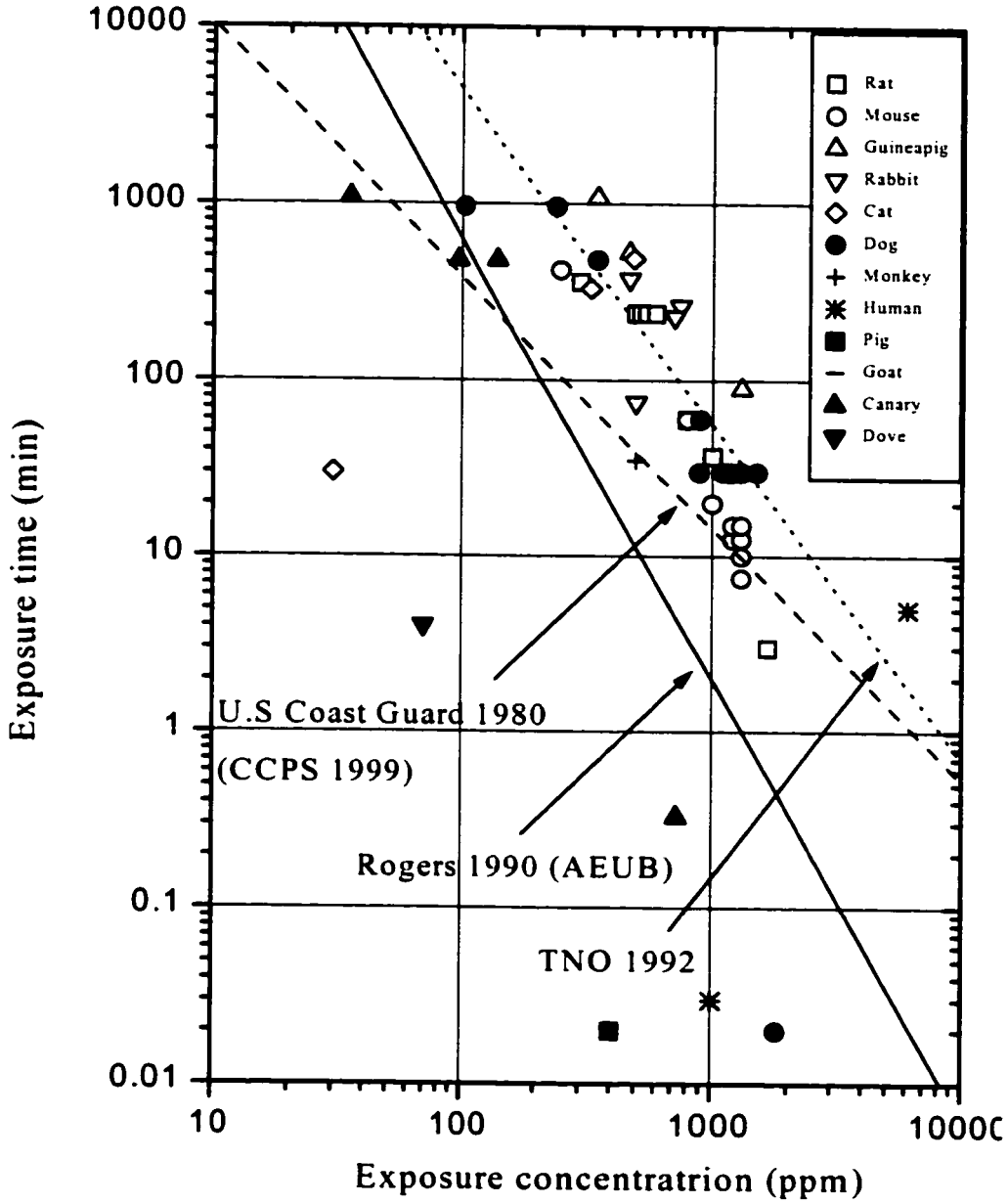


Figure 5.6 Comparison of different toxic load models for hydrogen sulfide

Toxic load equations for 90% fatality applied in Figure 5.6:

Rogers 1990 (AEUB)	$C^{2.5}t = 6.3 \times 10^7$ ppm$^{2.5}$min
U.S. Coast Guard 1980 (CCPS 1999)	$C^{1.43}t = 2.8 \times 10^5$ ppm$^{1.43}$min
TNO 1992	$C^{1.9}t = 2.8 \times 10^7$ ppm$^{1.9}$min

5.3.4.3 Intra-species Variability

Human

Although some factors are proposed for high-risk groups within a general population, little information is available in regard with inter-individual variability in response to lethal effect of hydrogen sulfide. Most victims in accidents are healthy male workers. There is no report on young children, seniors, or people with previous cardiopulmonary diseases regarding their susceptibility to hydrogen sulfide.

From Figure 5.5, it is noticeable that lethal response of humans was more variable than animals. The reasons for this significant variability are considered as: (1) The exposure concentrations and duration for human fatality are not as accurate as in the controlled animal experiments. Most of them are only estimated as described earlier in this chapter; (2) Humans may show more significant diversity in response to toxicants because of greater genetic diversity than experimental animal strains.

Animal

Regarding intra-species difference in lethal effects of hydrogen sulfide, an experiment on young male and female Sprague-Dawley, Long Evans and Fischer-344 rats demonstrated that LC_{50} and LC_{10} values for male, female, and strain were not different, but mortality in males was significantly higher than females (Prior et al. 1988). The reason for this difference was not discussed. This study also showed that LC_{50} and LC_{10} were time dependent (Prior et al. 1988). For 4 hours and 6 hours exposure, the concentration-time-mortality relationship obeyed Haber's rule, that is $C \times t$ is a constant for 50% and 10% mortality (40.3 ppm/34.1 ppm and 40.7 ppm/36.4 ppm for 4 and 6 h exposure, respectively). However, the value of $C \times t$ for 2 h exposure was significantly smaller, being approximately 40% less (Prior et al. 1988). The concentration-mortality curves for three exposure periods were steep which can be reflected by the very closed values for LC_{10} and LC_{50} (Table 5.5). This study suggests that inter-individual variability and strain difference in lethality were very small.

Table 5.5 Concentration-time-mortality relationships for H_2S in rats

Species	Time (hr)	LC_{10} (ppm)	LC_{50} (ppm)	Reference
Rat	2	549	587	Prior et al. 1988
	4	422	501	
	6	299	335	

In animal studies, the animals are usually chosen to be as similar as possible in conditions such as age, sex, body weight, strain, and diet, especially in the studies with mice and rats. Therefore, variability within same experimental group is expected to be small.

5.4 Variability in Response to Chlorine

There are numerous studies on the toxicity of chlorine gas in the literature. The earliest description of toxic effect of chlorine in animals and humans was provided by Lehmann (1887). But it was only after chlorine was used as a weapon in the World War I that the toxicity of chlorine has attracted extensive attention. Results of animal studies are consistent with the observation on humans that chlorine is a primary irritant of both the upper respiratory tract and the deeper structures of the lung (WHO 1982).

The mechanism of toxicity of chlorine is not completely understood. Molecular chlorine is a strong oxidizing agent. It reacts widely with many functional groups in cell components. Chlorine combines with water in the airway to form hydrochloric (HCl) and hypochlorous acid (HOCl). The latter is the oxidizing agent that can damage tissues by disrupting cellular proteins.

5.4.1 Irritation

Chlorine is a very potent irritant in both humans (Petri 1965, Rupp and Henschler 1967) and experimental animals (Barrow et al. 1977). It is a respiratory irritant acting as sensory irritant, bronchoconstrictor, and pulmonary irritant (Alarie 1973a). In other words, its action of irritation extends throughout the entire respiratory tract.

5.4.1.1 Human

A number of human case reports of accidental exposure to chlorine gas have shown that irritation of chlorine is mainly reflected by symptoms such as burning of the eye, the nose, and the throat, increased salivation, hoarseness, cough, choking sensation, substernal burning, pain and constriction. Loss of consciousness has also been observed in some casualties (Abhyankar et al. 1989, Beach et al. 1969, Berghoff 1919, Charan et al. 1985, Chasis et al. 1947.)

Acute pulmonary injury following exposure to chlorine gas includes acute bronchitis, pulmonary edema, and pneumonia (Chester et al. 1969). In an accidental release of chlorine affecting 418 persons, 33 of them were admitted by a hospital for treatment (Chasis et al. 1947). In this group, pulmonary edema developed in 23 patients (70%), pneumonia developed in 14 patients (42%), and acute tracheobronchitis occurred in all patients. Pulmonary edema persisted for a relatively short duration ranging from 12-36 hours; tracheobronchitis occurred 24-48 hours following exposure and subsided in most cases in 5 to 7 days; pneumonia was developed on the basis of pulmonary edema and the progress from pulmonary edema to pneumonia varied individually. In addition, 14 patients in this group had preexisting disease including pulmonary or heart diseases. Preexisting diseases are considered to affect the manifestations and course of chlorine intoxication. However, the most seriously injured patient had no previous disease, which suggests that the degree of exposure is the most important determinant of the degree of injury (Chasis et al. 1947).

The observation on war gas showed that the development of pulmonary edema occurred within minutes even with typical wartime dose, and began almost instantaneously after extremely high dose (Gerard 1948).

5.4.1.2 Animal

Experiments have been performed in rats, mice, cats, rabbits, and guinea pigs to investigate irritation effects of chlorine gas. Decrease in respiratory rate has been used as a measurement of sensory irritation. The respiratory rate of rats and mice has been observed to significantly decrease after short time exposure to chlorine (Barrow et al.

1977, Barrow and Steinhagen 1982, Chang and Barrow 1984). The response is concentration-dependent. RD_{50} (concentrations that produce 50% decrease in respiratory rate) in mice exposed to 0.7-34.8 ppm chlorine for 10 minutes was about 9.3 ppm. Respiratory rate would not further decrease when concentration was above 34.8 ppm (Barrow et al.1977) because the maximum response was about a 80%-85% decrease in respiratory rate from control values (Alarie 1973b). Mice appeared to be more sensitive to chlorine than rats when comparing their RD_{50}. For 10 minutes exposure, the RD_{50} was 25.4 ppm for rats (Barrow and Steinhagen 1982), and 9 ppm for mice (Barrow et al. 1977). In another study, reflex bronchial constriction, determined by pulmonary function testing, was observed in cats and rabbits exposed to chlorine at 100 and 200 ppm (Gunn 1920).

At much higher concentrations, chlorine induces severe effects in guinea pigs, rabbits, and cats (Lehmann 1887). Rats exposed to 1500 ppm chlorine for 10 minutes showed significant airway mucosal abnormality (Demnati et al. 1995). Dogs exposed to high concentrations of chlorine were found to have tracheitis, bronchitis with peribronchial inflammation and focal pulmonary necrosis, as well as extreme congestion and edema in the entire respiratory tract (Winternitz et al. 1920). Rabbits exposed to 100-200 ppm of chlorine for 30 minutes were also confirmed to have pulmonary edema by a frothy exudation in the trachea, and changes in lung function and histopathology (Barrow and Smith 1975). Investigation on chlorine induced acute lung injury in an isolated rabbit lung revealed that chlorine directly injured epithelial and endothelial membranes and increased permeability of capillary walls leading to increased transport of fluid and protein into the alveoli due to the alveolar epithelial injury (Menaouar et al. 1997).

5.4.1.3 Inter-species Variability

Mice are more sensitive than rats to sensory irritation, which is reflected by their more significant decrease in respiratory rate after exposure to chlorine gas. These data can be used to predict human response to chlorine (Barrow et al. 1977, see Table 5.7). Cats were observed to be extremely sensitive to irritation caused by chlorine compared to

rabbits and guinea pigs (Lehmann 1887, Wachtel 1941). Humans and animals have similar symptoms and pathological changes following acute exposure to chlorine.

Table 5.6 Prediction of human responses to Cl_2 from sensory irritation data obtained in mice

Concentration obtained in mice	Predicted sensory irritation responses in humans
RD_{50} = 9.3 ppm	Intolerable and rapidly incapacitating
1/10 RD_{50} = 0.90 ppm	Slightly irritating, some stinging or burning sensation of the eye, nose, and throat
1/100 RD_{50} = 0.09 ppm	Tolerable with very slight or no irritating sensation

Adapted from Barrow et al. 1977

5.4.1.4 Intra-species Variability

In one large accidental release of liquid chlorine, about 100 people were affected by a chlorine cloud and 15 were hospitalized (Joyner and Durel 1962). Ten of the hospitalized patients developed pulmonary edema (10%). Three children and an adult lost consciousness on admission. Concentrations of chlorine were measured three hours after spill to be 10 ppm in the fringes of and seven hours after the spill to be 400 ppm near the release center. This report indicates that children may be more susceptible to chlorine intoxication.

Accident reports suggest that acute chlorine intoxication may be related to some individual factors such as preexisting cardiopulmonary diseases (Chasis et al. 1947). Other factors involved in the outcome of pulmonary injury following short-term sublethal exposure to chlorine gas may include: 1) the intensity and duration of exposure; 2) the time between exposure and treatment; 3) The presence of secondary infection; 4) cigarette smoking; 5) individual variability in the inflammatory response; 6) the presence or absence of previous long-term exposure to chlorine and 7) type of treatment (Chester et al. 1977).

Experience in investigation of toxicity of chemical weapons including chlorine on animals showed considerable intra-species variability (Wachtel 1941), but detailed information was not available.

5.4.2 Long-term Effects after Acute Exposure to Chlorine

There are no systematic human pathological studies describing the late healing process caused by sublethal exposure to chlorine gas. Among chemical gases used in the World War I, only chlorine had the potential to cause chronic airway and parenchymal disease (Chester et al. 1969). The acute changes of the respiratory tract are followed by organization and cicatrisation (formation of a scar) of the parenchyma and obliterative bronchitis. It has been suggested that exposure to chlorine at sublethal concentration in humans is capable of producing lasting abnormalities of pulmonary gas exchange. Treatment with corticosteroid may have beneficial effects by preventing these long-term changes (Chester et al. 1977). Among individuals, smokers and asthmatics tend to demonstrate persistence of obstructive pulmonary defects (Jones et al. 1986).

Whether there are long-term effects after exposure to sublethal concentrations of chlorine gas remains controversial. Some investigators believed that a single sublethal concentration exposure to chlorine does not result in long-term pulmonary abnormality (Abhyankar et al. 1989, Beach et al. 1969, Chasis 1947, Chester et al. 1969, Hasan et al. 1983, Kaufmann and Burkons 1971, Jones et al. 1986). However, as early as 1967, Kowitz et al (1967) reported long-term lung after-effects occurring 2-3 years following acute chlorine exposure. Several recent reports also showed evidence that long-term asthmatic reactions and nonspecific bronchial hyperresponsiveness may be associated with acute exposure to high concentrations of chlorine (Donnelly and FitzGerald. 1990, Moore and Sherman. 1991, Schönhofer et al. 1996). In addition, a 12 years pulmonary function follow-up study suggested that short-term exposure to high concentrations of chlorine gas might lead to long-term respiratory complications. This was reflected by the progressive decline in residual volume (Schwartz et al. 1990).

The toxic effects of inhalation exposure to chlorine primarily focus on the respiratory tract and involve the entire respiratory tract. The most serious consequences include pulmonary edema and pneumonia with high mortality, and may progress to chronic pulmonary disease due to persistent pathological changes of small airways. Therefore, from the accidental release of chlorine gas, "pulmonary edema and pneumonia " can be considered as "serious and irreversible effects".

5.4.3 Death

5.4.3.1 Overview

Military reports of chemical attacks during the World War I and accidental reports demonstrated that chlorine caused deaths in humans (Berghoff 1919, Dixon and Drew 1968). Death at high exposure concentration is mainly from respiratory failure and cardiac arrest due to toxic pulmonary edema (Baxter et al. 1989). Bronchopneumonia is considered to be a common and potentially lethal complication of pulmonary edema that may be responsible for delayed deaths.

Animal experiments demonstrated that animals died from exposure to high concentration chlorine had severe pulmonary edema accompanied by hemorrhagic infections (Lehman 1887, Winternitz et al. 1920).

5.4.3.2 Inter-species Variability

LC_{50}

The sensitivity of different species to lethal effect of chlorine is compared by values of LC_{50} (Figure 5.7). The values of LC_{50} indicate that mice are more sensitive than rats and dogs, and the sensitivity of dogs seems to be similar to rats. The ratio of LC_{50} for rats to LC_{50} for mice ranges from 1.4 to 3.0 depending on exposure time. Similarly, the ratio of LC_{50} for dogs to LC_{50} for mice is about 1.4.

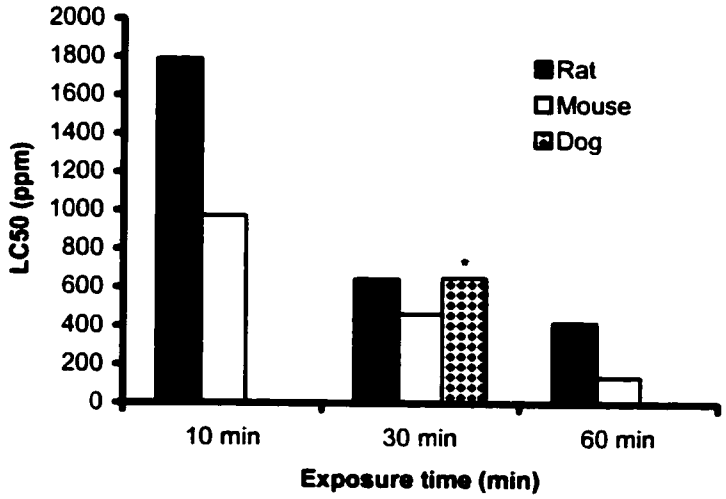


Figure 5.7 LC_{50} values of different species for chlorine
(Data from MacEwen & Vernot 1972, Underhill 1920 (*), Zwart et al. 1988)

Lethal Toxic Load

Figure 5.8 to 5.10 provide graphic summaries of the lethal doses of chlorine in different species. In these figures, the concentrations of chlorine in air are plotted against exposure time on a log-log scale. The combination of these two variables, that is toxic load, produces different levels of fatality. Figure 5.8 shows the toxic load for 10% fatality (ranging from 1% to 20%), Figure 5.9 for 50% fatality (ranging from 40% to 60%) and Figure 5.10 for 90% fatality (ranging from 80% to 100%). All data points on the graphs are from animal experiments existing in the literature (See Table B in Appendix).

Figure 5.8 and 5.9 indicate that, at 10% and 50% lethality level, mice are more sensitive than rats and dogs to acute chlorine intoxication. Figure 5.10 shows that, at 90% fatality level, mice are most sensitive species to chlorine. Cats, Guinea pigs, and rabbits are less sensitive than rats.

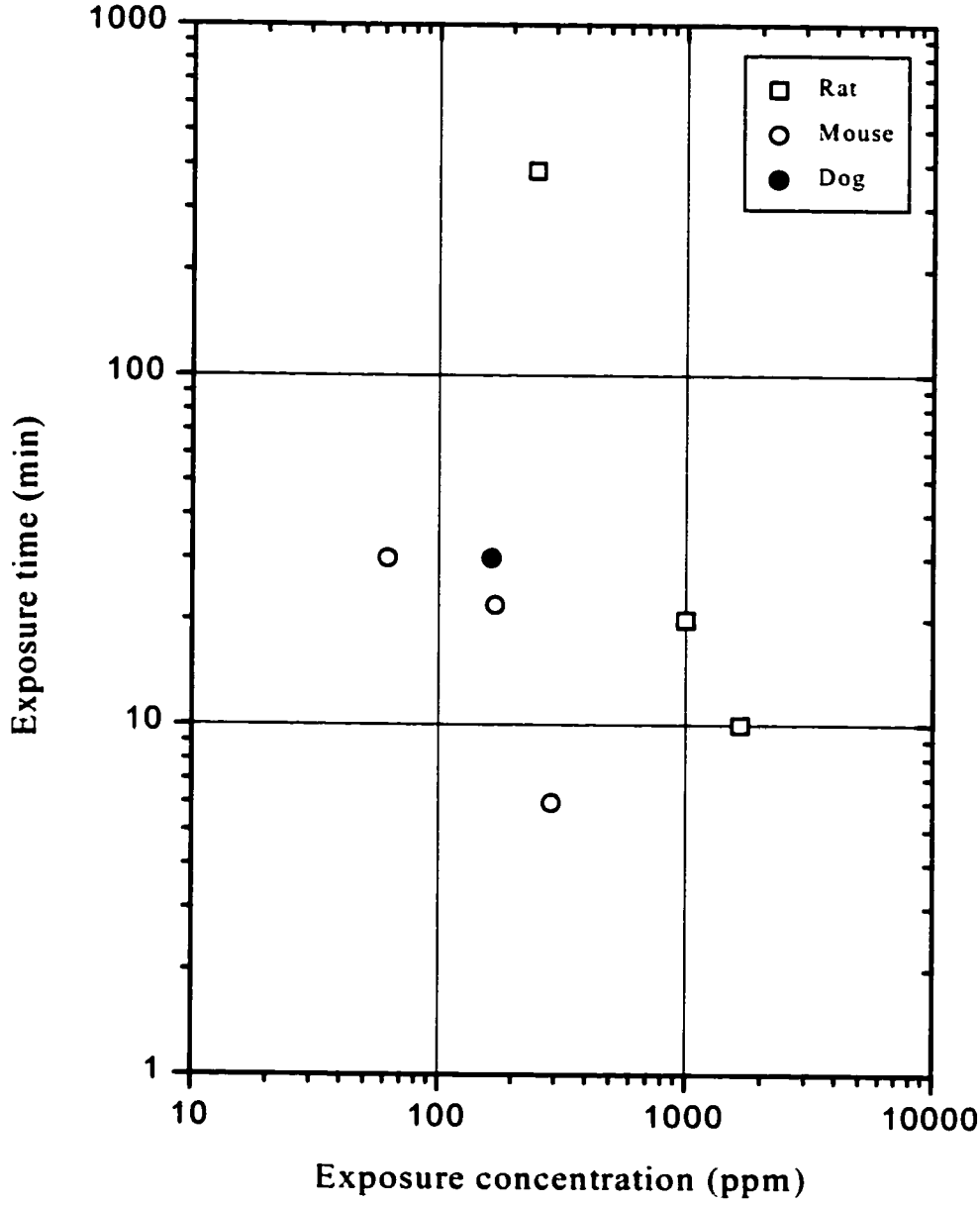


Figure 5.8 Lethal effect (10% fatality) of chlorine

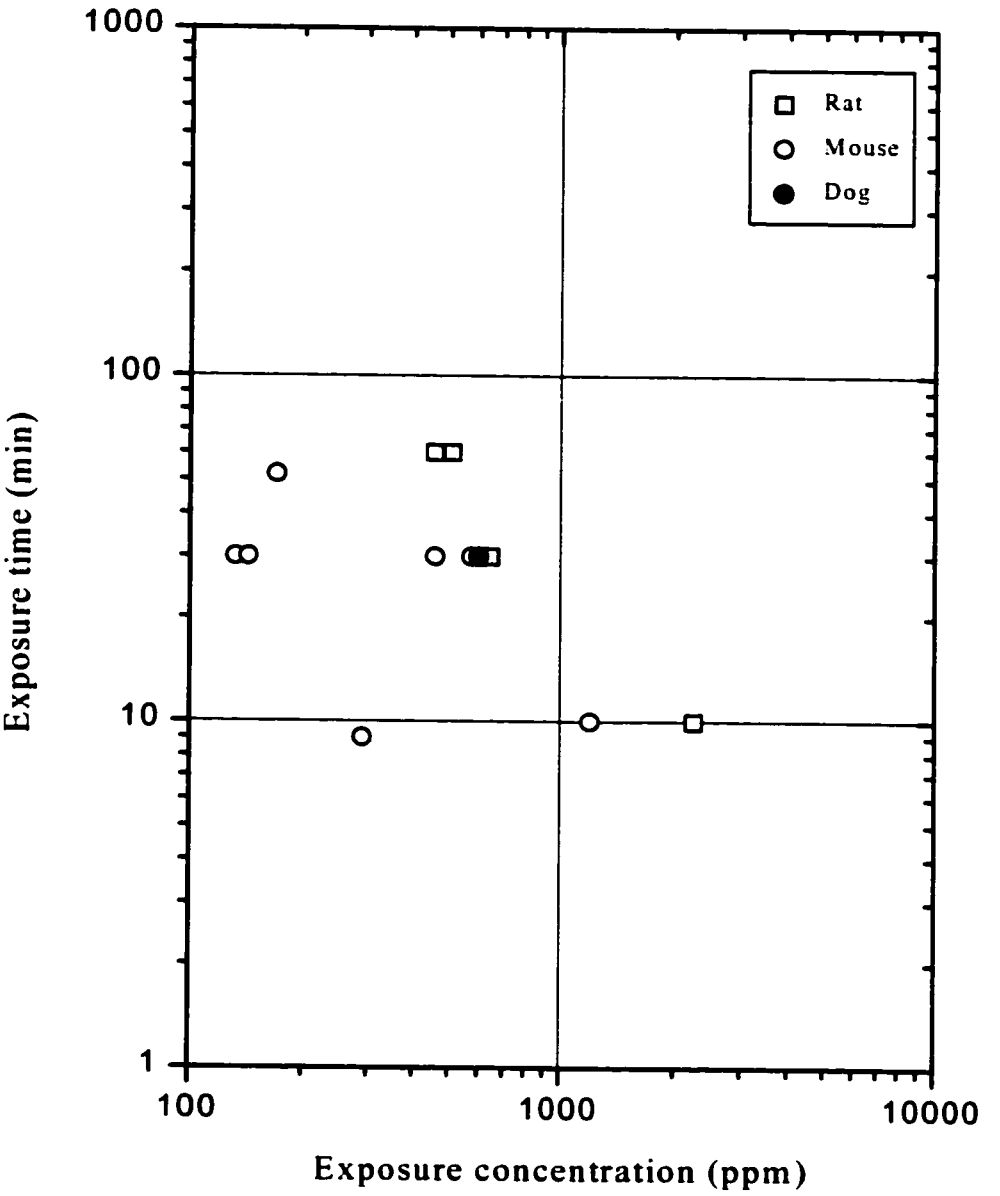


Figure 5.9 Lethal effect (50% fatality) of chlorine

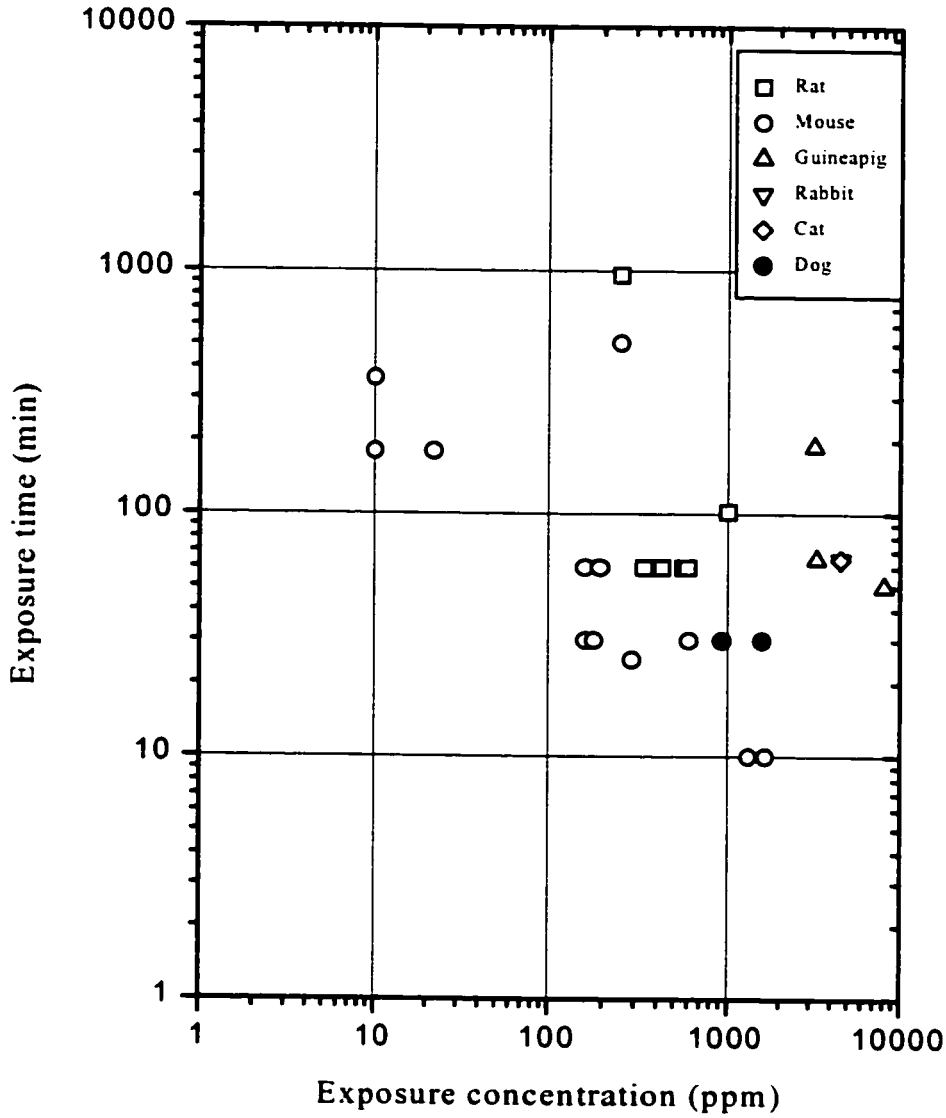


Figure 5.10 Lethal effect (90% fatality) of chlorine

In Figure 5.8, one point for a human is based on a report of an accident in which the chlorine concentrations were measured three to seven hours after a large liquid chlorine spill (Joyner and Durel 1962). The only victim in this accident was an 11-month infant who might have been exposed to at least 400 ppm chlorine for 15-20 minutes. Based on this estimate, human infants seem to be more sensitive than rats.

In the foregoing accident, a lot of animals including 49 hogs, 320 chickens, 60 ducks, 4 cats, 4 mules, 11 dogs, 2 cows, and 1 horse died. Among one hundred people who were exposed, three children and one adult lost consciousness in addition to the infant who died (Joyner and Durel 1962). Studies of chlorine as a weapon of war show that dogs are less sensitive than cats, and little more or almost equal with humans (Wachtel 1941). Monkeys are about the same as dogs. However, detailed evidence supporting this comparison was not provided (Wachtel 1941).

Overall, mice may be the most sensitive species to chlorine gas, while rats, dogs, cats, guinea pigs and rabbits are less sensitive. Human sensitivity to acute chlorine poisoning may be similar to dogs and monkeys, and vulnerable populations such as infants are more sensitive than regular population.

In order to compare different probit equations proposed for humans, four probit equations for 90% lethality of chlorine proposed by TNO (1992), Withers & Lees (1985b), and World Bank (1988, cited by CCPS 1999) are plotted in Figure 5.11 on the background of Figure 5.10. The reason for choosing 90% fatality for the plot is that, compared to 10% and 50% fatality, more data points for 90% fatality are available from the literature. Figure 5.11 indicates that four equation lines are well within the range of data points. The equation by Withers & Lees (1985b) for vulnerable population is most conservative, while the equation by World Bank (1988) is the least conservative one. However, there are more data points that fall to the left of the most conservative toxic load model.

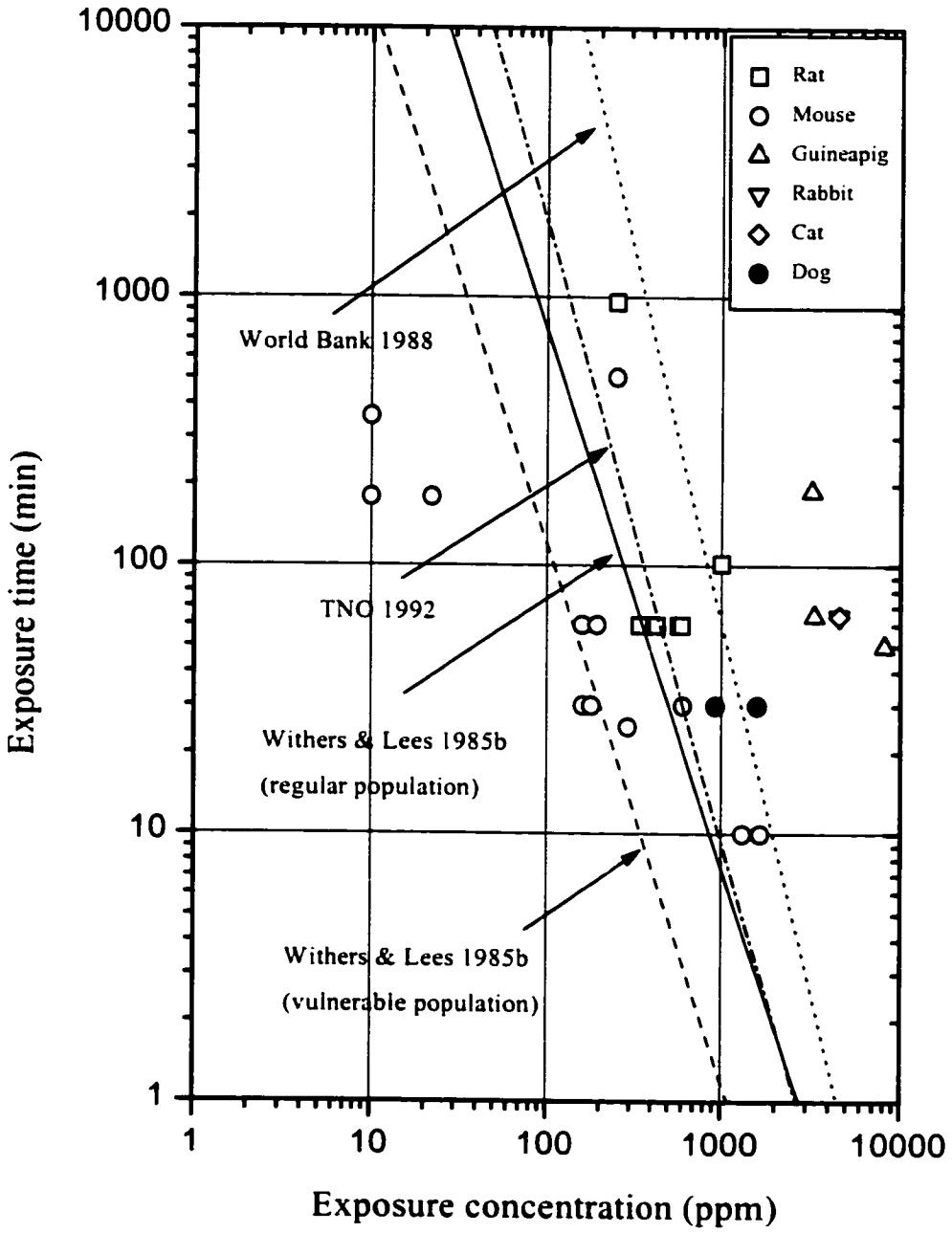


Figure 5.11 Comparison of different toxic load models for chlorine

Toxic load equations for 90% fatality applied in Figure 5.11:

TNO 1992	$C^{2.3}t = 2.4 \times 10^4$ ppm$^{2.3}$min
World Bank 1988 (CCPS 1999)	$C^{2.75}t = 6.8 \times 10^7$ ppm$^{2.75}$min
Withers & Lees (1985b)	
For regular population:	$C^2t = 4.7 \times 10^5$ ppm^{2}min
For vulnerable population:	$C^2t = 7.5 \times 10^4$ ppm^{2}min

5.4.3.3 Intra-species Variability

Human

In an accidental spill of large amount chlorine liquid occurred in a rural area, about 100 people were exposed to the chlorine cloud to different extent (Joyner and Durel 1962). The only victim was an 11-month-old infant who was estimated to be exposed to at least 400 ppm chlorine for 15-20 minutes. (Joyner and Durel 1962). Infants and seniors are considered to be more vulnerable to acute chlorine toxicity.

Animal

Wide variability in response to chlorine has been observed among cats, depending on breed, age, food, and domestication (Wachtel 1941). Intra-species variability in susceptibility is different for each species. For example, the ratio of lethal index ($C \times t$) LI_{90} to LI_{10} for chlorine is postulated as 4 for cats and 7.5 for dogs (Withers and Lees 1985a), which indicates that the intra-species variability of dogs may be greater than cats.

An experimental study on dogs (Underhill 1920) with relatively large sample size (9-23 per experiment) can be used to demonstrate intra-species variability. As shown in Table 5.7, the ratio of LC_{90} (928 ppm) to LC_{10} (164 ppm) for 30 minutes in experimental dogs is about 5.7. Furthermore, the ratio of the highest lethal concentration (1583 ppm) to lowest one (164 ppm) is about 9.7. Combine with the ratio of LI_{90} to LI_{10} being 7.5 mentioned above, intra-species difference in susceptibility to chlorine is between 5-10 fold in dogs.

Table 5.7 Concentration-time-mortality relationships in dogs for 30 min exposure duration

Species	Concentration (ppm)	Mortality (%)	LC_{50} (ppm)	Reference
Dogs	164	11	650	Underhill 1920
	491	29		
	600	40		
	710	67		
	819	61		
	928	91		
	1583	93		

5.5 Variability in Response to Ammonia

The outstanding features of ammonia gas poisoning are acute inflammation of the upper respiratory tract and conjunctiva, pulmonary edema and toxic changes in the vital organs such as the kidneys (Boyd et al. 1944).

5.5.1 Irritation

5.5.1.1 Human

Due to its high water solubility, ammonia is primarily an upper respiratory irritant in humans. Exposure to 50 ppm ammonia can cause immediate irritation of the nose and throat. Acute exposure to a higher concentration of 500 ppm resulted in a significant increase of respiratory minute volume ranging from 50% to 250% over controls in human volunteers (Silverman et al. 1949).

Accidental exposure to much higher concentrations of ammonia resulted in severe irritation of eyes, mouth, nasopharynx and trachea causing airway obstruction and respiratory distress. Pulmonary edema develops within a few hours and may be followed by bronchitis, bronchiolitis, pneumonia, and atelectasis (incomplete expansion of a lung or a portion of a lung) (Burns et al. 1985, Caplin 1941, Close et al. 1980, Hatton et al. 1979, Heifer 1971, Price et al. 1983, Taplin et al. 1976). The clinical manifestations are determined by exposure concentration and duration. For exposure to high concentrations for a short time, the upper airway obstruction can be life-threatening if not treated immediately (e.g., emergency intubation). However, once the airway is re-established, patients may recover with no significant pulmonary sequelae (Close et al. 1980). On the other hand, exposure to lower concentration for longer time can lead to extensive alkali burns of the entire tracheobronchial tree and alveolar area without severe upper airway obstruction. Pulmonary edema and infection of lower respiratory tract may also develop and can be fatal (Caplin 1941, Close et al. 1980). This type of acute intoxication can result in moderate to severe chronic pulmonary disease due to injury to the small airways (Close et al. 1980).

5.5.1.2 Animal

Animal experiments have shown that acute exposure to low concentrations less than 1000 ppm resulted in irritation to the eyes and the upper respiratory tract in rats, guinea pigs, cats, rabbits, and dogs, while exposure to concentrations higher than 4000 ppm, caused severe damage of both upper and lower respiratory tract and alveolar capillaries (Coon et al. 1970, Dood and Gross 1980, Kapeghian et al. 1982, Lehmann 1899, Mayan and Merilan 1972, Richard et al. 1978 a, b, Schaerdel et al. 1983, Stombaugh et al. 1969). Pathological changes include acute vascular congestion and diffuse intra-alveolar hemorrhage (Kapeghian et al. 1982).

5.5.1.3 Inter-species Varaibility

Available data indicate that the action of ammonia on the respiratory tract is similar in humans and animals. Ammonia mostly affects the upper respiratory tract. However, exposure to high concentrations for prolonged duration can also induce damage of the lower respiratory tract including pulmonary edema and secondary pulmonary infection.

5.5.2 Long-term Effects after Acute Exposure to Ammonia

There are some reports on the long-term effects after acute exposure to ammonia gas. A two- month to two-year follow-up study revealed different consequences resulted from different exposure conditions (Close et al. 1980). Patients exposed to high concentrations for short periods of time mainly developed upper airway obstruction which required immediate intubation or tracheotomy. These patients recovered well with few pulmonary sequelae. Other patients exposed to lower concentrations for prolonged periods of time did not manifest upper airway obstruction but developed damage in deeper structures of the lungs including pulmonary edema and pneumonia. Patients in this group have shown a grave prognosis with long-term pulmonary disorders (Close et al. 1980). In this study, the expressions of higher or lower concentrations were mainly determined by the distance from exposure location to the center of release but not by actual measurement.

Brill et al. (1957) also reported the follow-up of two patients with severe accidental exposure to ammonia gas and found that some inflammation persisted, causing permanent

narrowing of the airways, with chronic obstructive airway disease. Six months follow-up of a patient showed abnormal pulmonary arterial perfusion (Taplin et al. 1976). In this study, radionuclide lung-imaging procedures revealed partial airway obstruction, which is characteristic of the chronic bronchitic type of obstructive airway disease (Taplin et al. 1976).

In another report, permanent blindness was observed in a 17-year-old boy following acute exposure to anhydrous ammonia (Levy et al. 1964). Although eye injury is not life-threatening, it is a serious permanent disability. The mechanism of damage is that ammonia can produce liquefaction of tissues and penetrate to the deeper tissues with increased damage and scarring.

Based on the information given above, it is clear that acute exposure to ammonia can cause permanent damage. Patients with initial effects of acute ammonia poisoning involving the lower respiratory tract (pulmonary edema and pneumonia) tend to develop chronic respiratory dysfunction. In addition, severe eye injury can also cause permanent damage. Therefore, for the purpose of predictive modeling, "pulmonary edema and pneumonia" and "severe eye injury" may be considered as "serious and irreversible effects" of acute ammonia intoxication.

5.5.3 Death

5.5.3.1 Overview

There are many case reports in the literature regarding human death caused by inhalation of ammonia (Anonymous 1995, Burns et al. 1985, Caplin 1941, Close et al. 1980, Heifer 1971, Price et al. 1983, Yang et al. 1987). Exposure to 5000-10000 ppm ammonia for a short time can be immediately fatal (Henderson and Haggard 1927). Immediate deaths after acute exposure to ammonia may be caused by airway obstruction. If this initial stage is survived, pulmonary edema and /or infection of the respiratory tract can cause delayed deaths (Caplin 1941, Close et al. 1980).

Mice, rats, rabbits, and cats have been used to study the lethal toxicity of inhaling ammonia gas (Appleman et al. 1982, Boyd et al. 1944, Carpenter et al. 1949, Hilado et al. 1977, Kapeghian et al. 1982, Prokop'eva 1973, Silver and McGrath 1948).

5.5.3.2 Inter-species Variability

LC_{50}

Figure 5.12 provides a comparison of LC_{50} values for several species. Mice are more sensitive than rats, cats, and rabbits with one exception for 30 minutes exposure. These data show an extremely high LC_{50} of mice for 30 minutes exposure. Those data are from the study of Hilado et al. (1977). They used a static gas exposure system and the actual ammonia concentration is likely to be lower than reported because of absorption and adsorption of the gas by body fluids, animal hair, and chamber surfaces. The ratio of LC_{50} for rats to that for mice is about 1.4 to 4. LC_{50} for rabbits and cats are very close to that for rats. It is generally agreed that mice appear to be extremely sensitive to ammonia toxicity (NAS 1979).

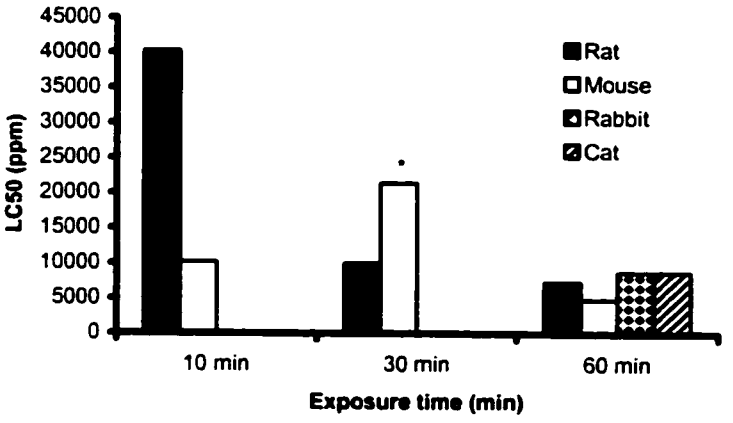


Figure 5.12 LC_{50} values for ammonia in animals
(Data from Appelman et al. 1982, Boyd et al. 1944, Hilado et al. 1977, MacEwen & Vernot 1972, Prokop'eva et al. 1973, Silver & McGrath 1948)
* data from Hilado et al. 1977 in which a static exposure system was used.

Lethal Toxic Load

Figure 5.13 to 5.15 provide graphic summaries of the lethal doses of ammonia in different species. In these figures, the concentrations of ammonia in air are plotted against exposure time on a log-log scale. The combination of these two variables, that is toxic load, produces different levels of fatality. Figure 5.13 shows the toxic load for 10% fatality (ranging from 1% to 20%), Figure 5.14 for 50% fatality (ranging from 40% to 60%) and Figure 5.15 for 90% fatality (ranging from 80% to 100%). All data points on the graphs are from animal experiments existing in the literature (See Table C in

Appendix). As shown in Figure 5.13 to15, data are only available for three animal species including rats, mice, and cats because lethal data from other species do not fall in the range of selected percentage fatality (10%, 50%, or 90%). Figure 5.13 indicates that cats are more sensitive than rats. Figure 5.14 and Figure15 show that mice are more sensitive than rats.

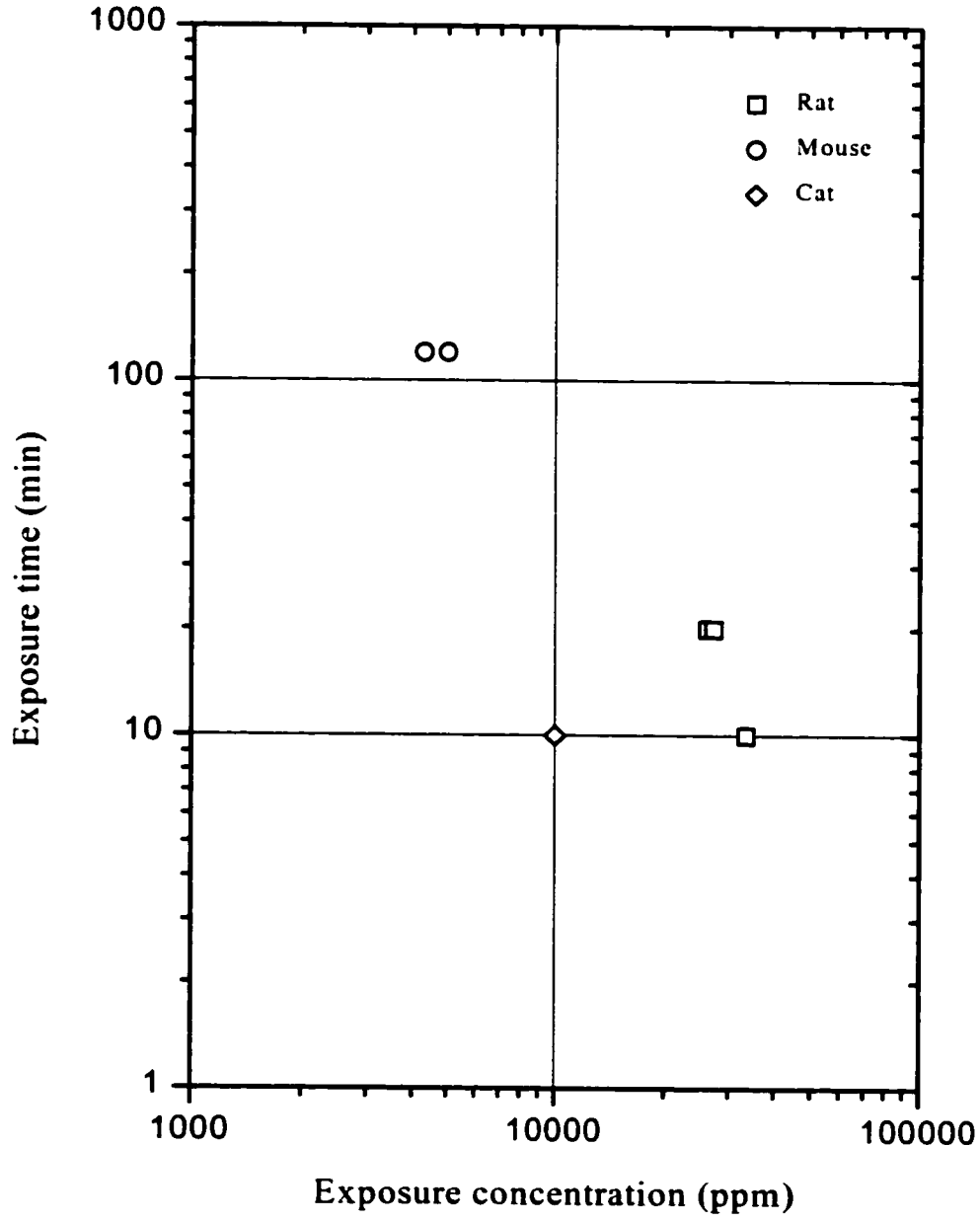


Figure 5.13 Lethal effect (10% fatality) of ammonia

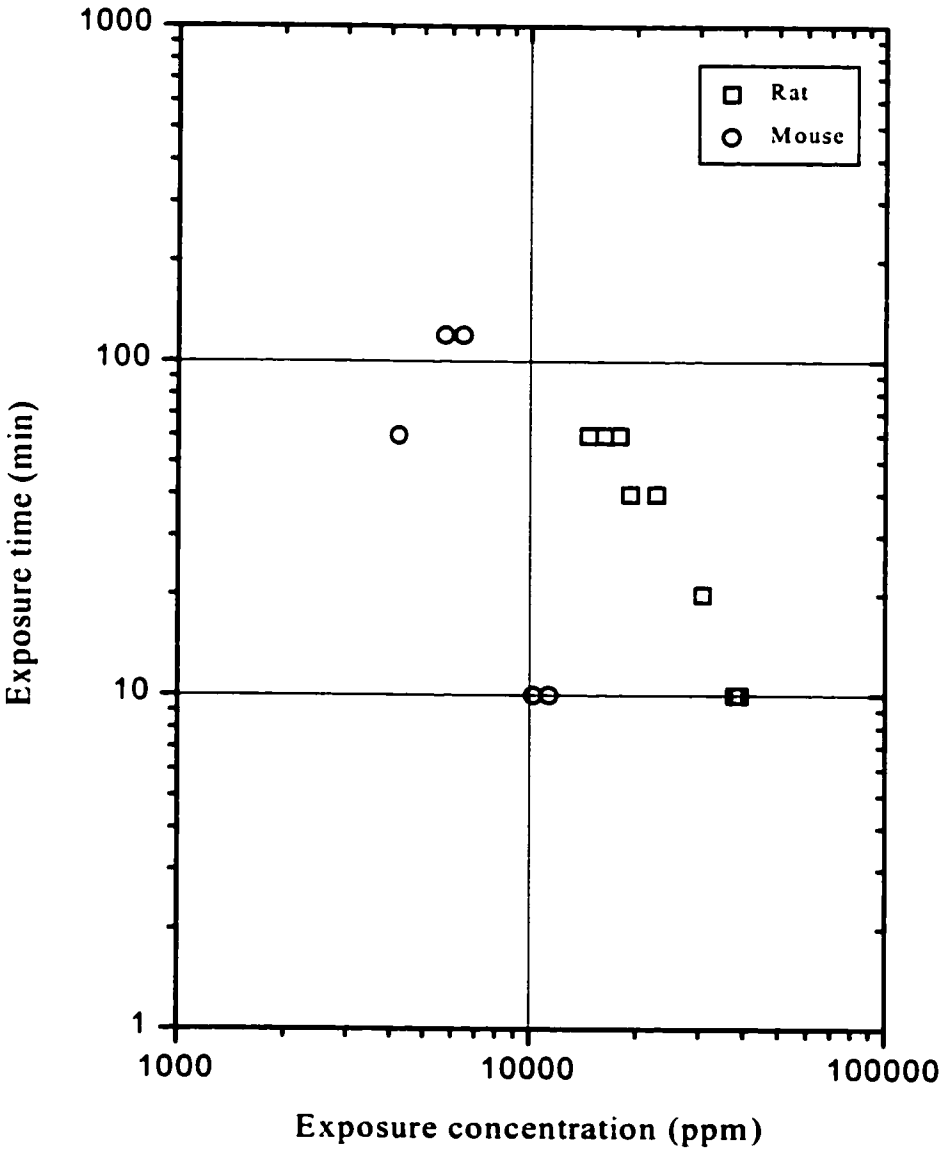


Figure 5.14 Lethal effect (50% fatality) of ammonia

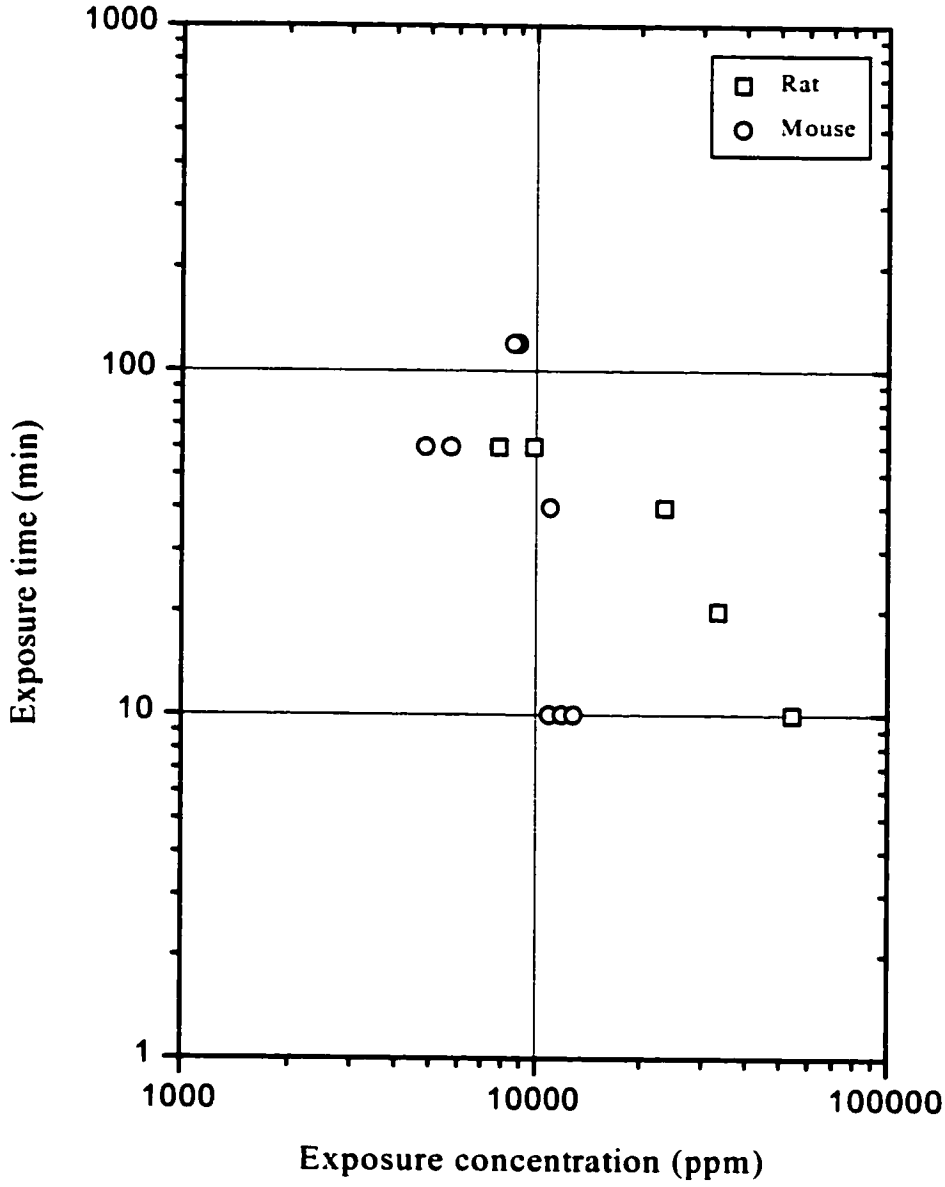


Figure 5.15 Lethal effect (90% fatality) of ammonia

Almost all reports of human deaths following accidental acute ammonia poisoning provided no information on exposure concentrations, not even estimated ones. Exposure time has been estimated in some cases ranging from a few minutes to more than 30 minutes. Human experiments usually applied concentrations lower than 500 ppm. Thus, it

is very difficult to compare the difference in sensitivity between humans and animals in terms of the fatal effects of ammonia.

Humans exposed to high concentrations of ammonia for a short period of time develop severe upper airway obstruction caused by laryngeal edema or spasm which can be rapidly fatal. Whether this characteristic of immediate death also happened in animals is not certain. From this point, humans may be more sensitive than other animal species to lethal concentrations of ammonia.

In order to compare different probit equations proposed for humans, four probit equations for 90% lethality of ammonia proposed by Eisenberg et al. (1975), TNO (1992), U.S Coast Guard (1980, cited by CCPS 1999), and World Bank (1988, cited by CCPS 1999) are plotted in Figure 5.16 on the background of Figure 5.15. As shown in Figure 5.16, Probit equation proposed by Eisenberg et al. (1975) is the most conservative one, which other three are less conservative and close to each other.

5.5.3.3 Intra-species Variability

Human

Little information is available regarding individual sensitivity to ammonia poisoning. Exposure conditions are of great importance. In an accidental report, a 6-month-old infant exposed to high concentration of ammonia for a short time developed upper airway obstruction. After immediate intubation this boy recovered very well without any abnormality. In contrast, a 26-year-old healthy man exposed to a lower concentration but for a longer time died from massive hemorrhage and bronchopneumonia (Close et al. 1980). The high or lower concentrations stated in this report are according to the distances from exposure location to the release center but not based on actual measurements.

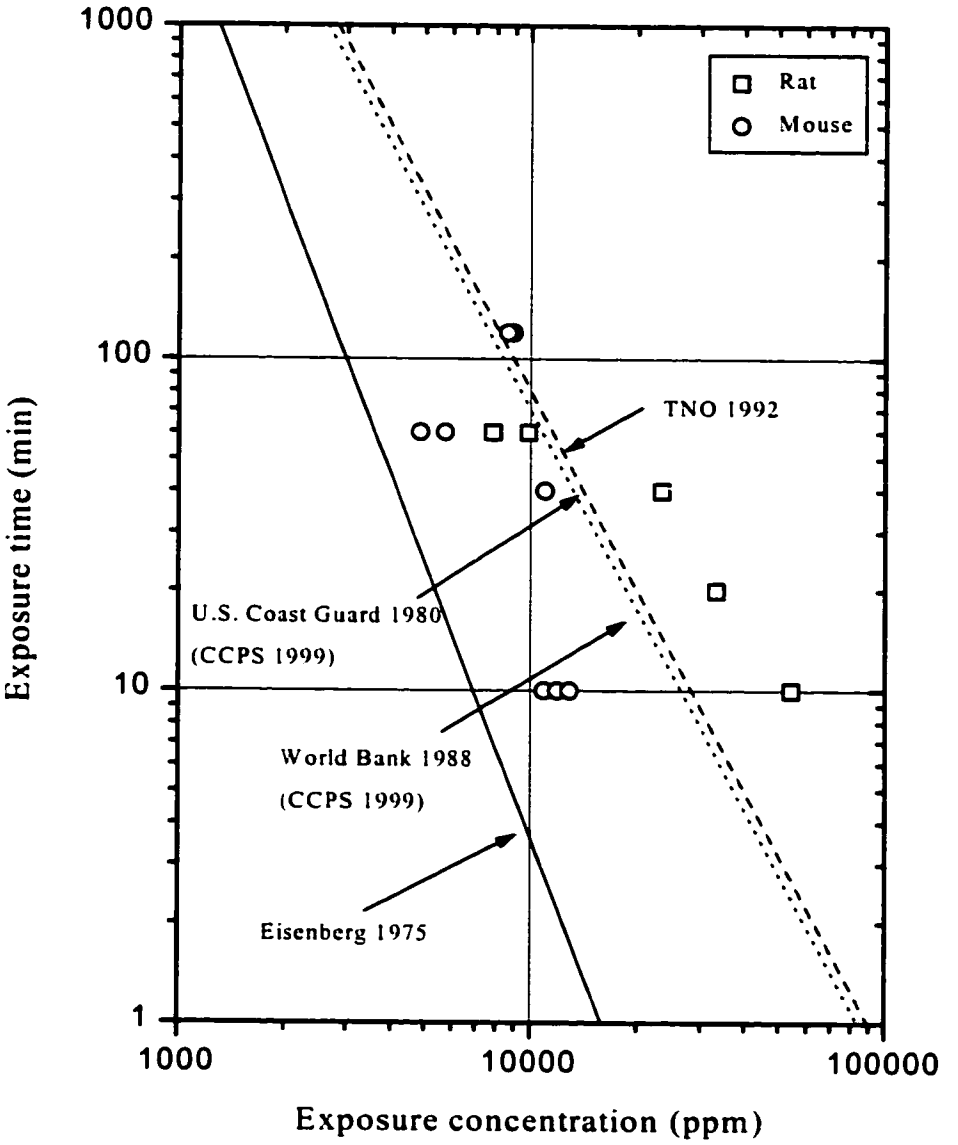


Figure 5.16 Comparison of different toxic load models for ammonia

Toxic load equations for 90% fatality applied in Figure 5.16:

Eisenberg et al. 1975	$C^{2.75}t = 3.6 \times 10^{11} ppm^{2.75} min$
U.S. Coast Guard 1980 (CCPS 1999)	$C^{2}t = 8.0 \times 10^{9} ppm^{2} min$
World Bank 1988 (CCPS 1999)	$C^{2}t = 7.0 \times 10^{9} ppm^{2} min$
TNO 1992	$C^{2}t = 8.0 \times 10^{9} ppm^{2} min$

Animal

Table 8 shows the ammonia concentration-time-mortality relationship in rats. The ratio of LC_{90} to LC_{10} for rats is about 1.6 indicating small intra-species variability in response to ammonia. Comparison of intra-species variability for large animals is not possible because of lack of available data

Table 5.8 Concentration-time-mortality relationships for NH_3 in rats

Species	Exposure time (min)	Concentration (mg/m^3)	Mortality (%)
Rats	10	20,950	0
		23,380	10
		26,410	60
		27,220	50
		37,820	90

Data from Appelman et al. 1982

5.6 Toxic Effects of Hydrogen Fluoride

5.6.1 Irritation

5.6.1.1 Human

Exposure to hydrogen fluoride mainly causes irritation of eyes and the nose at relatively low concentrations (38-62 ppm), and respiratory irritation at higher concentration (122 ppm) in two human volunteers (Machle et al. 1934). Table 5.9 lists the effects of various concentrations of hydrogen fluoride on human volunteers (Largent 1952).

Table 5.9 Effects of hydrogen fluoride in human volunteers

Concentration (ppm)	Effect
3	No local and immediate systemic effects;
10	Many subjects experienced discomfort;
30	All subjects complained and objected seriously to staying in the environment;
60	At brief exposures, definite irritation of conjunctiva, nasal passages, tickling and discomfort of pharynx and trachea;
120	The highest concentration tolerated less than 1 min by 2 male subjects), smarting of skin in addition to the above effects was noted.

From: Largent 1952

Data of human exposure to high concentrations of hydrogen fluoride are very limited in the literature. Most cases related to accidental exposure to hydrofluoric acid were accompanied by dermal exposure (Chan et al. 1987, Chela et al. 1989, Dieffenbacher and Thompson 1962, Tepperman 1980). The exposure concentrations were not provided in any of these reports. Acute inhalation of hydrogen fluoride following facial splashes with hydrofluoric acid can cause bronchiolar ulceration, pulmonary hemorrhage, pulmonary edema, and death (ATSDR 1993). Postmortem findings showed that tracheal and bronchial membranes were significantly injured and hemorrhagic in places; bronchi contained thick mucus with blood, and lungs were edematous, with hemorrhagic patches (Dieffenbacher and Thompson 1962, Braun et al. 1984).

5.6.1.2 Animal

At concentrations ranging from 29 – 9784 ppm for 5 minutes to 41 hours, rabbits and guinea pigs showed irritation of the respiratory tract and decrease in the respiratory rate which was especially noticeable in rabbits (Machle et al. 1934). The severity and duration of symptoms varied directly with increases in concentrations and time of exposure. Pathologic data indicated two types of characteristic changes of the respiratory tract: 1) those resulting from the chemical injury including hemorrhage, edema, emphysema, bronchitis and congestion, and 2) those changes resulting from secondary infection in the

injured areas with bronchopneumonia being the most common and rabbits being more susceptible than guinea pigs. Congestion of the liver and the kidneys has been also observed in some exposed animals, but changes in the lungs were most constant (Ronzani 1909, Machle et al. 1934).

A recent study used both mouth-breathing (MB) and nose-breathing (NB) patterns in rats revealed that damage caused by a high concentration of hydrogen fluoride are mainly limited in the nose of NB groups, while in MB groups the injuries extended to more distal regions (Dalbey and Bannister 1998, Dalbey et al. 1998).

5.6.1.3 Inter-species Variability

Available data indicate that hydrogen fluoride irritation of the respiratory tract is similar in animals and humans. They also showed consistency in basic pathological changes of the respiratory tract followed acute hydrogen fluoride poisoning between humans and animals. Difference in the breathing pattern between rodents and humans should be taken into consideration when comparing sensitivity of species.

5.6.2 Long-term Effects after Acute Exposure to Hydrogen Fluoride

The prognosis following hydrogen fluoride inhalation is generally believed to be poor. A study reported that a 38-year-old man with upper airway burns by hydrofluoric acid fumes had persistent symptoms such as hoarseness, coughing, pain in the nasopharynx, and severe nose bleeding during a one-year follow-up (Braun et al. 1984). There is not much information on the long-term effects following acute exposure to hydrogen fluoride in the literature.

Inhalation of high concentrations of gaseous fluorides can cause substantial damage to lung tissues ranging from modestly impaired pulmonary function to severe pulmonary edema with secondary infection (Braun et al. 1984, Dalbey and Bannister 1998, Dalbey et al. 1998, WHO 1984,). Based on this information, pulmonary edema caused by acute inhalation of hydrogen fluoride is the most serious effect leading to high mortality. Survivors may develop chronic pulmonary disease since the damage of small airways may not be completely repaired. Thus, "pulmonary edema" is suggested as a "serious and

irreversible" effect of acute hydrogen fluoride intoxication, but there is no strong supportive evidence for this suggestion.

5.6.3 Death

5.6.3.1 Overview

Accidental reports revealed that acute inhalation of hydrogen fluoride fumes in combination with dermal exposure to hydrofluoric acid can cause death in humans (Braun et al. 1984, Dieffenbacher and Tompson 1962, Gosselin et al. 1984, Kleinfeld 1965, Mayer and Guelich 1963, O'Neil 1994, Tepperman 1980). Actual exposure concentrations are not available in these reports. Death was generally caused by pulmonary edema resulted from irritation of the respiratory tract (Kleinfeld 1965), or cardiac arrythmias with hyperkalemia, hypocalcemia, and hypomagnesemia (Tepperman 1980).

The lethal concentration of hydrogen fluoride has been investigated in mice, rats, guinea pigs, rabbits, and monkeys.

5.6.3.2 Inter-species Variability

LC_{50}

Figure 5.17 provides a comparison of LC_{50} values for hydrogen fluoride in several species. This figure indicates that among animal species, mice appear to be most sensitive to lethal toxicity of hydrogen fluoride. Sensitivity of rats seems to be similar to that of monkeys. Guinea pigs are less sensitive than rats. The ratios of LC_{50} for rats to that for mice are about 2.5 to 3. The ratio of LC_{50} for monkeys to that for mice is about 3.5.

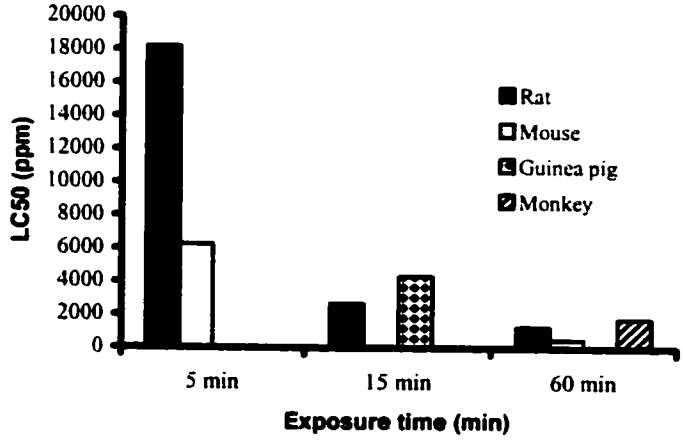


Figure 5.17 LC_{50} values for hydrogen fluoride in animals
(Data from Darmer et al. 1972, Higgins et al. 1972, Rosenholtz et al. 1963)

One very old study showed that rabbits were more resistant than guinea pigs to the inhalation exposure to hydrogen fluoride (Ronzani 1909). No experimental study has been found on dogs in regards to the lethal effects of hydrogen fluoride.

Lethal Toxic Load

Figure 5.18 to 5.20 provide graphic summaries of the lethal doses of hydrogen fluoride in different species. In these figures, the concentrations of hydrogen fluoride in air are plotted against exposure time on a log-log scale. The combination of these two variables, that is toxic load, produces different levels of fatality. Figure 5.18 shows the toxic load for 10% fatality (ranging from 1% to 20%), Figure 5.19 for 50% fatality (ranging from 40% to 60%) and Figure 5.20 for 90% fatality (ranging from 80% to 100%). All data points on the graphs are from animal experiments existing in the literature (See Table D in Appendix). Figures 5.18 to 5.20 show that mice are more sensitive to hydrogen fluoride than rats. Figure 5.20 also indicates that guinea pigs are more sensitive than mice and rats, while rabbits seem to be more resistant than mice.

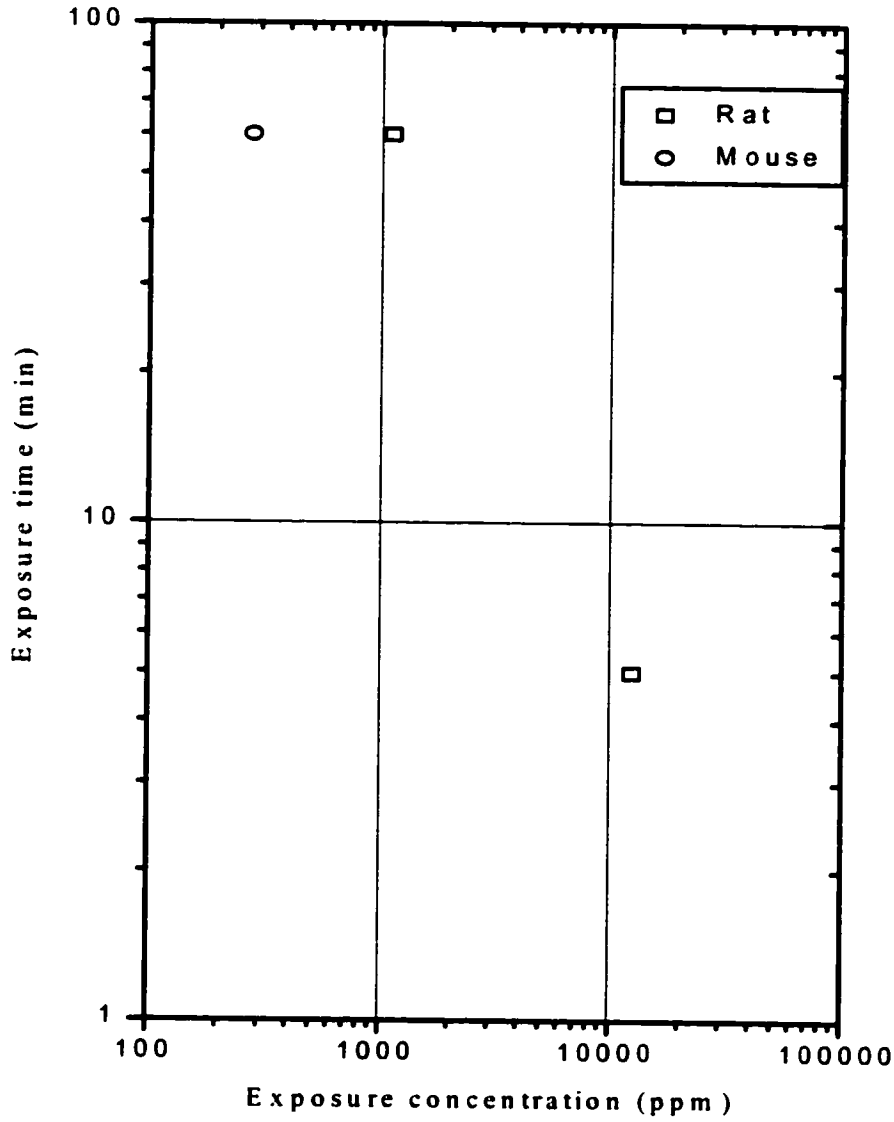


Figure 5.18 Lethal effect (10% fatality) of hydrogen fluoride

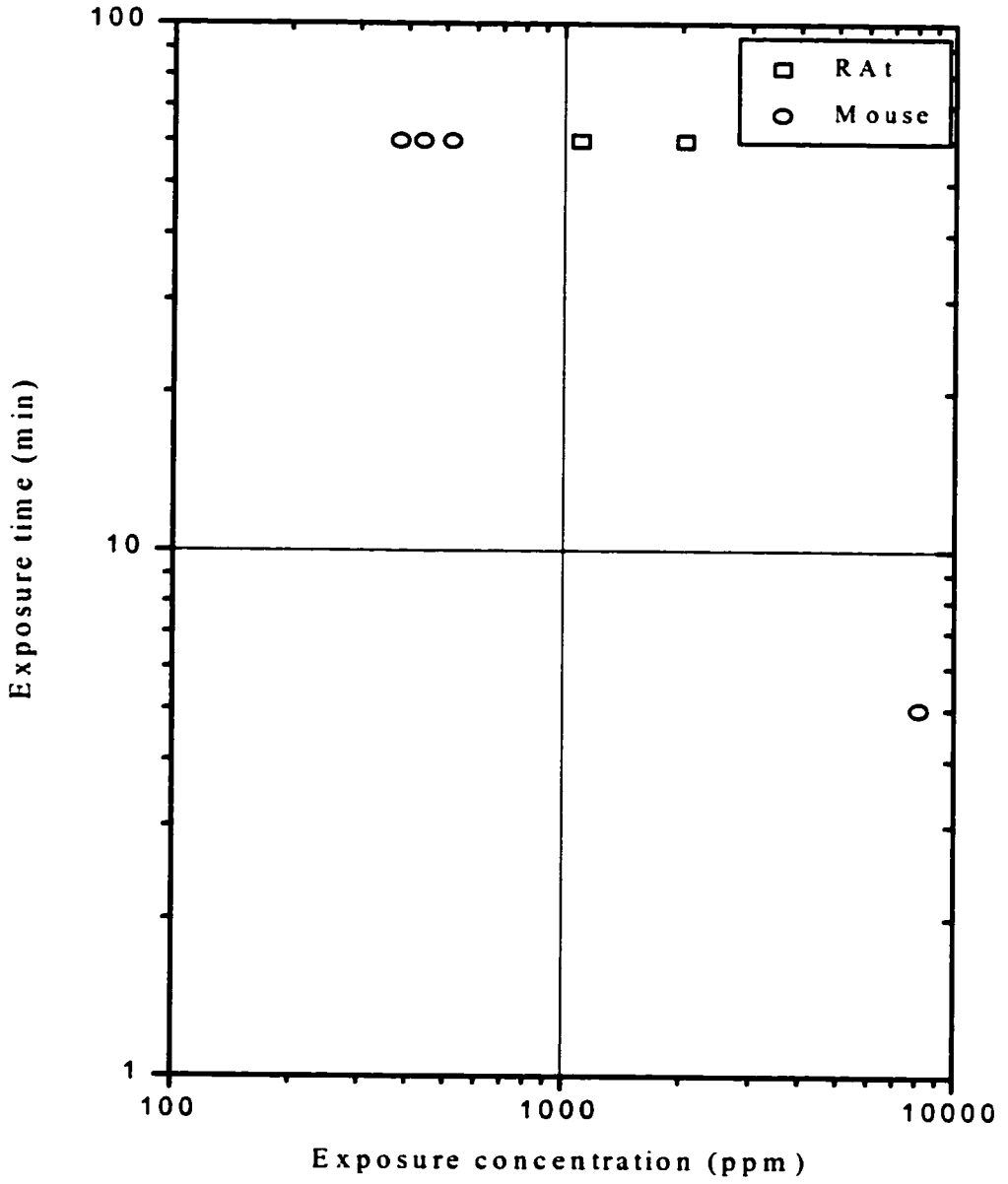


Figure 5.19 Lethal effect (50% fatality) of hydrogen fluoride

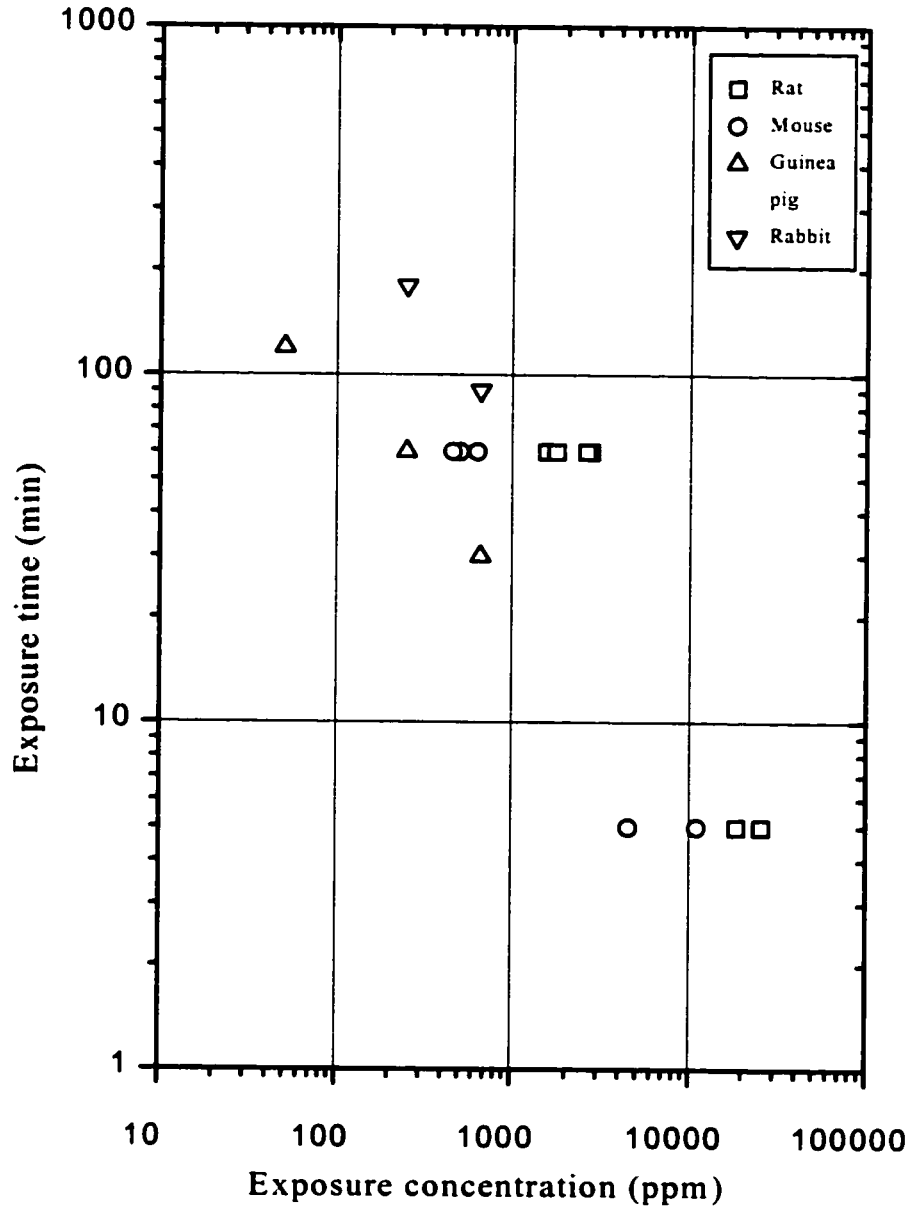


Figure 5.20 Lethal effect (90% fatality) of hydrogen fluoride

In order to compare different probit equations proposed for humans, three probit equations for 90% fetality of hydrogen fluoride proposed by TNO (1992), U.S Coast Guard (1980, cited by CCPS 1999), and World Bank (1988, cited by CCPS 1999) are plotted in Figure 5.21 on the background of Figure 5.20 As shown in Figure 5.21, Three

equation line are within the range of data points. Equations proposed by U.S Coast Guard (1980) and World Bank (1988) are very close and more conservative than TNO (1992).

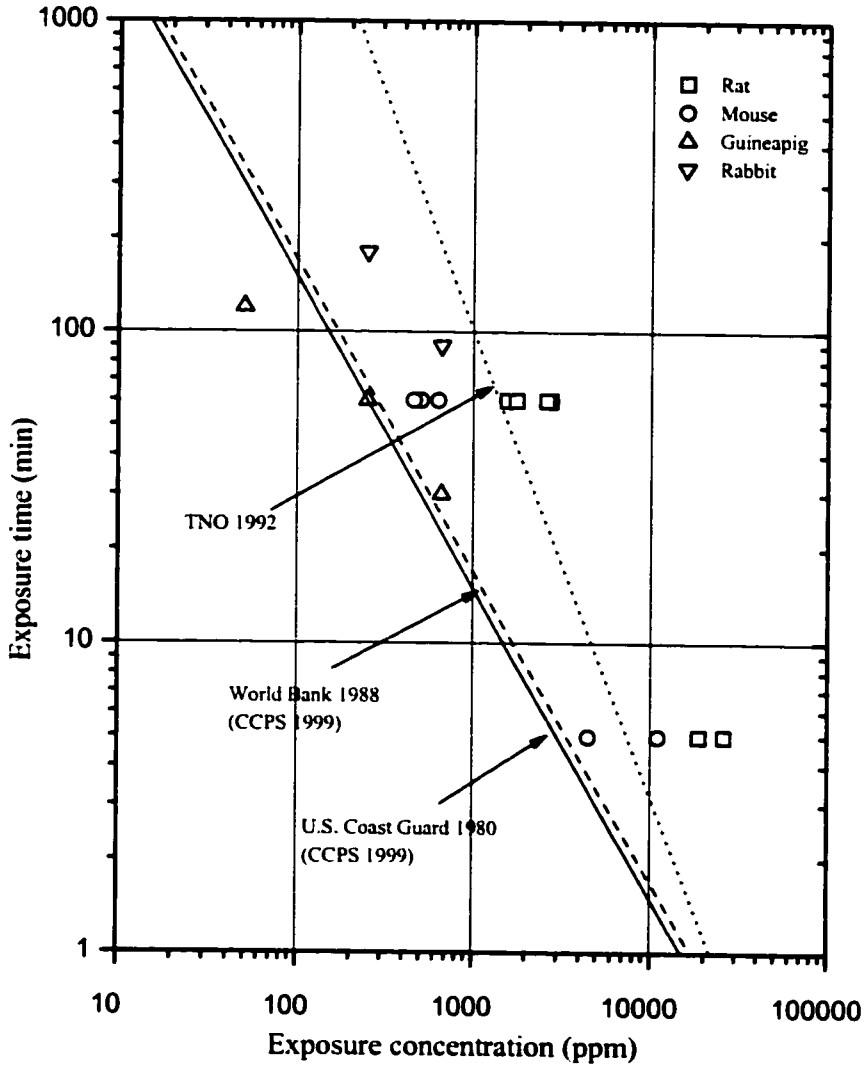


Figure 5.21 Comparison of different toxic load models for hydrogen fluoride

Toxic load equations for 90% fatality applied in Figure 5.21

TNO 1992	$C^{1.5}t = 3.2 \times 10^6$ ppm$^{1.5}$min
U.S. Coast Guard 1980 (CCPS 1999)	$Ct = 1.5 \times 10^4$ ppm min
World Bank 1988 (CCPS 1999)	$Ct = 1.5 \times 10^7$ ppm min

5.6.3.3 Intra-species Variability

Human

There are few data available for a comparison of human inter-individual differences in susceptibility to lethal effects of hydrogen fluoride. The large accidental release of hydrogen fluoride that occurred in 1987 in Texas City resulted in 1037 hospitalizations. However no one died in this serious accident (Selcraig 1992). Many lethal cases only involved a few individuals, mostly one individual.

Animal

Table 5.10 shows the HF concentration-time-mortality relationship in rats and mice. The ratio of LC_{90} to LC_{10} for rats and mice is about 1.5, which means the intra-species variability for both rats and mice is very small.

Table 5.10 Concentration-time-mortality relationships for HF in rats and mice

Species	Exposure time (min)	Concentration (ppm)	Mortality (%)
Rats	60	1087	0
		1108	20
		1406	30
		1567	80
		1765	100
Mice	60	263	0
		278	10
		325	70
		383	60
		458	90

Data from Wohlslagel et al. 1976

5.7 Summary

The principal characteristics of the acute toxicity of the four gases are similar in mammalian animals and humans. The major target site of toxicity of the four gases is the respiratory tract, while the nervous system is the major target site for the systemic effects of hydrogen sulfide. Irritation to the eyes and the respiratory tract is the primary action of these four irritants with pulmonary edema and secondary pneumonia being the most

severe effects. Results of acute toxicity of the four gases obtained in animals can be applied to humans with some justification and judgment.

Some studies have investigated the long-term effects after acute exposure to hydrogen sulfide, chlorine, and ammonia and provided supportive evidence of long-term sequelae following acute exposure to these three gases. The long-term effects include permanent brain damage, chronic pulmonary dysfunction and disorder, and permanent blindness. Accordingly, pulmonary edema and pneumonia are considered to be serious and irreversible effects for all four gases. In addition, loss of consciousness caused by hydrogen sulfide and severe eye injury caused by ammonia are also considered to be serious and irreversible effects.

The sensitivity to the acute toxicity of the four gases varies among animal species. Generally, mice appear to be most sensitive to all four gases among mammalian animals. Studies on hydrogen sulfide and chlorine suggested that human sensitivity was similar to that of dogs. Limited data also indicate that the susceptibility of rats is similar to dogs.

Information on human individual natural variability in sensitivity to these four gases is almost absent in the literature. Most data of human fatality come from reports of accidents in which the actual exposure conditions can only be estimated afterwards. Very young or old people, smokers, people with asthma or other preexisting cardiopulmonary diseases or lack of nutrition are generally believed to be more sensitive to these chemicals than the general population.

Chapter 6

Assessment of Human Variability in Odor Perception

6.1 Odor Perception

6.1.1 Olfactory System and Sensation of Smell

The human olfactory system is very complex and consists of the peripheral and the central parts. The peripheral olfactory system is called the olfactory epithelium, which is a yellow-tinged patch of nasal mucosa located at the top of each nasal cavity. Each olfactory epithelium contains about five million olfactory receptor cells, surrounded by columnar supporting cells. The olfactory receptor cells are bipolar neurons and each cell has some hairs on its tip. These hairs lie in the moist layer directly exposed to the air stream and respond to airborne gases and vapors (Marieb 1989). The complex structure of receptor proteins makes olfactory system extremely specific and sensitive. Because the location of olfactory epithelium is out of the main air stream, normally only 5% of inspired air passes through the olfactory area. However, this may be increased as much as 20% by sniffing which flushes more air across the olfactory epithelium (Qu 1999).

The central olfactory structure includes olfactory bulb and olfactory cortical areas. The olfactory bulb is a cortically organized structure with six concentric layers including glomerular layer and mitral cell layer (Kratskin 1995). Olfactory receptor cells are connected by a nerve fiber into the olfactory bulb of the brain. The olfactory bulb transfers information received from receptors to various central structures of the brain.

The process of olfaction is not fully understood. One proposed process is as follows: when air that contains odorants is inhaled through the nostrils, molecules of odorants move along the nasal passageways and stimulate olfactory receptors by producing specific bioelectric discharges. The information is transferred through the olfactory nerve to the reception center of the brain. After processing, the message is delivered to the evaluation center in the brain, and is compared with stored experience (the memory). The interpretation of the information is then relayed back along other nerve paths. Because the interpretation of sensory stimuli only exists in the brain, odor is purely an individual (subjective) response to odorants (Summer 1971).

6.1.2 Odor Measurement

Parameters used to describe odor sensation include threshold, intensity, character, and hedonic tone (ASHRAE 1997). Threshold and intensity reflect the quantitative aspect of odor while character and hedonic tone are qualitative descriptions of odor.

Threshold refers to the minimum concentration of an odorant that is necessary for perception in some specified percentage of the population. There are two types of thresholds: (1) the detection threshold, and (2) the recognition threshold. The *detection threshold* is defined as the lowest concentration of odorant at which the odor can be detected by a specified percentage (usually 50 percent or the median), of the population of a group being tested. The *recognition threshold* is the minimum concentration at which a characteristic odor quality can be recognized by a specified percentage of the population (usually 50 percent or the median) (AIHA 1989). On average, recognition of an odor requires about three to five times the detection threshold concentration (Hellmann and Small 1974).

It should be noted that a threshold value is not a fixed physiological or physical constant; it should be viewed as a statistical value representing the best estimate of response from a group of individual scores (Rafson 1998).

The odor detection threshold is measured by determining the mass concentration of a pure odorous substance or odor dilution-to-threshold ratio of mixtures of odorants, which represent environmental odors. The method used to measure odor threshold is called olfactometry consisting of a human panel and an olfactometer. Usually, a panel contains five to ten individuals with average odor sensitivity. Methods to determine odor threshold include single-sample methods and multiple-sample methods (Shustermann 1992).

When using single-sample methods, concentrations of odorants are presented in ascending, descending, or random order, and subjects are asked to respond whether an odor is present or not. Two major sources of bias exist within this method. First, the order of stimulus presentation may affect results; ascending series tend to produce an anticipatory response while descending series tend to produce olfactory fatigue and blunt perception at low concentration. Secondly, differences in subjects' 'decision criteria' may also affect results; subjects may tend to be either more conservative or more liberal in

reporting the presence of an odor under conditions of uncertainty (Doty and Kobal 1995, Shustermann 1992).

When using multi-sample methods, subjects are presented with multiple odor dilution ports (e.g., one with odorant and others without odorant) and are asked which port contains the odorant. This "forced-choice" procedure can provide more reliable results because of less variability determined by each subject's decision criteria.

Intensity is the relative strength of the odor above the detection threshold (suprathreshold). There are three ways to measure the magnitude of odor including magnitude estimation, category scales, and intensity matching (Doty and Kobal 1995).

Magnitude estimation is the most widely used psychological scale, a so-called ratio scale. This procedure requires subjects to assign numbers proportional to perceived magnitude. For example, an odor magnitude of 30 is three times as strong as magnitude of 10. Data from several observers are then normalized to a common reference point.

A category scale includes number category (0, 1, 2, 3, 4, 5, etc) and word category (none, threshold, slight, moderate, strong, etc). Numerical values on this scale do not reflect a ratio relationship among odor magnitudes. For example, a value of 10 does not mean a perceived magnitude twice as great as a value of 5. The limitations of category scaling are that there are a finite number of categories to choose from and they are open to bias through subjective number preferences or aversions.

Intensity matching for measuring odor intensity has been standardized as the American Society for Testing and Materials (ASTM) Standard Practice E 544 (ASTM 1993). This method proposes a standard reference odorant, 1-butanol, set in a series of known concentrations. A subject should choose one that matches most closely the intensity of an unknown odorant. This method has several advantages: (1) it allows comparison of subjective odor intensities between laboratories; (2) it allows for odor control regulations to be expressed in terms of perceived intensity rather than odor thresholds, (3) it allows cross-modality comparisons (i.e., sound and odor). One disadvantage is that some find it difficult to compare odors that have a different odor character from a reference standard (Rafson 1998).

Character defines the odors as similar to some familiar smell, for example, fishy, earthy, flowery, sour, etc. Odor character is also known as the odor quality which is useful in air pollution control to describe the source or process responsible for community odors because different odor characters are associated with various processes and industries.

Hedonic tone, also referred to as *acceptability* is a judgment of the relative pleasantness or unpleasantness of an odor. Hedonic judgments include both category judgments (pleasant, neutral, unpleasant) and a magnitude judgment (very unpleasant, slightly pleasant). Acceptability of the odor is based on the combination of frequency of occurrence, odor character, and odor intensity (Rafson 1998).

The characteristics of odor from the four gases investigated in this study are summarized in Table 6.1.

Table 6.1 Odor of the four gases

Chemical	Detection threshold (ppm)	Odor character	Hedonic tone
Hydrogen sulfide	0.0094[a]	Rotten eggs [a]	unpleasant
Chlorine	0.080 [a]	Suffocating, bleach [a]	unpleasant
Ammonia	17 [a]	Pungent, irritating [a]	unpleasant
Hydrogen fluoride	0.036 [b]	Sharp penetrating [b]	unpleasant

[a] AIHA 1989
[b] ATSDR 1993

6.1.3 Odor and Health Effect

An unpleasant odor persistently existing at industrial sites or the surrounding's environment can raise many health concerns. For the average person, a bad smell is likely to be judged as unhealthy unless the individual is informed by convincing evidence to the contrary (Rafson 1998). This phenomenon may partly contributes to the frequency of complaints about odor from the public.

From an environmental health point of view, professionals should be clearly aware of the relationship between odor and health effects. Many chemicals have an odor threshold far below the toxic concentration level. For example, hydrogen sulfide has an extremely

low detection threshold of 0.01 parts per billion (ppb) while its threshold limited value (TLV) for 8-hour exposure is 10 parts per million (ppm) (ACGIH 1993) - six orders of magnitude greater. Therefore, detection of the 'rotten egg' character of hydrogen sulfide does not necessarily indicate a potential adverse health effect. In contrast, some chemicals, such as vinyl chloride monomer, have an odor threshold that is far above their TLV (ACGIH 1993). Indeed, these types of chemicals that are very dangerous because there are no warning signs indicating their existence before a dangerous concentration level is reached.

Table 6.2 shows odor safety factors, the ratio of TLV to the odor threshold, for some chemicals. For chemicals with a ratio greater than one, such as hydrogen sulfide and chlorine, most people can detect odor and take action to avoid the hazard before chemical compounds reach their toxic level. Conversely, if the ratio is much less than one, such as that for ammonia and hydrogen fluoride, the detection of odor of that compound would indicate that a safe vapor concentration has already been exceeded making a dangerous scenario. As the ratio increases, the safety factors provided by the odor also increase (ASHRAE 1997).

Table 6.2 Odor thresholds and safety factors (ratio with TLV) of selected gases

Chemical	Odor threshold (mg/m^3)	Safety factor
Hydrogen sulfide*	0.007	2000
Chlorine	0.007	430
Hydrogen fluoride	2.7	0.9
Ammonia	33	0.5
Vinyl chloride monomer	1400	0.007
Carbon monoxide	infinite	0

* Some report lower odor thresholds for H_2S

On the other hand, even at very low concentration of chemicals, odor itself, especially originated from chemicals like methylmerceptan and hydrogen sulfide, can cause symptoms such as headache, sleep disturbance, lack of appetite, nausea (Georgieff and Turnovska 1999). There is still debate whether these symptoms are purely psycho-

emotional or have any physiological basis (Shusterman 1992, Ziem and Davidoff 1992). Current knowledge is far from providing satisfactory answers to this question. To deal with this issue, further investigation needs to be done on the measurement of biological changes when collecting evidence indicating symptoms caused by odor pollution.

6.2 Human Variability in Odor Threshold

6.2.1 Overview

Values of odor threshold for an odorant obtained by different investigators have been found to disagree (Leonardos et al. 1969, Amoore and Hautala1983). For example, the values of the detection threshold for hydrogen sulfide reported by different authors are between 0.0005 ppm and 0.13 ppm (WHO 1981). Two major sources are considered to account for this wide variation of odor threshold data: (1) the error in odor measurement, and (2) true human individual variability in odor perception.

From the introduction of odor measurement, it is not surprising that the diversity of procedures and techniques used to determine odor threshold could significantly influence the result of odor measurement. Factors such as the mode of presentation of the odorant to the observer, stimulus delivery system, sniffing style (e.g., strong or weak), the type of observer used, the definition of the odor response, the treatment of the data obtained, and the chemical purity of the odorant may contribute to the variation of odor threshold values from different sources (Leonardos 1969, Stevens et al. 1988). For example, Adams et al. (1968) found that detection thresholds for hydrogen sulfide are significantly lower for ascending orders of presentation than for descending, and both ascending and descending are significantly higher than the random presentation. However, these presentation factors may account for only a small fraction of the total variability (Stevens et al. 1988).

Human variability in sensitivity to odorants is believed to be the most important contributor to the variation in odor threshold. It is generally accepted that human odor sensitivity varies markedly from one person to another within the general population. Also, for a given individual, the odor sensitivity can differ greatly over a period of time. The ability of a person to detect a given odor is influenced by their innate variability of

olfactory acuity, their prior experience with that odor, age, sex, occupation, and health status (Amoore and Hautala 1983, Rabin and Cain 1986, Stevens et al. 1988).

6.2.2 Distribution of Odor Detection Threshold

6.2.2.1 Distribution Form

The odor threshold of a sample of human population is commonly observed to follow a normal (Amoore and Hautala 1983) or lognormal distribution (Adams et al. 1968, Brown et al. 1968). For example, Adams et al. (1968) found that the distribution of detection thresholds for hydrogen sulfide among 987 20-year old students was skewed toward the higher concentrations when plotted linearly but a logarithmic plot of the distribution appeared normal.

Figure 6.1 to Figure 6.3 provide a few examples of the lognormal distribution of odor threshold by using a probit plot as described in Chapter 3. Figure 6.2 to Figure 6.3 were obtained by replotting the raw data from a single study in which the thresholds for several different odorants were detected in the same sixty volunteers. A similar distribution form can be observed for different odorants. Figure 6.3 shows the probit plot for ammonia. Unfortunately, there are no raw data available for such plot for hydrogen sulfide, chlorine, and hydrogen fluoride. The data fitting a straight line in such plot indicates the lognormal distribution of odor detection threshold.

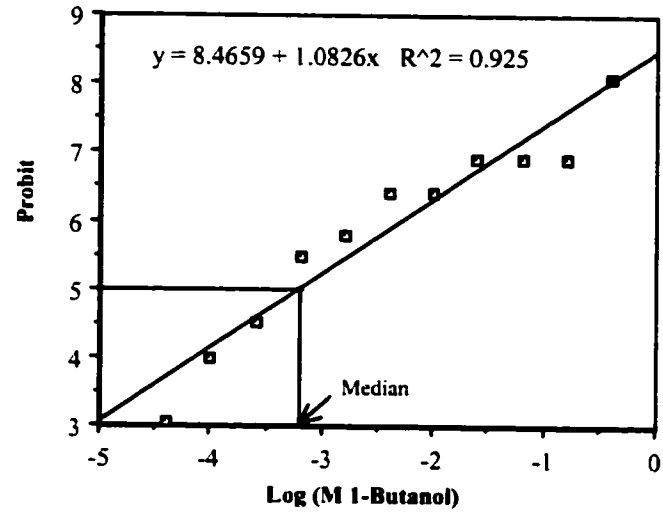


Figure 6.1 Log probit plot of the detection threshold for 1-butanol (Data from Hertz et al. 1975, Table 1)

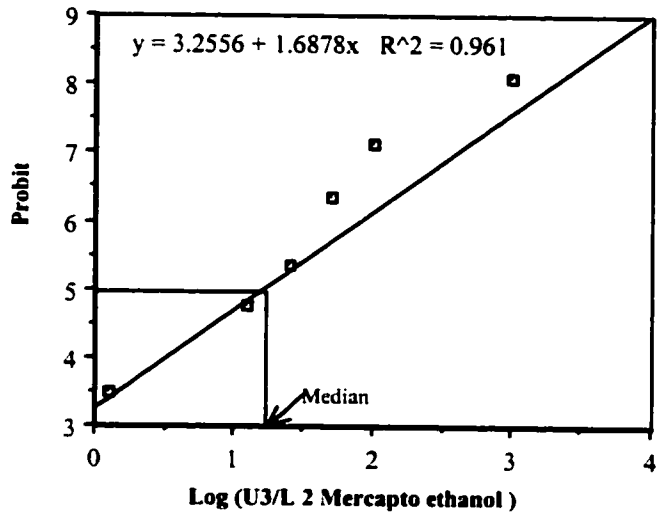


Figure 6.2 Log probit of the threshold for 2 Mercapto ethanol (Data from Brown et al. 1968, Table 1)

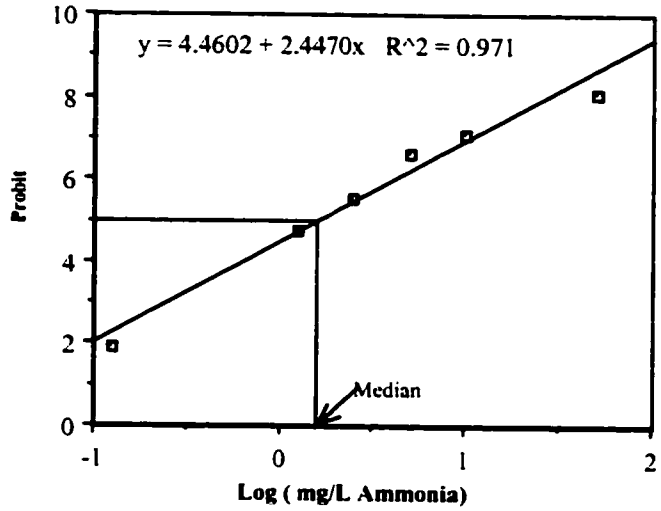


Figure 6.3 Log Probit plot of the threshold for ammonia (Data from Brown et al. 1968, Table 1)

6.2.2.2 Range of Inter-individual Variability

How big is the inter-individual variability in the human sense of smell? Different studies give different answers. Based on his own studies, Amoore (1980) suggested that about 96% of the population have sensitivities between 16 times and 1/16th of the mean (or median) threshold for an odorant; that is, within a range of 256 fold. However, larger ranges of odor threshold among subjects have been reported in the literature, and the extent of variation differs from one odorant to another (see Table 6.3).

Table 6.3 Range of odor threshold reported in the literature

Chemical	No of subject	Range of threshold	References
Iso-amyl butyrate	20	1:5	Stevens & Cain 1987
Benzaldehyde	20	1:300	Stevens & Cain 1987
Hydrogen sulfide*	789	1:400	Adams et al. 1968
D-limonene	20	1:4000	Stevens & Cain 1987
Skatole	1000	16 decades	Yoshida 1984

* The range of threshold was calculated for this thesis based on the data provided in this study.

Most studies of odor threshold detection have been conducted on selected and trained panelists. Usually, these selected panelists have average odor sensitivity; that is, they are not too sensitive or too insensitive to the odorant tested. Obviously, data obtained from such small groups are unable to reflect the real variability in odor sensitivity of the general population. However, studies intended to address the issue of human variability in odor sensitivity are very limited.

A few studies conducted on untrained subjects provide a basic description of human inter-individual variability in odor sensitivity by measuring the detection threshold of odorants (Brown et al. 1968). In Brown's study (1968), sixty volunteers were employed to detect odor threshold for eight different odorants. The ranges of odor thresholds for seven out of eight odorants were quite similar, from 1: 80,000 to 1: 250,000, average about 1:100,000. Yoshida (1984) provided another observation of odor thresholds for 10 different odorants on a large sample of 450 to 1000 "normal subjects" and showed wide variation of odor thresholds ranging from 8 decades (10^8) to 16 decades (10^{16}) for the different odorants. The latter extremes are well outside the range reported by any other researchers.

Some investigators doubt whether such huge variation only comes from inter-individual variability. Stevens et al. (1988) carried out a study on threshold detection for three odorants by three young subjects. Odor measurements were repeated different testing days and also at different times (morning and afternoon) within a day. The result showed significant variability in the threshold across sessions for a given subject, and the magnitude of variability for a given subject is comparable to the variability across subjects reported by other studies mentioned above. In this study, a subject's range of thresholds across sessions was typically ~2000-fold, and in one case closed to 10,000-fold. Furthermore, the within-day variability of the odor threshold was larger than that among days. In contrast, the mean thresholds of three subjects were almost uniform across subjects. The biggest difference in mean thresholds among these subjects was only a factor of 5. Stevens et al. (1988) therefore concluded that the fluctuation in sensitivity of a given subject from session to session is the major source of human variability in odor

threshold, and the variability in odor sensitivity across the population is not as significant as reported by the earlier studies.

Rabin and Cain (1986) conducted a similar study and they indicated that differences in odor threshold obtained by repeated measurements tend to be much smaller than that obtained by single brief testing because the latter will underestimate olfactory sensitivity and overestimate individual differences.

The variability in odor sensitivity from individual to individual, and from time to time for the same person may be caused by changes within the central nervous system, alteration in the sensitivity of the olfactory system itself or changing conditions within the nasal cavity. Nasal mucous membrane function including swelling, wetness and color of the membrane, and nasal obstruction can influence the odor sensitivity (Schneider and Wolf 1960).

6.2.3 Factors Affecting Human Odor Sensitivity

6.2.3.1 Age

The human sense of smell normally increases with age, reaches its peak at puberty, and then remains at its most sensitive for a further 30 years (Callan 1993). It is generally agreed that olfactory sensitivity declines with increasing age after 45 years. Anosmia, the total lack of olfactory perception, occurs in 5% of young people at 26, but increases with age, affecting about one-third of all people aged 78 (Summer 1971).

Several studies on the changes in olfactory abilities of humans at different ages have shown a significant decline in olfactory sensitivity as determined by measurements of thresholds to various odorants (Meisami 1994, Murphy 1983, Stevens and Cain 1987, Venstrom and Amoore 1968). Average thresholds are elevated in the elderly subjects when compared to that of young subjects. The magnitude of threshold rise differs from one odorant to another ranging from two-fold to nine-fold (Stevens and Cain 1987). Also, the inter-individual variability of odor sensitivity among elderly subjects is larger than that among young subjects, the latter show a relatively uniform distribution in odor sensitivity (Stevens and Cain 1987).

The olfactory structure including olfactory receptor cells, bulb, and tract changes with age. Significant decline in human bulb size with aging has been reported by some investigators (Smith 1942, Bhatnagar et al.1987) and confirmed by recent noninvasive magnetic resonance imaging (MRI) studies of the olfactory bulb and tract (Geckle et al. 1997). Meisami et al. (1999) have found that the number of mitral cells and glomeruli declined markedly with age (Table 6.4).

Table 6.4. Decline in the total number of mitral cells and glomeruli of the human olfactory bulbs with age

	Young adult	Middle age	Old age
No. of mitral cells	38,000	18,000	11,000
% of cell loss		51 %	70 %
No. of glomeruli	7,800	4,900	2,100
% of glomeruli loss		37%	74%

Data from Meisami et al. 1999

The progressive, life-long loss of fibers in the olfactory nerve and structures in bulb are considered to be responsible for the decline in odor sensitivity with the age (Meisami et al. 1999).

6.2.3.2 Sex

Although it seems to be a common belief that women generally have higher odor sensitivity than men, conclusions of different studies are conflicting. Some studies found a distinct difference in odor sensitivity in favor of females, whereas other investigators concluded that males were more sensitive (see Koelega and Köster 1974). Also, some studies have shown no sex difference in odor sensitivity (Venstrom and Amoore 1968, Cowart 1989).

The reasons for these controversial results may include small number of subjects in total or subjects in each age group, small number of stimulus intensities, inadequate procedures, and different methods (Koelega and Köster 1974). To address this issue, Koelega and Köster (1974) investigated the olfactory responses of different subject groups regarding both sex and age (sexual maturity vs sexual immaturity) to different

odorants (biological vs neutral). They found differences in sensitivity between men and women in favor of the women for most odorants. The sex difference was found most significant for odors that may be considered biologically meaningful, and such differences were not found in the group of prepubescent children and adolescents. Furthermore, the inter-individual variability in odor threshold is very similar between two sexes.

The sensitivity of women was found to vary during the menstrual cycle and reach a peak right before and during ovulation. Smell thresholds were significantly elevated during menstruation. In addition, postmenopausal women or younger women with ovaries removed for therapeutic reasons usually have poor olfactory sensitivity, and olfactory sensitivity can be significantly improved by estrogen replacement (Schneider 1974).

6.2.3.3 Occupation

Occupational exposure to certain chemicals in the workplace can evidently affect human olfactory sensitivity to those chemicals due to exposure-induced adaptation; which is a decrease in sensitivity to a stimulus resulting from prior exposure to that stimulus. This aspect will be discussing further below. Wysocki et al. (1997) reported a significant difference in acetone odor threshold between acetone-exposed workers and unexposed control subjects. The odor detection threshold for workers was twenty times of that for unexposed subjects. The study also indicates that exposure to acetone does not affect the sensitivity to another odorant, butanol.

6.2.3.4 Health Status

Temporary anosmia is a well-known characteristic of certain diseases. Recovery of the sense of smell may take a few days to several months (Summer 1971). For people suffering from a cold, swelling of the middle turbinate will cause a partial or complete blocking of the upper turbinate, resulting in a reduced ability to smell. A study on the effect of the common cold on olfactory function has shown the decrease of the volume of the anterior nasal cavity and an increase of odor threshold of butanol after the onset of the

acute rhinitis. The olfactory sensitivity increased as the subjects recovered (Hummel et al. 1999). Nasal allergy and other conditions can have the same results (Summer 1971).

6.2.3.5 Smoking

The effects of cigarette smoking on smell are not conclusive. Some studies reveal that compared to groups of non-smokers, odor detection thresholds were elevated in smokers for phenol (Joyner 1964), acetone (Kittel 1970), isobutyric acid, and 2-sec-butyl-cyclohexanone (Hubert et al. 1980). Conversely, some investigators found no effect of smoking on odor threshold for some other chemicals (Venstrom and Amoore 1968, Hubert et al. 1980). These controversial results might be explained by the selected effect of tobacco smoking, that is, the adverse effect occurs for odors perceptually similar to those of cigarette smoke (Moncrief 1968). In order to test this hypothesis, Berglund and Nordin (1992) examined the effect of formaldehyde, an important component in cigarette smoke, on odor detection threshold. The results indicate that odor detection thresholds for smokers are significantly higher than that of non-smokers.

The mechanisms by which smoking affects odor sensitivity remain unclear. The study by Berglund and Nordin (1992) suggests that the effects of smoking on sense of smell are more likely to be long-term rather than short-term effects. The possible biological explanation for these long-term effects is that the chemical components in cigarette smoke may have an adverse effect on the olfactory receptor cells or on the olfactory bulb. Another explanation is that the high CO-level in cigarette smoke may cause a degeneration of cells that are sensitive to an oxygen deficit (Berglund and Nordin 1992).

6.3 Variability in Suprathreshold Odor Intensity

6.3.1 Individual Variability in Psychophysical Function

Generally, perceived odor magnitude increases as a function of odorant concentration. The relationship between perceived odor magnitude and concentration was expressed by Stevens (1957) as a psychophysical power function:

$$S = k\,C^{n} \qquad (6.1)$$

Where S is perceived intensity of odor sensation, C is odorant concentration, n is exponent, and k is constant. When the above equation is expressed in logarithms,

$$\log S = \log k + n \log C \qquad (6.2)$$

then n is slope and k is y intercept which can be estimated by using linear regression techniques.

The value of exponent n varies from one odorant to another, but is usually less than one, ranging from less than 0.2 to about 0.7 (Berglund et al. 1971a, Cain 1969, 1970, Engen 1965, Hellman and Small 1974). A small exponent n is reflected by a flat slope on a log-log plot, whereas a steeper slope indicates a higher exponent n in the logarithmic form of the equation. The significance of a small exponent n is that a specified decrease in odor intensity requires a much greater decrease in odorant concentration compared to odorants with larger exponent n. This is shown in Table 6.5 with hypothetical numbers of exponent n.

Table 6.5 Different decrease level in concentrations required for decrease in intensity with different exponent n

Decrease in odor sensitivity	Required decrease in concentration of odorant			
	n = 0.13	n = 0.2	n = 0.5	n = 0.7
2-fold	156-fold	24-fold	4-fold	3-fold
5-fold	180,000-fold	2300-fold	25-fold	10-fold
10-fold	37,000,000-fold	760,000-fold	100-fold	27-fold

For some odorants, the values of exponent n agree well between studies, while other odorants have quite different values (Patte et al. 1975). The factors such as inter-individual variability in perceived odor intensity, odorant presentation procedures, ranges of concentrations examined, and diluents might account for these discrepancies (Doty 1975). A study by Berglund et al. (1971a) indicated that the size of the individual exponents for a given odorant varies significantly, ranging from 0.12 to 1.02 for acetone (0.54±0.32), for example. There are two possible explanations proposed for this inter-individual variation in the exponent of the psychophysical function. The inter-individual variability in perceived odor intensity may be caused by genuine perceptual differences or

by response bias related to the subjects' conception of numerical quantity. Individual differences have been suggested to be largely due to differences in handling numbers (Jones and Marcus 1961).

Differences in exponent n for various odorants have an important implication for malodor control in industrial practice. A low slope value indicates that the odor requires greater relative dilution for it to dissipate below detectable levels while a high slope value indicates that the odor can be reduced to non-detectable levels by less dilution. Compounds with low slope values include hydrogen sulfide, butyl acetate, and amines, whereas compounds with high slope values include ammonia and aldehydes (Rafson 1998). This explains why hydrogen sulfide, butyl acetate, and amines can be detected far away from the odor source, while ammonia and aldehydes can only be detected near the odor source. The exponent n for hydrogen sulfide was reported to be 0.13 (Patte et al. 1975). The relationship between decrease in intensity and required decrease in concentration for hydrogen sulfide shown in Table 6.5 indicates it will be extremely difficult to reduce odor intensity of hydrogen sulfide when considering its very flat slope. No values of exponent n for chlorine, ammonia, and hydrogen fluoride could be found in the literature.

6.3.2 Factors Affecting Suprathreshold Odor Intensity

Factors such as age, occupational exposure to odorants, health status, and smoking can also influence perceived odor intensity. For example, the decline of human sense of smell with advancing age can be reflected by significantly reduced suprathreshold intensity perception (Cowart 1989, Murphy 1983, Stevens and Cain 1987). Murphy (1983) found that the rate of growth of intensity of the odorant as a function of concentration was twice as great for young subjects as for elderly subjects. The median slope for the old group was half that for the young group, which means that a 10-fold increase in odorant concentration produced a four-fold increase in perceived intensity for young subjects and only a two-fold increase in perceived intensity for elderly subjects. There was also a correlation between odor threshold and perceived odor intensity. People with high odor thresholds also showed a low slope value for that odorant (Murphy 1983).

This indicates that the ability in both odor detection and perception of suprathreshold intensity declined in elderly people.

Regarding health status, acute rhinitis can result in decrease of perceived odor intensity (Hummel et al. 1999). Smoking can also affect odor intensity. A study by Berglund and Nordin (1992) found that smokers perceive formaldehyde odors as less intense than non-smokers. Moreover, there are no significant differences in intra- and inter-individual variability in magnitude estimates for perceived odor intensity.

6.4 Olfactory Adaptation

Olfactory sensitivity decreases over a period of time following stimulation of the sense of smell. This phenomenon is described as olfactory adaptation and is unique for the smell sense. There are two types of adaptation: (1) self-adaptation, and (2) cross-adaptation. Self-adaptation is a change in perceived intensity of an odorant after one has been exposed to it. In contrast, cross-adaptation is the decrease in sensitivity to one odorant after exposure to a different odorant. The assumption for cross-adaptation is that different odorants may involve the same olfactory receptors and thus adapt to each other (Engen 1982). Olfactory adaptation can result in increases in odor thresholds and decrease in perceived odor intensity (Cometto-Muñiz and Cain 1995).

6.4.1 Effects of Adaptation on Odor Thresholds

Olfactory adaptation has been usually investigated by measuring detection thresholds. The typical increase of the threshold with increasing time of constant exposure to stimulation indicates a decrease of intensity (Ekman et al. 1967). The extent of this effect mainly depends on two factors, adaptation time and concentration of the adapting stimulus. The increase in threshold with adaptation time is more significant with high concentrations. Thresholds increase rapidly at first, and then the rate of change decreases (Cometto-Muñiz and Cain 1995).

Berglund et al. (1971b) measured effects of adaptation on odor detection and response bias for hydrogen sulfide (H_2S) by using weak stimulus, i e., concentrations that are around classically measured thresholds for H_2S. The concentration affects the increase

of threshold in agreement with other studies, but adaptation duration has no effect on the change of thresholds. In addition, the response bias of subjects may account for the different results of a variety of studies.

6.4.2 Effect of Adaptation on Suprathreshold Odor Intensity

Many studies deal with the effects of concentration and duration of the adaptation stimulus on the time-course function during self-adaptation (Ekman et al. 1967, Cain 1974) and on the psychophysical function during self- and cross-adaptation (Cain 1970, Berglund et al. 1978). A study by Ekman et al. (1967) showed an exponential decay of perceived intensity of hydrogen sulfide (H_2S) for constant concentrations ranging from 0.7 to 6.4 ppm (see Figure 6.4). This figure shows that the rate of adaptation tended to decrease with increasing concentration. An asymptote about 30-40% of the initial perceived magnitude was reached within approximately 10 minutes of adaptation. Recovery was fast at first, but remained incomplete even 3- 4 minutes after cessation of adaptation. The results also indicated that the odor sensation did not disappear completely even after 15 minutes of stimulation.

Adaptation to odors takes place more rapidly during the initial stages of exposure. Cain (1974) compared rates of adaptation across the odorants propanol, ozone, eugenol, and butyl acetate. Perceived odor intensity declined exponentially similar to Ekman's work. Rate of decline varied little from one odorant to another, and tended to decrease with increasing odorant concentration. For weak stimuli, on the average, perceived magnitude of an odorant decays about 2.5% per second toward an asymptote that is about 30-40% of initial magnitude by about 4 minutes. For strong stimuli this asymptote is somewhat greater than 30%. Furthermore, the perceived magnitude does not decay to zero sensation level.

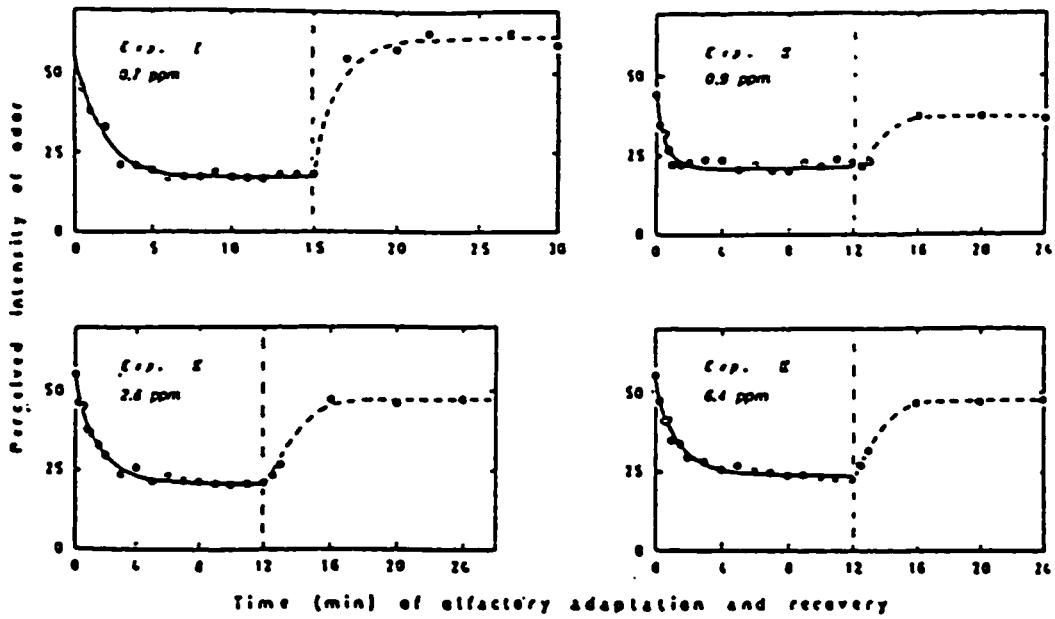


Figure 6.4 Group data describing adaptation and recovery to H_2S. The solid lines represent the exponential adaptation function fitted to these data. The dashed lines have been drawn to indicate the general trend of the recovery process. (From Ekman et al. 1967)

The olfactory adaptation has a significant implication in industrial practice. Workers exposed to an odorant for a long period may have significant increase of odor threshold and decrease in perceived odor intensity for that odorant compared to people who are never exposed to the same odorant. These trends lead to increased tolerance to exposure because the odor nuisance is reduced.

6.5 Summary

Among the four gases selected in this thesis, information of human inter-individual variability in odor perception is mostly available for hydrogen sulfide but not for the other three. Some general information on other odorants was discussed in this chapter to compensate the lack of required data.

Because the malodor of hydrogen sulfide is of high industrial and public concern, and hydrogen sulfide is a main focus of this analysis, the further discussion on odor of hydrogen sulfide is necessary to provide some insight for the developing a predictive model.

Based on the information provided above, knowledge of the nuisance odor of hydrogen sulfide can be summarized as:

(1) Hydrogen sulfide has an extremely low odor detection threshold at parts per billion and below levels;

(2) The range of human variability in detection thresholds for hydrogen sulfide is estimated to be about 400 fold;

(3) The relationship between perceived odor magnitude (S) and concentration (C) of hydrogen sulfide follows a psychophysical power function, that is

$$S = k C^{n} \qquad (6.1)$$

(4) The value of exponent n for hydrogen sulfide is reported to be 0.13. This very small exponent n indicates that very large decreases in concentration are required in order to reduce odor intensity even by 2- or 5- fold (see Table 6.5).

(5) The perceived odor intensity decays exponentially with time during self-adaptation for hydrogen sulfide. An asymptote of about 30-40% of the initial perceived magnitude was reached within approximately 10 min of adaptation. Thus, the relationship between perceived odor intensity and exposure time should be

$$S \propto 1/T^{m} \qquad (6.3)$$

Where S is perceived intensity, T is exposure time, and m is an exponent.

(6) It was also known that odor of hydrogen sulfide will completely disappear when concentration is above 150 ppm because of the olfactory paralysis (WHO 1981). Such exposure concentrations are clearly dangerous from a toxicity perspective and are no longer relevant as an odor nuisance.

(7) Combining the information described above, the relationship between perceived odor intensity, concentration, and exposure time can be proposed as:

$$S \propto \frac{aC^n}{bT^m} \quad (\text{when } C < 150 \text{ ppm}) \tag{6.4}$$

where S is perceived intensity;

C is concentration;

T is exposure duration;

a and b are constants;

m and n are exponents.

and $$S \rightarrow 0 (\text{when } C \geq 150\text{ppm, and } T = 2\text{-}15 \text{ min}) \tag{6.5}$$

because of odor paralysis at high concentrations of hydrogen sulfide.

Chapter 7
Discussion

7.1 Variability in Response to the Four Gases

In this analysis three different levels of responses to the four gases are chosen to deal with variability in response. These are: odor perception, non-fatal respiratory and neurological injuries, and death. All data regarding odor perception are based on human experiments; thus the human inter-individual variability is the only focus in the study of odor. In contrast, dealing with the other two more serious effects has to be dependent on both animal and human data. Accordingly, both inter-species and intra-species variability are investigated for non-fatal respiratory and neurological effects and fatality.

7.1.1 Variability in Odor Perception

An unpleasant odor emitted from an industrial site is often the source of public complaints. Thus, dealing with an odor problem is important even if it does not cause more serious toxic effects. Basic understanding of characteristics of odorants is required for the purpose of odor modeling and treatment. Although abundant information regarding odors of industrial chemicals exists in the literature, only a few studies have intentionally focused on the human inter-individual variability in odor perception. Furthermore, information on the four gases selected in this analysis is very scarce and scattered in the literature. This analysis made a first attempt to systematically describe and discuss inter-individual variability in odor perception of these four industrial gases.

The human sense of smell varies significantly; but the true basis for this variability is unclear. This analysis gathered most relevant data on odor threshold and odor intensity to describe human inter-individual variability in odor perception. Significant variation in the reported values of odor detection threshold for the four gases has been found in the literature, for example several orders of magnitude for hydrogen sulfide. In addition to several factors affecting this value, human inter-individual variability in odor perception is believed to be the major contributor for this huge variation.

The odor detection threshold has commonly been used to measure inter-individual variability in odor perception. Some studies demonstrated that population odor detection threshold follows a normal (AIHA 1989, Amoor 1980) or a lognormal (Adams et al. 1968, Brown et al. 1968) distribution. In the normal distribution, the relationship of the mean (μ) and the standard deviation (SD) is expressed in "addition and subtraction", that is, $\mu \pm$ SD. Accordingly, the inter-individual variability is relatively small. In contrast, in lognormal distribution, the relationship between geometric mean (GM) and geometric standard deviation (GSD) is expressed in "multiplication and division", that is GM×/÷GSD. Thus, this distribution indicates much larger inter-individual variability in the parameters measured. As discussed in Chapter 6, the inter-individual variability in odor detection threshold differs from one odorant to another, ranging from 1:5 to $1:10^{16}$ (see Table 6.3).

Hydrogen sulfide has commonly been used as a stimulus in odor measurements. The detection threshold of hydrogen sulfide follows a lognormal distribution and 1-99% range of detection threshold is about 1:400. This result comes from the study on 789 20-year-old students (Adams et al. 1968). Since human olfactory sensitivity declines after age of 45 years, the result of this experimental age group can be used to represent the variability of a healthy occupational population. For the general population including young children, old seniors, and people who are anosmic (unable to smell) or hyperosmic (very sensitive to smell), the inter-individual variability might be far greater.

Regarding the odor intensity of hydrogen sulfide, it is very important to know that above 100-150 ppm, odor will disappear because of olfactory paralysis. This condition removes a vital warning to workers exposed to potentially high concentrations of hydrogen sulfide and may cause prolonged exposure. The other characteristic of the odor of hydrogen sulfide is that it is very difficult to control its detectability because of its very flat slope in the concentration-odor intensity relationship. These two characteristics have to be taken into consideration in modeling and controlling odor problem with hydrogen sulfide.

Little information is available for chlorine, ammonia, and hydrogen fluoride in terms of individual variability in odor detection.

7.1.2 Variability in Non-lethal Injury

One difficult part in comparing inter- and intra-species differences in non-lethal injuries is to select endpoints. Because the four gases are local irritants and hydrogen sulfide also acts on the neurological system, respiratory irritation and neurological effects have been chosen in this analysis. Because of the lack of quantitative data, comparisons were mainly made from qualitative aspects. The purpose of this comparison is to clarify whether the action of the four gases and the mechanisms of action are similar in animals and humans. According to the findings in this thesis, symptoms and pathological changes following sublethal exposure to the four gases are similar in the common laboratory animals and humans. This similarity provides an opportunity to apply animal data to humans with some confidence in their relevance.

Regarding the sensitivity of animal species and humans to the non-lethal injuries, some very old studies made substantial contributions. Among them, Lehmann's works on hydrogen sulfide, chlorine and ammonia (1887, 1892, 1893, 1899), although done a century ago, are still widely quoted in the literature. His studies usually involved both large animals and humans and thus made it possible to directly compare sensitivity of animals and humans. For example, he observed that the sensitivity of dogs and humans to hydrogen sulfide were similar at non-lethal levels under the same exposure condition, and thus he postulated that the sensitivity to lethal effects might also be similar in dogs and humans. Mitchell and Yant (1925) also directly compared the sensitivity of humans and several animal species to hydrogen sulfide. The concentration-time-mortality relationship of hydrogen sulfide for humans has been established on the combination of their observations (see Table 5.4). These old studies are still of great value in risk assessment because these are the only studies that contained information on various animal species, especially large animals like dogs, and humans. The absolute measurements of exposure variables such as exposure concentrations in these studies have to be used with caution because of the limitations in experimental conditions so long ago.

Based on the current knowledge, it is not possible to develop a probit equation for non-lethal injury, although many attempts have been made in the past.

7.1.3 Variability in Fatality

Death is a definite quantal response to the lethal dose of toxic gases. Thus it is commonly used as an endpoint to compare inter- and intra-species variability in response. Numerous human fatality reports regarding accidental exposure to the large amounts of the four gases exist in the literature. However, all of these reports failed to provide actual ambient concentrations of released gases. Several studies made an attempt to measure concentrations after exposure, but most of them have been done several days to two weeks later when the weather conditions might be significantly changed. Only two studies measured concentrations several hours after accidental release (Hsu et al. 1987, Joyner and Durel 1962). These are of greater value. In these two studies, the hydrogen sulfide concentration was 429 ppm four hours after the accident (Hsu et al. 1987), and the chlorine concentration was 10 ppm in the fringe of the release center three hours and 400 ppm near the release center seven hours after spill. These concentrations might be close to the lower bound of actual concentrations during the exposure if atmospheric conditions were extremely stable (no wind, inversion conditions). Otherwise they would substantially underestimate concentrations unless the source was still releasing the toxic gas at rates similar to the accidental conditions.

Human accident reports have not been able to provide sufficient data for a quantitative description of the concentration-time-mortality relationship. Animal experimental data must be used to compensate for the lack of human data. LC_{50}, a measure of lethal effect, is commonly used to compare inter-species sensitivity.

In the literature, there is a considerable variation in reported LC_{50} values for the same species (Table 7.1). As shown in Table 7.1, the difference of LC_{50} values ranges from 1 to 8-fold among different sources. Because LC_{50} is a key value for deriving toxic load relationship, the diversity of reported LC_{50} values makes it difficult to select a key study.

Sources for the variation in reported results may include real biological variability in susceptibility, different exposure system, different experimental condition, and different observation periods. As introduced earlier in Chapter 3, different gas delivery systems and exposure chambers may affect experimental results. Static gas delivery system and small whole-body chamber, often used in studies that have been done before 1940, can result in significant decline of exposure concentration during experiments and thus

produce higher LC_{50} estimates. After 1940, most studies used dynamic gas delivery system and large exposure chamber (see Table 7.1). The concentrations in exposure chambers are usually continuously monitored. Different sampling apparatus can also introduce variation in the reported values. For example, the 1-hour LC_{50} value for hydrogen fluoride reported by Haskell Laboratory (1990) is 2-fold higher than other studies (Table 7.1). One possible explanation for this difference is that this study used Teflon®-lined impingers to collect air sample, while other studies used glass impingers. The use of glass impingers underestimated the hydrogen fluoride concentration by about 26% due to reaction with SiO_2 groups in glass (Haskell Laboratory 1990). With Teflon®-lined impingers there was little loss of fluoride. Thus, the 1-hour LC_{50} value for hydrogen fluoride reported by Haskell Laboratory (1990) is considered to be more accurate than other values.

Table 7.1 Compilation of LC_{50} values for the four gases

Gas	Species (No)	Exposure time (min)	LC_{50} (ppm)	Gas delivery	Exposure chamber	Observation period (d)	Reference
H_2S	Rat (144)	240	501	dynamic	whole-body	14	Prior et al. 1988
	Rat (80)	240	444			14	Tansy et al. 1981
Cl_2	Mouse (80)	30	127				Schlagbauer & Henschler 1967
	Mouse (50)	30	463	dynamic	whole-body	14	Zwart et al. 1988
NH_3	Rat	60	7340	dynamic	whole-body	14	MacEwan & Vernot 1972
	Rat	60	11300	unknown	unknown	14	Prokop'eva 1973
	Rat (50)	60	16600	dynamic	whole-body	14	Appelmann et al. 1982
HF	Rat	60	1307	dynamic	whole-body	14	Rosenholtz et al. 1963
	Rat (50)	60	1395	dynamic	whole-body	14	Wohlslagel et al. 1976
	Rat (40)	60	2240	dynamic	head-only	14	Haskell Laboratory 1990

There is a question about how to measure or how to express the difference in sensitivity to the toxic gases. The origin of 100-fold safety factor comes from the investigations on food additives (Lehman 1954, Lu 1988), in which only the dose (e.g.,

20 mg/kg) administrated is considered in the uncertainty factor. For example, if toxic dose is 10 mg/kg for rats and we expect a toxic dose of 1 mg/kg for humans, we can simply put an uncertainty factor of 10 to account for this difference. The comparison of species sensitivity for toxic gases and vapors is complicated by the three dimensional non-linear relationship between exposure concentration, time, and response. As discussed in Chapter 2, three concepts, namely LC_{50}, Lt_{50}, and toxic load can be used to represent "dosage" of toxic gases. The value of LC_{50} is most commonly available in acute toxicity studies, while only a few studies provided values of Lt_{50}. Studies designed to obtain LC_{50} usually contain a range of exposure time, e.g., 10 min, 30 min, 60 min or 4 hr, which makes it possible to compare LC_{50} for different species from different studies if the exposure time is stated. In contrast, there is no common concentration used for the purpose of obtaining Lt_{50} and this parameter is not commonly reported. Thus, the difference in LC_{50} values between species is convenient to use to measure species sensitivity and is easy to understand. Theoretically, difference in toxic load is a more appropriate measure of species sensitivity because of its characteristic of integrating exposure concentration, time, and response. However, in practice, there is not much opportunity to compare toxic load of different species because of the lack of data or at least the lack of full reporting of data. Therefore, ratio of LC_{50} values of different species is the most pragmatic measure of species sensitivity. The uncertainty factor obtained by the comparison of LC values should be applied to the concentration in the context of toxic load provided that there are not vastly different exposure time involved.

From the comparison of LC_{50} values provided in Chapter 5, inter-species difference in sensitivity to the four gases ranges from 1 to 4-fold (Table 7.2). Mice appear to be the most sensitive species for chlorine, ammonia and hydrogen fluoride. One exception is that mice seem to be slightly less sensitive than rats to hydrogen sulfide. The sensitivity of rats and dogs are similar in most studies. A few studies on monkeys showed similar sensitivity as dogs. The difference between small animals (rats) and large animals (dogs and monkeys) seems to be similar to that between small animals (rats, mice, and guinea pigs). On average, 3-fold might reasonably represent inter-species variability based on a comparison of the available and somewhat reliable LC_{50} values.

Table 7.2 Inter-species variability in susceptibility to the four gases

Chemical	Ratio of LC_{50} values					
	Rat/mouse	Dog/mouse	Monkey/mouse	Dog/rat	Rabbit/cat/rat	Guinea pig/rat
H_2S	1 - 0.8	–	–	–	–	–
Cl_2	1.4 - 3.0	1.4	–	1	–	–
NH_3	1.4 - 4	–	–	–	1	–
HF	2.6 - 3	–	3.5	–	–	1.6

Intra-species variability in sensitivity calculated by ratio of LC_{90}/LC_{10} is about 1.5 fold in rats and mice, and 5-10 fold in dogs. Obviously, inter-individual variability in dogs is greater than rats and mice in part because experimental rats and mice are usually specific strains bred for experimental purposes whereas dogs used in research are more genetically diverse. It is generally believed that human inter-individual variability in response should be greater than animals. TNO (1992) suggested a ratio of LC95/LC5 should be 27-fold for humans. Taken all these factors into account, a maximum 30-fold uncertainty factor is suggested to represent this large variability.

There is no human experimental study that can confirm this estimate of human variability in lethal effects. For hydrogen sulfide the vulnerable populations have been suggested to include (a) growing infants; (b) alcoholics; (c) individuals with eye or respiratory tract problems; (d) individuals with certain cardiac conditions; (e) individuals with neurological or psychiatric disorders; (f) individuals with haematological disorders (Greenhill 1978, Illinois 1974). For all four gases, very young children, very old people, and individuals with cardiopulmonary diseases are considered to be susceptible populations.

Eisenberg et al. (1975) suggested that chlorine concentration causing 3% and 50% fatality in general population would cause 50% and 100% fatality in vulnerable population. This is purely an estimate without any confirmating evidence. Abundant reports of accidents contain little information with regard to the vulnerable population. One valuable study (Joyner and Durel 1962) provided actual measurement of chlorine concentrations (although 3 hours after accidental release) and reported a 1% fatality rate (one death of an infant out of 100 affected people). Although the extent of exposure was

not likely to be the same for each individual, this fatality rate suggests the possibility of a significant inter-individual variability in susceptibility to chlorine.

7.2 Possible Explanations for Biological Variability in Response

The reasons for the inter-species variability in response to the four gases selected in this study may include differences in breathing pattern, minute volume per unit bodyweight or per unit area of the lungs, and susceptibility of different parts of the respiratory tract to the four gases.

7.2.1 Nose- vs Mouth-breathing

Rodents are obligate nose breathers. This characteristic can prevent toxic gases reaching deeper structures of lungs, on one hand. On the other hand, when exposed to highly water-soluble substances such as ammonia, it can cause complete blockage of the upper respiratory tract and thus result in immediate death due to lack of oxygen. Humans and dogs can breath through both nose and mouth; this allows more toxic gases to bypass the "scrubber" effect of the upper airways and enter the lower respiratory tract. From this point of view, humans and dogs may receive more damage in the lower respiratory tract by breathing through the mouth.

7.2.2 Breath-in Dose

When exposed to the same ambient concentration of toxic gases for a specified duration, the actual breath-in dose differs from one species to another. As shown in Figure 4.2, the minute volume per unit bodyweight in mice is the highest among different species, and about 10 times higher than humans. The minute volume per unit area of the lungs in rats and mice are also higher than humans. This means, rats and mice may receive toxic gases much more than humans relative to their body size. This can partly explain why mice appear to be the most sensitive species to the four gases.

7.2.3 Susceptibility of the Respiratory Tract

To some chemicals, different parts of the respiratory tract have different susceptibility. For example, the lower respiratory system is less susceptible to ammonia

than the upper airways. It has been reported that LC_{50} for 10 minutes is 4,110 ppm in normal mice and 12,600 ppm in mice breathing through a tracheal cannula (IchemE Working Party 1988). This may be due to the blockage of the upper airways as mentioned above. In addition, the respiratory bronchioles are absent in mice, thus the lower respiratory tract may be less susceptible to ammonia. Similar information has not been found in the literature for hydrogen sulfide, chlorine, and hydrogen fluoride.

7.3 Toxicokinetics

Information on the toxickinetics of the four gases is very limited. The principal processes of absorption, distribution, metabolism and excretion are known to be similar in animals and humans. This means the underlying mechanisms of toxicity of the four gases may be similar in animals and humans. Therefore, animal data can be applied to humans with some adjustments based on informed judgements.

One consideration to deal with concentration fluctuation in toxic load model is to integrate some parameters representing absorption and elimination processes into the model (Hilderman et al. 1999). However, toxicokinetic studies of the four gases only provide qualitative descriptions of processes involving absorption, distribution, metabolism and excretion. There are no data available for the rate of absorption or excretion. One paper reported that the biological half-life for the four gases are all less than 20 minutes, but did not provide the source of this number (Saltzman 1988). Under this situation, it is difficult to provide a reasonable estimate of the biological parameters to improve toxic load model.

7.4 Human Actions

When applying animal data to humans, in addition to the inter- and intra-species variability, two other aspects have to be considered.

One aspect is the difference in the pattern of response between humans and experimental animals. During experiments, animals have to be passively exposed to the supplied toxicants. Thus the exposure concentration and duration are well defined. However, in real accidents, humans may take some actions to avoid exposure. For example, people may stay indoors where concentrations of toxic gases or vapors are

much lower than outdoors, or people may try to escape the exposure. In either case, human fatalities are expected to be less than experimental animals under the same exposure conditions.

The other aspect is medical intervention that might be involved in the human exposure, which is especially important for delayed death. In animal experiments, animals stay in exposure chambers until the experiments finish. If they do not die during the experiments, they are usually further observed for at least 14 days without any medical treatment. However, in the real world, when an accidental release occurs, especially when the massive exposure in the work place involves many casualties, medical intervention usually takes place as quickly as possible after exposure. For example, unconscious victims will be removed from the toxic atmosphere resulting in regaining consciousness. Patients with pulmonary edema will receive intensive medical treatment. The prognosis will be completely different with or without medical intervention. In other words, even though concentrations and duration of exposure are the same, the fatality rate of humans after accidental exposure should be less than that of experimental animals if effective medical interventions are pursued.

Two reports of accidental exposure to hydrogen sulfide of oil and gas industrial workers revealed the decrease in fatality rate from 6.0% to 2.8% after one decade which was attributed to improved first-aid training and increased awareness of the danger of hydrogen sulfide (Arnold et al. 1985, Burnett et al. 1977). These estimates do not allow separation of accident rates from intervention but they do suggest some possible benefits from human actions.

Medical intervention is of extreme importance in reducing mortality followed by exposure to high concentrations. Exposure to high concentrations of ammonia can cause complete blockage of the upper airway due to laryngeal edema or spasm and thus rapid death. However, emergency intubation or tracheotomy can produce dramatic change of the life-threatening conditions, and the prognosis will be completely different with good recovery and few long-term sequelae. Therefore, accidental exposure occurring close to hospitals or other medical facilities may cause considerably fewer deaths than where medical intervention is not available. The difference between these two conditions is a reality to consider in judging the likely consequences of a toxic gas release.

7.5 Toxic Load Model and Probit Equation

Although several probit equations have been proposed for humans by different authors (see Table 2.5), there is no consistent procedure regarding how to derive probit equation as discussed in Chapter 2. When the basic required data are available, the probit equation is derived for a specific substance. The Netherlands Organization of Applied Scientific Research (TNO 1992) proposed a systematic approach to derive probit equations for the toxic substances where little data are available. Generally, three major steps in this process include (1) obtaining LC_{50} for human, (2) deriving exponent n, and (3) setting coefficients a, and b. Since most basically required data are available for the four gases selected in this thesis, the probit equations can be derived on the case-specific basis.

7.5.1 Estimating an LC_{50} for Humans

7.5.1.1 Criteria for Choosing Key Study

Up to now, there are no agreed criteria for selecting a key study to derive a LC_{50} for humans. Therefore, for the same chemical, different animal studies have been chosen by different authors according to available data and their own considerations. This analysis proposes the following criteria to select the key study.

(1) Suitable Animal Species Employed in the Experiments

The first consideration in using animal data is to determine the ideal animal model for humans. From animal studies conducted in different species, mice appear to be the most susceptible species to the four gases. Mice are not considered to be a good animal model for humans because of significant difference in the breathing pattern, structure of the respiratory tract and minute volume per unit bodyweight between mice and humans.

Studies on the toxicity of hydrogen sulfide and chlorine revealed that the sensitivity of dogs is similar to humans. This is understandable because of the similarity in the respiratory anatomy and physiology between dogs and humans. Generally, for the study of effects of irritants on the respiratory tract, dogs may offer more advantages because the anatomy of the respiratory tract of dogs resembles that of humans closely, and the conditions of pulmonary infection and the reaction of the lungs to bacteria and other

injuries are much the same as in humans (Ireland 1926, cited by Withers and Lees 1985a). Therefore, data obtained from dogs are likely of greater value to determine LC_{50} for humans.

There is no study on ammonia and hydrogen fluoride that provides the comparison of sensitivity of animals and humans to these two gases. No studies have been done on dogs for ammonia and hydrogen fluoride. Only one LC_{50} value on monkeys has been located for hydrogen fluoride.

The sensitivity of rats is similar to dogs for chlorine, and to monkeys for hydrogen fluoride according to the comparison of LC_{50} values. The respiratory anatomy and physiology of rats seems to be close to mice but not to dogs and monkeys. However the difference between mice and rats are even greater than that between rats and dogs or monkeys. One explanation is that the results have been obtained in different studies. But for hydrogen fluoride, the LC_{50} values for rats and monkeys for 60 minutes came from one single study (Darmer et al. 1972). Thus, for some unknown reasons, the sensitivity of rats may truly be similar to dogs and monkeys. This fact brings more confidence when applying data from rats to humans.

(2) Reliable Experimental Quality

The quality of animal experiments is extremely importance when results have to be extrapolated to humans. Some differences have been noticed when comparing old studies (before 1940) to recent ones. First, sample size was smaller in older studies. For example, Lehmann (1892) usually used one or two animals for one experiment. In contrast, recent well-designed studies usually use 10-20 animals per group for certain exposure concentration and duration. Secondly, the exposure systems are different. Most old studies used small whole-body exposure chambers and static exposure system. The big disadvantage of this exposure system is that the desired concentrations cannot be maintained during experiments. Concentrations decreased considerably due to absorption of the gas by animal hair, excreta, and chamber walls, especially when the exposure duration was longer than 1 hour. This is one reason for the higher values of lethal or sublethal concentrations reported in older studies. For example, in Lehmann's work (1887), cats that are believed to be extremely sensitive to chlorine died at a concentration

of 2,800-6,300 ppm for one hour (2,800 ppm for 30 minutes and followed by 6,300 ppm for 30 minutes). Guinea pigs were even more resistant dying after exposure to 8,100 ppm for 50 minutes. Such high lethal concentrations are obviously not comparable with other studies.

Recent animal studies usually use head- or nose-only exposure chambers and dynamic exposure systems with continuous monitoring of actual concentrations. Therefore, desired concentrations are well maintained during experiments and more accurate data can be obtained from animal studies.

(3) Realistic Duration of Exposure

In animal experiments, animals have to stay in the exposure chambers during the whole experiment to passively receive toxic gases or vapors. However, in a real situation when accidental release of toxic gas occurs, humans certainly will not passively stay in the toxic atmosphere but will try to escape or be removed from release center. This can be confirmed by the large number of accident reports in which most exposure durations were less than 30 minutes. Thus, longer exposure time (a few hours) applied in the animal experiments do not resemble realistic exposures. Exposure duration of 10 minutes has been previously selected by US EPA as a more general approach to accidental release of a compressed gas (EPA 1987, 1996). Therefore, animal studies with shorter exposure times (10-60 minutes) are thought to be more realistic for determining human LC_{50} for accidental exposures.

(4) Available Selection of a Key Study

Unfortunately, there is no single study that meets all of the above criteria according to the literature search. In regard with animal species, the reality is that before 1940 animal experiments often used large animals, while since then small animals such as rodents are almost universally employed in the toxicological studies because they are inexpensive, rich in supply, and easy to handle (Barrow 1986, panel discussion). For the four gases selected in this analysis, almost all well designed animal experiments have been conducted on the rodents. Under this situation, available data becomes the first

consideration. Based on available data, the other three criteria can be applied in order to choose the most representative animal study for humans.

7.5.1.2 Key Studies for the Four Gases

According to the criteria discussed above, key studies are suggested for the four gases (Table 7.3).

Table 7.3 Suggested key studies for obtaining LC_{50} for humans

Gas	Species (No.)	Gas delivery	Exposure time (min)	Conc. (ppm)	Mortality (%)	LC_{50} (ppm)	Reference
H_2S	Rat (10)	Dynamic	60	400	0	712	MacEwen & Vernot 1972
				504	0		
				635	10		
				800	90		
Cl_2	Dog (9-23)	Dynamic	30	164	11	650	Underhill 1920
				491	29		
				600	40		
				710	67		
				819	61		
				928	91		
				1583	93		
NH_3	Rat (10)	Dynamic	60	9870	30	16600	Appelman et al. 1982
				10230	40		
				11300	50		
				12500	60		
				13240	70		
HF	Rat (10)	Dynamic	60	1087	0	1395	Wohlslagel et al. 1976
				1108	20		
				1405	30		
				1565	80		
				1765	100		

These four studies are relatively well designed with sample sizes of at least 10 for an experiment. Continuous gas delivery system and large exposure chambers have been used in these studies with continuous monitoring gas concentrations. The exposure time ranges from 30 to 60 minutes, which resembles the real accidental exposure. Most studies have been conducted on rats, while the study on chlorine has been done on dogs. As discussed above, in case data from large animals are not available, rats are thought to be the better animal model than mice.

7.5.1.3 LC_{50} for Humans

Once the LC_{50} from animals is determined, LC_{50} for humans can be obtained by applying uncertainty factor to this concentration. Usually LC_{50} for animals is divided by an uncertainty factor accounting for the inter- and intra-species difference in response to toxicants. The Netherlands Organisation of Applied Scientific Research (TNO 1992) proposed an extrapolation factor (f_d) that combines both a scaling factor and a safety factor accounting for the inter-species differences. A scaling factor represents the difference in the inhaled dose per unit lung surface area (or per unit body weight) between small animals and humans. A safety factor that is arbitrarily assigned to be 5 for local irritants and 10 for systemic acting agent is to account for the species difference in response. The safety factor is then multiplied by 2 to account for the possible increase in breathing-minute-volume due to stress in accidents. According to the different animal species, the extrapolation factor ranges from 0.2 to 0.5. In other words, the total uncertainty factor for inter-species difference ranges from 2 to 5-fold. This is close to the range of inter-species variability of 1-4-fold found in this analysis.

7.5.2 Deriving Toxic Load Exponent n

In order to derive exponent n, various exposure concentrations and durations have to be available in one study. If a study only contains one single exposure time (say 30 minutes), then it is impossible to derive a toxic load. Extensive literature review shows that majority of studies only investigate toxicity for one fixed time. ten Berge et al. (1986) selected about 20 available studies which contain various exposure concentration and duration to derive toxic load for about 20 chemicals. Some very old studies such as studies of Lehmann (1892) have to be used because there are few alternatives. The reliability of the exponent n derived from such old studies that only used one or two animals for an experiment is questionable. For example, exponent n for hydrogen sulfide derived from experiments on cats and rabbits (Lehmann 1892) by ten Berge et al. (1986) was 2.2, while another author calculated an n value of 8.2 from experiments on rats (Arts et al. 1989, Zwart et al. 1990). Recently, US EPA obtained an n of 4.36 by putting all available data on rats together (EPA 2000). What do these different n mean? How will different n values affect the estimate of risk after accidental release of toxic chemicals?

Compared to C × t relationship, $C^n t$ reflects the greater importance of concentration in relation to exposure time. In other words, C × t relationship indicates the effect of accumulative dose, while $C^n t$ relationship emphasizes the effect of dose rate. The biological mechanisms behind these two relationships are considered to relate to the detoxification process. Theoretically, for systemically acting agents such as hydrogen sulfide, the $C^n t$ relationship should be more dominant since it is rapidly detoxicified through oxidation after being absorbed into the blood. Primary local irritants should be more likely to have C × t relationship. However, according to ten Berge et al. (1986), there seems no difference in exponent n between local irritants and systemically acting substances.

Another interesting finding in the study of ten Berge et al. (1986) is that for the same chemical, the exponent n can be different when derived from different animals species. One example for this is that exponent n for hydrogen cyanide ranges from 1.6 to 4.3 according to different animal species used. In addition, the size of n seems not to be corresponding to the animal size. For example, n is 1.6 from rats, 1.8 from monkeys, 3.1 from dogs, and 4.3 from rabbits. Thus it raises a question whether exponent n is chemical-specific or animal-specific. If n is chemical-specific, then the n derived from animals can be directly used for humans. If n is animal-specific, application of n derived from animals to humans should involve some justification. However, current knowledge seems insufficient to answer these questions. More studies may need to be better designed to provide convincing explanations.

Although the choice of n is simply a mathematical data fitting process, the factors governing the size of n are not clear. In the case of hydrogen sulfide, which n should be chosen for human, 2.2, 4.36, or 8.0? This will be discussed in the next session.

7.5.3 Deriving Coefficient a and b

There is no general rule for setting coefficient a, and b in the probit equations. The process involved in the derivation of a, and b is usually not clearly provided by different authors. The Netherlands Organization of Applied Scientific Research (TNO 1992) provided a systemic approach for deriving a, and b. Their hypothesis is that the species difference in sensitivity can be expressed as a factor that influences the concentration C

or the exposure duration. From a calculation point of view, this factor always appears in the a-term. Thus, the difference in sensitivity between humans and animals only influences a-term, but not the b-term or the n-term in the probit equation. On the other hand, intra-species variation in response is expressed by the steepness of the probit function, which can be measured by the ratio of the toxic load ($C^n t$) corresponding to a response percentages as follows (TNO 1992):

$$(C^n t)_{95}/(C^n t)_5 = \exp (Y_{95}-Y_5)/b \qquad (7.1)$$

where Y is probit.

From equation (7.1), higher b indicates smaller intra-species difference in response. For the four gases selected in this thesis, b derived from animal studies ranges from 0.71 to 2.89 (ten Berge et al. 1986) and b values proposed for humans range from 0.5 to 3.354 (see Table 2.4). According to equation (7.1), if b = 0.5, the LL_{95}/LL_5 = 706; if b = 1.0, the LL_{95}/LL_5 = 27, and if b = 3.354, the LL_{95}/LL_5 = 2.6. From animal studies on the four gases, the ratio of LL_{90}/LL_{10} ranges from 1.5 to 5 dependent on different animal species, as discussed earlier in this chapter. The ratio of LL_{95}/LL_5 should be greater than this range although the relatively small sample size precludes demonstrating the size of this difference by animal experiments. Furthermore, human inter-individual variability in response is considered to be greater than animals. Thus, as suggested by TNO (1992), the ratio of 27 may reasonably represent human variability in response corresponding to value of b equal to 1.0 for humans.

Once the toxic load ($C^n t$) and b are determined from an analysis of the experimental data, the coefficient a is easy to calculate according to the probit function, that is,

$$a = Y - b(\ln C^n t) \qquad (7.2)$$

As mentioned earlier in this thesis, exponent n of 2.2 was derived from an old study on cats and rabbit (Lehmann 1892). The exposure durations in this study ranged from 3 to 8 hours for fatality. When considering the static gas delivery system employed in this study, for such long period of exposure, the desired concentrations obviously could not

be maintained. Thus, the inaccurately reported exposure concentrations are expected to distort the real relationship between concentration and time. In addition, in this study, only one or two animals were used for one experiment. This small sample size will cause wider confidence interval of the estimate. An exponent n of 4.36 proposed by EPA (2000) was derived from relatively recent studies, which have larger sample size and much improved experimental conditions. Furthermore, an exponent n of 4 was also proposed by HSE (Turner and Fairhurst 1990c) for hydrogen sulfide based on the studies on rats. An exponent n around 4 for hydrogen sulfide is considered to be a reasonable choice based on the limited animal experimental database available.

Several probit equations have been proposed for humans by different authors, which are presented in Table 2.5 and Figure 5.6 (for hydrogen sulfide), 5.11 (for chlorine), 5.16 (for ammonia), and 5.21 (for hydrogen fluoride). The plotting of proposed toxic load on the background of lethal dose of animals in Figure 5.6, 5.11, 5.16, and 5.21 is actually to use real animal lethal data to measure the validity of proposed toxic load relationship.

Rogers (1990) did the similar plotting for hydrogen sulfide (Figure B-1, B-2, B-3). In his background plotting, however, the data points were not from the same levels of fatality. The percentages of fatality on the graph ranged from 10% to 100% (Rogers 1990, Table B-1). The toxic load equations plotted on the background graph were L_1, L_{50}, and L_{99} (Rogers 1990, Figure B-2). In this thesis, the data points from different fatality percentages were plotted separately, e.g., 10% fatality in Figure 5.3, 50% fatality in Figure 5.4, 90% fatality in Figure 5.5, for hydrogen sulfide. Accordingly, the toxic load equations for 90% fatality were plotted on the 90% fatality graph. As discussed in Chapter 2, the toxic load plot has to be demonstrated at the fixed level of fatality, e.g., 10%, 50%, or 90%. Therefore, compared to Rogers' study, the approach for the toxic load plotting in this thesis is much more meaningful. The toxic load figures in this thesis provide more informative pictures of fatal effects of the four gases on the different animal species.

As shown in Figure 5.6, 5.11, 5.16, and 5.21, all proposed toxic load equations for humans are within the range of available animal data. Some are more conservative, while others are less conservative. In the industrial practice, when the previous equations did not satisfy the industrial need, some other new equations were developed. However, it is

difficult to judge the quality of these equations. Ultimately, the choice for which equation to use cannot be made on strictly scientific grounds, this choice depends on how cautious the risk management is intended to be. Choosing how cautious to be with any risk is not a choice that scientific analysis can resolve.

Chapter 8

Conclusions and Recommendations

8.1 Conclusions

(1) Studies dealing with variability in susceptibility of different species to toxic gases are very limited and weak. Some old data on experimental animals have been inaccurately represented in more recent publications. For example, lethal concentrations and exposure times obtained from dogs have been reported as human data (Table 5.4).

(2) Based on the limited data of acute toxicity, inter-species variability in response to the four gases selected in this thesis is about 3-fold. This relatively small difference among species may be due to the similarity in local irritation effects of the four gases which may cancel out other factors involved in sensitivity. The default 10-fold uncertainty factor may overestimate the inter-species difference in response to the four toxic irritants. An uncertainty factor of 3 is considered to be reasonable to account for differences between animals and humans.

(3) Intra-species variability, indicated by LC_{90}/LC_{10} ratio, varies from one species to another. Intra-species variability is smaller in rats and mice, and greater in dogs, which ranges from 1.5 for lab rats to 10-fold for dogs with larger genetic difference than lab rats. Human inter-individual variability is supposed to be greater than that in animals. The default 10-fold uncertainty may underestimate human inter-individual variability. A maximum uncertainty factor of 30 is suggested to represent maximum human natural variability in response to the four toxic gases.

(4) There is a great variation in odor detection threshold, a measure of human olfactory sensitivity. The variation can be from several orders of magnitude up to a reported 10^{16}-fold for different odorants. Variability of odor detection threshold of hydrogen sulfide among young adults has been calculated in the present study to be 400-fold. Odor data on the other three gases are very limited.

(5) Toxicokinetic studies on the four gases are very limited. There is no quantitative description on the process of absorption, distribution, metabolism, and excretion of the four gases. Thus, there is no experimental evidence on which to base any biological parameters to modify the current toxic load model.

(6) It is important to recognize that LC values are a special case of toxic load for comparing species sensitivity and applying uncertainty factor. The ratio of LC values for different species is the measure of inter-species variability for toxic loads at the same exposure time. The ratio of different percentage LC values (LL_{90}/LL_{10}) is one measure of intra-species variability.

(7) This analysis proposed several criteria to choose key animal studies to obtain LC_{50} values for humans based on the animal species used in the experiments, quality of studies, and similarity of exposure conditions in the experiments to the real exposure scenario. Other relevant animal studies also need to be considered in terms of inter-species variability.

(8) Differences in the size of exponent n in the toxic load relationship is simply the result of data fitting to experimental data and the validity of any n value is determined by the validity of the data used. An n of 4 is suggested for hydrogen sulfide in this analysis because that value is derived from an analysis of the best available experimental data.

(9) Coefficient a is affected by inter-species variability, while b is influenced by intra-species variability. The coefficient b is suggested to be 1 from this analysis, which corresponds to a 27-fold range of human inter-individual variability.

(10) Current knowledge does not provide an evidentiary basis to propose a toxic load relationship and probit equation for non-lethal injuries. Previous approaches have been based on judgements about appropriate degree of caution applied to quantitative relationships for fatality and those approaches likely remain as the only viable basis for choosing an equation for non-lethal injuries.

8.2 Recommendations for Future Research

Although the four gases selected in this analysis are heavily used in the industry and are demonstrably toxic to humans having caused fatalities, information of inhalation toxicology of these four gases are still surprisingly lacking. Risk assessment and management of four industrial gases have been done based on these insufficient data for several decades. Rather than continuing to talk about lack of data, targeted research should be undertaken to develop the required data.

With respect to toxicokinetics, quantitative data on the extent and rate of absorption, distribution, metabolism, and excretion of the four gases are very limited. This makes it impossible to take biological processes into consideration in terms of modification of the toxic load model. In regard to toxicodynamics of the four gases, most recent studies on acute toxicity have been done in small animals such as rodents, while some old studies have been conducted in large animals such as dogs and monkeys. Few studies have been done in both small animals and large animals under the same experimental conditions. Comparison of species difference in responses based on the studies done under different conditions is obviously not reliable because factors other than real biological variability also contribute to the difference in parameters measured (e.g., LC_{50}). In addition, in many animal studies only exposure concentrations are variable, while exposure durations are usually fixed, thereby limiting the choice of available studies to derive a toxic load relationship. Therefore, more well designed studies need to be done to bridge the huge gap between required basic data and demands for more rational regulation of these four gases. The following recommendation for future research is primarily focused on improving the toxic load model.

8.2.1 Toxicokinetic Study

8.2.1.1 Human Study

Human experimental studies on the toxicokinetics of the four gases need to be done in the following areas:

- The extent and rate of absorption of hydrogen sulfide after inhalation exposure

- Continuous measurement of the concentration of hydrogen sulfide or sulfide in the blood and other tissues following inhalation exposure
- Continuous measurement of the concentration of thiosulfate or sulfate in urine following inhalation exposure
- Estimate of biological half-life of hydrogen sulfide

8.2.1.2 Animal Study

The parameters need to be measured are similar to that of humans as mentioned above, but animal experiments need to be conducted under different exposure conditions. Exposure concentrations should cover a wide range from low level that will be encountered in the normal occupational sites to the high level that is possible to see in an accidental release. Exposure durations also need to include short time (a few minutes) to longer time (few hours to 24 hours). The combination of different exposure conditions could more efficiently describe the whole biological processes, and provide reasonable estimate for humans to compensate for the practical and ethical constraints on acquiring better human data.

Animal species should include small animals such as rodents as well as large animals such as dogs and monkeys.

8.2.2 Acute Toxicity Study

8.2.2.1 Human Study

Numerous human accident reports document that humans have been exposed to high levels of toxic gases. Unfortunately the actual ambient concentrations of toxicants and exposure durations are usually unknown. Further research should explore how exposure concentrations can be reconstructed after accidents. The equipment for immediately collecting air samples should be developed and placed in the occupational sites or residential areas where accidental release is possible. The most practical possibility is to collect information on the mass of gas releases and on the weather conditions and to simulate the release in a model. Even if we can estimate a few such real exposure concentrations, the confidence about the estimate of human risk following accidental exposure will be substantially increased.

8.2.2.2 Animal Study

Most recent animal toxicity studies have been conducted in rodents because of some advantages mentioned in the previous chapters. Although these studies are well designed, and experimental conditions are well controlled, it always remains in doubt how useful and reliable the data obtained from rodents are for of application to humans. To make more accurate estimates of human risk following acute exposure to these chemicals, some studies on large animals, especially dogs or monkeys are needed because of the similarity in the respiratory anatomy and physiology between humans and dogs or monkeys. Obviously practicality does not allow always using large animals to do animal experiments. However, for each chemical selected in this analysis, at least one well-designed large animal study needs to be done. The components of such study include:

- Application of wide range of animal species from small animals to large animals including mice, rats, guinea pigs, cats, rabbits, dogs, monkeys, and goats in different age, sex, strains, diet and health status to reveal the true species variability in response and find out vulnerable subpopulation;
- Application of large animal sample size with at least 100 animals of each species are needed to demonstrate the distribution form of the response of different species, calculate response rate more accurate, and provide better estimate of human response;
- Application of a wide range of exposure concentrations and durations to demonstrate the true concentration-time-mortality relationship and to provide a solid basis for deriving a toxic load model;
- Application of the most advanced experimental equipment to ensure accurate results, which include large head- or nose-only exposure chambers, dynamic gas delivery system, continuous concentration monitoring system, inert sample collecting devices, and advanced analytical methods.

The results of such studies would substantially improve the current level of knowledge to provide risk assessors and decision makers with a much better basis for managing the acute risks from these toxic industrial gases.

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Appendix

Lethality Data for the Four Gases

Table A Lethality data for hydrogen sulfide

Species	# Exposed	% Fatality	Conc (ppm)	Time (min)	Time (sec)	Reference
10% (1-20%) fatality						
Rat	10	10	635	60	3600	MacEwen & Vernot 1972
	8	12.5	250	1074	64440	Weedon et al. 1940
Mouse	20	5	800	30	1800	Clanechan 1979
	20	10	900	15	900	Clanechan 1979
	46	20	1000	10	600	Clanechan 1979
	20	5	1100	2.5	150	Clanechan 1979
	20	20	1100	5	300	Clanechan 1979
	20	10	1200	2.5	150	Clanechan 1979
	20	15	1300	2.5	150	Clanechan 1979
	10	20	400	60	3600	MacEwen & Vernot 1972
Human	10	10	1000	< 1	60	Prouza 1970
50% (40-60%) fatality						
Rat	2	50	450	240	14400	Mitchell & Yant 1925
Mouse	20	60	1000	30	1800	Clanechan 1979
	20	40	1100	7.5	450	Clanechan 1979
	46	54	1100	10	600	Clanechan 1979
	20	60	1300	5	300	Clanechan 1979
	10	50	635	60	3600	MacEwen & Vernot 1972
Guinea pig	2	50	1500	30	1800	Mitchell & Yant 1925
Dog	2	50	800	60	3600	Mitchell & Yant 1925
90%(80-100%) fatality						
Rat	12	100	300	360	21600	Alberta Environmental Centre 1986
	5	100	1665	3	180	Lopez et al. 1989
	10	90	800	60	3600	MacEwen & Vernot 1972
	10	80	500	240	14400	Tansy et al. 1981
	10	80	525	240	14400	Tansy et al. 1981
	10	90	554	240	14400	Tansy et al. 1981
	10	100	600	240	14400	Tansy et al. 1981
	8	100	1000	37	2200	Weedon et al. 1940
Mouse	20	85	1100	30	1800	Clanechan 1979
	20	85	1200	12.5	750	Clanechan 1979
	20	95	1200	15	900	Clanechan 1979
	20	100	1200	30	1800	Clanechan 1979
	20	85	1300	7.5	450	Clanechan 1979
	46	95	1300	10	600	Clanechan 1979

Table A Lethality data for hydrogen sulfide (Continued)

Species	# Exposed	% Fatality	Conc (ppm)	Time (min)	Time (sec)	Reference
Mouse	20	100	1300	12.5	750	Clanechan 1979
	20	100	1300	15	900	Clanechan 1979
	20	100	1300	30	1800	Clanechan 1979
	10	80	800	60	3600	MacEwen & Vernot 1972
	4	100	1000	20	1200	Weedon et al. 1940
	4	100	250	420	25200	Weedon et al. 1940
Guinea pig	1	100	470	530	3180	Lehmann 1892
	1	100	1300	90	5400	Lehmann 1892
	3	100	350	1080	64800	Mitchell & Yant 1925
Rabbit	1	100	500	75	4500	Biefel & Polek 1880
	1	100	470	375	22500	Lehmann 1892
	1	100	750	265	15900	Lehmann 1892
	1	100	710	230	13800	Lehmann 1892
Cat	1	100	1100	30	180	Eulenberg 1865
	1	100	720	330	1980	Lehmann 1892
	1	100	710	489	29340	Lehmann 1892
Dog	1	100	900	< 60	3600	Haggard 1925
	1	100	1500	30	1800	Haggard 1925
	1	100	1800	0.02	1	Haggard 1925
	2	100	103	960	5760	Mitchell & Yant 1925
	2	100	240	960	5760	Mitchell & Yant 1925
	2	100	350	480	28800	Mitchell & Yant 1925
	3	100	890	30	1800	Mitchell & Yant 1925
	8	100	1140	30	1800	Mitchell & Yant 1925
	4	100	1280	30	1800	Mitchell & Yant 1925
	9	100	1500	30	1800	Mitchell & Yant 1925
Monkey	1	100	500	35	2100	Lund & Wieland 1966
Human	1	100	1000	0.03	2	NIOSH 1977
	1	100	6100	< 5	300	Winek et al. 1968
Pig	1	100	400	0.02	1	O'Donoghue 1961
Goat	4	100	1100	30	1800	Mitchell & Yant 1925
	4	100	1330	30	1800	Mitchell & Yant 1925
Canary	2	100	35	1080	64800	Mitchell & Yant 1925
	6	100	97	480	28800	Mitchell & Yant 1925
	4	100	140	480	28800	Mitchell & Yant 1925
	?	100	730		20	Mitchell & Yant 1925
Dove	1	100	70	4	240	Eulenberg 1865

Table B Lethality data for chlorine

Species	# Exposed	% Fatality	Conc (ppm)	Time (min)	Reference
10%(1-20%) fatality					
Rat	8	12.5	1000	20	Weedon et al. 1940
	8	12.5	250	384	Weedon et al. 1940
	10	10	1654	10	Zwart & Woutersen 1988
Mouse	14	2	290	6	Bitron and Aharonson. 1978
	14	13	170	22	Bitron and Aharonson. 1978
	10	10	62	30	Schlagbauer & Henschler 1967
Dog	9-23	11	164	30	Underhill 1920
50%(40-60%) fatality					
Rat	10	60	2248	10	Zwart & Woutersen 1988
	10	50	606	30	Zwart & Woutersen 1988
	10	60	645	30	Zwart & Woutersen 1988
	10	40	457	60	Zwart & Woutersen 1988
	10	60	508	60	Zwart & Woutersen 1988
Mouse	14	40	290	9	Bitron and Aharonson. 1978
	14	49	170	52	Bitron and Aharonson. 1978
	10	60	132	30	Schlagbauer & Henschler 1967
	10	60	143	30	Schlagbauer & Henschler 1967
	10	40	1202	10	Zwart & Woutersen 1988
	10	40	458	30	Zwart & Woutersen 1988
	10	60	574	30	Zwart & Woutersen 1988
Dog	9-23	40	600	30	Underhill 1920
90%(80-100%) fatality					
Rat	10	80	338	60	MacEvan & Vernot 1972, Vernot et al. 1977
	10	100	427	60	MacEvan & Vernot 1972, Vernot et al. 1977
	8	87.5	1000	102	Weedon et al. 1940
	8	87.5	250	960	Weedon et al. 1940
	10	80	569	60	Zwart & Woutersen 1988
	10	100	595	60	Zwart & Woutersen 1988

Table B Lethality data for chlorine (Continued)

Species	# Exposed	% Fatality	Conc (ppm)	Time (min)	Reference
Mouse	14	93	290	25	Bitron and Aharonson. 1978
	10	80	159	60	MacEwan & Vernot 1972, Vernot et al. 1977
	10	100	193	60	MacEwan & Vernot 1972, Vernot et al. 1977
	10	80	160	30	Schlagbauer & Henschler 1967
	10	100	179	30	Schlagbauer & Henschler 1967
	10	100	22	180	Schlagbauer & Henschler 1967
	10	80	10	180	Schlagbauer & Henschler 1967
	10	90	10	360	Schlagbauer & Henschler 1967
	4	100	250	504	Weedon et al. 1940
	10	100	1319	10	Zwart & Woutersen 1988
	10	100	1654	10	Zwart & Woutersen 1988
	10	90	606	30	Zwart & Woutersen 1988
Rabbit	1	100	2800-6300	65	Lehmann 1887
Guinea pig	1	100	3200	190	Lehmann 1887
	1	100	3300	65	Lehmann 1887
	1	100	8100	50	Lehmann 1887
Cat	1	100	2800-6300	65	Lehmann 1887
Dog	9-23	91	928	30	Underhill 1920
	9-23	93	1583	30	Underhill 1920

Table C Fatality data for ammonia

Species	# Exposed	% Fatality	Conc (ppm)	Time (min)	Reference
10%(1-20%) fatality					
Rat	10	10	33500	10	Appelman et al. 1982
	10	20	26200	20	Appelman et al. 1982
	10	10	27265	20	Appelman et al. 1982
Mouse	20-25	5	4310	120	Alpatov & Michailov 1963
	20-26	20	5020	120	Alpatov & Michailov 1963
Cat	5	20	10000	10	Dodd & Gross 1980
50%(40-60%) fatality					
Rat	10	60	37840	10	Appelman et al. 1982
	10	50	39000	10	Appelman et al. 1982
	10	60	30690	20	Appelman et al. 1982
	10	50	19210	40	Appelman et al. 1982
	10	50	22740	40	Appelman et al. 1982
	10	40	14660	60	Appelman et al. 1982
	10	50	16190	60	Appelman et al. 1982
	10	60	17900	60	Appelman et al. 1982
Mouse	20-27	40	5740	120	Alpatov & Michailov 1963
	20-28	45	6460	120	Alpatov & Michailov 1963
	12	41	4220	60	Kapeghian et al. 1982
	20	55	10200	10	Silver & McGrath 1948
	20	55	11340	10	Silver & McGrath 1948
90%(80-100%) fatality					
Rat	10	90	54200	10	Appelman et al. 1982
	10	90	33240	20	Appelman et al. 1982
	10	80	23340	40	Appelman et al. 1982
	10	80	7820	60	MacEwan & Vernot 1972
	10	90	9840	60	MacEwan & Vernot 1972
Mouse	20-30	84	8900	120	Alpatov & Michailov 1963
	20-31	100	8620	120	Alpatov & Michailov 1963
	12	100	4860	60	Kapeghian et al. 1982
	12	83	4860	60	Kapeghian et al. 1982
	10	90	5720	60	MacEwan & Vernot 1972
	20	85	10910	10	Silver & McGrath 1948
	20	80	11920	10	Silver & McGrath 1948
	20	80	12920	10	Silver & McGrath 1948
	20	84	11000	40	Stupfel et al. 1971

Table D Fatality data for hydrogen fluoride

Species	# Exposed	% Fatality	Conc (ppm)	Time (min)	Reference
10%(1-20%) fatality					
Rat	10	10	12440	5	Higgins et al. 1972
	10	20	1108	60	Wohlslagel et al. 1976
Mouse	10	10	278	60	Wohlslagel et al. 1976
50%(40-60%) fatality					
Rat	4	50	2036	60	Haskell Laboratory 1990
	5	60	1097	60	MacEwan & Vernot 1976, Vernot et al. 1977
Mouse	15	53	8140	5	Higgins et al. 1972
	10	50	438	60	MacEwan & Vernot 1976, Vernot et al. 1977
	10	60	518	60	MacEwan & Vernot 1976, Vernot et al. 1977
	10	60	381	60	Wohlslagel et al. 1976
90%(80-100%) fatality					
Rat	4	100	2734	60	Haskell Laboratory 1990
	4	100	2617	60	Haskell Laboratory 1990
	10	80	18580	5	Higgins et al. 1972
	10	100	25690	5	Higgins et al. 1972
	5	100	1576	60	MacEwan & Vernot 1976, Vernot et al. 1977
	10	80	1565	60	Wohlslagel et al. 1976
	10	100	1765	60	Wohlslagel et al. 1976
Mouse	15	33	4500	5	Higgins et al. 1972
	15	100	11010	5	Higgins et al. 1972
	10	90	505	60	MacEwan & Vernot 1976, Vernot et al. 1977
	10	100	633	60	MacEwan & Vernot 1976, Vernot et al. 1977
	10	90	458	60	Wohlslagel et al. 1976
Guinea pig	5	100	660	30	Ronzani 1909
	5	100	250	60	Ronzani 1909
	5	100	50	120	Ronzani 1909
Rabbit	5	100	660	90	Ronzani 1909
	5	100	250	180	Ronzani 1909